INTERNATIONAL SYMPOSIA VIRTUAL

Stem Cells: From Basic Science to Clinical Translation

27 - 29 October 2021

ISSCR

TOKYO JAPAN

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ISSCR DIGITAL SERIES

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Stem Cells and Regenerative Medicine
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Translating Pluripotent Stem Cell Discoveries to the Clinic: Preclinical, Manufacturing, and Regulatory Strategies for Success
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Stem Cells: From Genes to Organs
30-31 March 2022

ISSCR/ASGCT MADISON INTERNATIONAL SYMPOSIUM
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ISSCR

Learn more at **isscr.org/meetings**

Welcome

Dear Colleagues,

On behalf of the International Society for Stem Cell Research (ISSCR) and the Japanese Society for Regenerative Medicine (JSRM), we warmly welcome you to the International Symposium, “Stem Cells: From Basic Science to Clinical Translation.” We look forward to showcasing the research that is taking place in Japan, a driving force in stem cell research that is transforming the field of regenerative medicine, and around the world during this virtual meeting.

Stem cell science is rapidly advancing and shows great potential to transform human health. This exciting symposium will highlight progress along the continuum of stem cell science that is helping progress stem cell therapies to the clinic. Over the course of three days, leading scientists will present breakthroughs in stem cell research that span from basic science to clinical application, all with an eye towards therapeutic goals.

During this symposium you will learn about advances in stem cell modeling and technology that reveal novel insights into developmental plasticity, cell identity, and tissue organization, hearing from experts who are using innovative methods to dissect development from the earliest stages of embryogenesis to the acquisition of exceptional cellular complexity. Speakers will explore progress in research using organoids, tissue engineering, and new disease models that may uncover novel insights into disease mechanisms and identify potential new treatments. Discover groundbreaking work that is pushing forward gene therapy, genome editing, and cellular therapies, culminating in a lively panel discussion on how to bring these new therapies to market. Throughout the meeting, prominent ethicists will discuss the related ethical considerations of scientific advances from chimeras to organoids to genome editing.

Take part in the Early Career Investigator Session, organized by the JSRM Diversity Committee to promote the exchange of information and ideas between young researchers in Japan and abroad. In breakout rooms, early career researchers can ask questions, exchange ideas, and network. Continue these conversations when browsing over 200 posters and engaging with your peers during two Poster Sessions. You can also set up meetings with other attendees in the meeting platform to network with stem cell researchers, discuss specific research areas, or explore potential collaborations. Don’t miss the latest scientific tools and products that can enhance your research projects at the virtual Exhibit Hall.

Thank you for joining us for this virtual International Symposium. You are a valued member of the global stem cell community, and together we can advance the therapeutic potential of regenerative medicine in Japan and worldwide.

Sincerely,

The Tokyo Organizing Committee

Nissim Benvenisty, MD, PhD, *Hebrew University, Israel*

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Shinya Yamanaka, MD, PhD, *Gladstone Institutes, USA and Center for iPS Cell Research & Application, Kyoto University, Japan*

ABOUT THE ISSCR

The mission of the International Society for Stem Cell Research (ISSCR) is to promote excellence in stem cell science and applications to human health.

The ISSCR is the largest society in the world dedicated to the advancement of responsible stem cell research–a field that strives to advance scientific understanding, treatments, and cures that better human health. We foster junior scientists, give voice and visibility to scientific advancement, and encourage a positive global environment for future discovery and treatment. Our promise is to help the field of stem cell research reach its potential.

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ABOUT JSRM

The mission of the Japanese Society for Regenerative Medicine is Orchestrating Wisdom to Innovate, Universalize (Regenerative Medicine), for the Happiness and Future of All Human Beings.

The JSRM is a comprehensive regenerative medicine organization comprised of basic researchers, clinicians, and engineers from academia, industry, and government, as well as experts in ethics, regulation, and law. The JSRM not only promotes research, but also actively engages in activities such as policy advocacy and human resource development etc.

Contact Us

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CODE OF CONDUCT

The ISSCR is committed to providing a safe and productive meeting environment that fosters open dialogue and discussion and the exchange of scientific ideas, while promoting respect and equal treatment for all participants, free of harassment and discrimination.

All participants are expected to treat others with respect and consideration during the virtual event. Attendees are expected to uphold the standards of scientific integrity and professional ethics.

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ISSCR prohibits any form of harassment, sexual or otherwise. Incidents should immediately be reported to ISSCR/JSRM at **tis2021@congre.co.jp**.

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By registering for this meeting, you agree to ISSCR/JSRM's Recording Policy. The ISSCR/JSRM strictly prohibits the recording (photographic, screen capture, audio, and/or video), copying, or downloading of scientific results from the sessions, presentations, and/or posters at the ISSCR/JSRM Tokyo International Symposium.

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Table of Contents

BOARD OF DIRECTORS AND ORGANIZERS 4-5

PROGRAM SCHEDULE 7-13

Wednesday, 27 October 7-8

Thursday, 28 October 9-10

Friday, 29 October 11-13

SPONSORS AND EXHIBITORS 15-18

SPONSORED CONTENT: MINI-SYMPOSIA 19

SPEAKER ABSTRACTS 21-38

Wednesday, 27 October 21-24

Thursday, 28 October 25-31

Friday, 29 October 31-38

POSTER ABSTRACTS 39-132

AUTHOR INDEX 133-135

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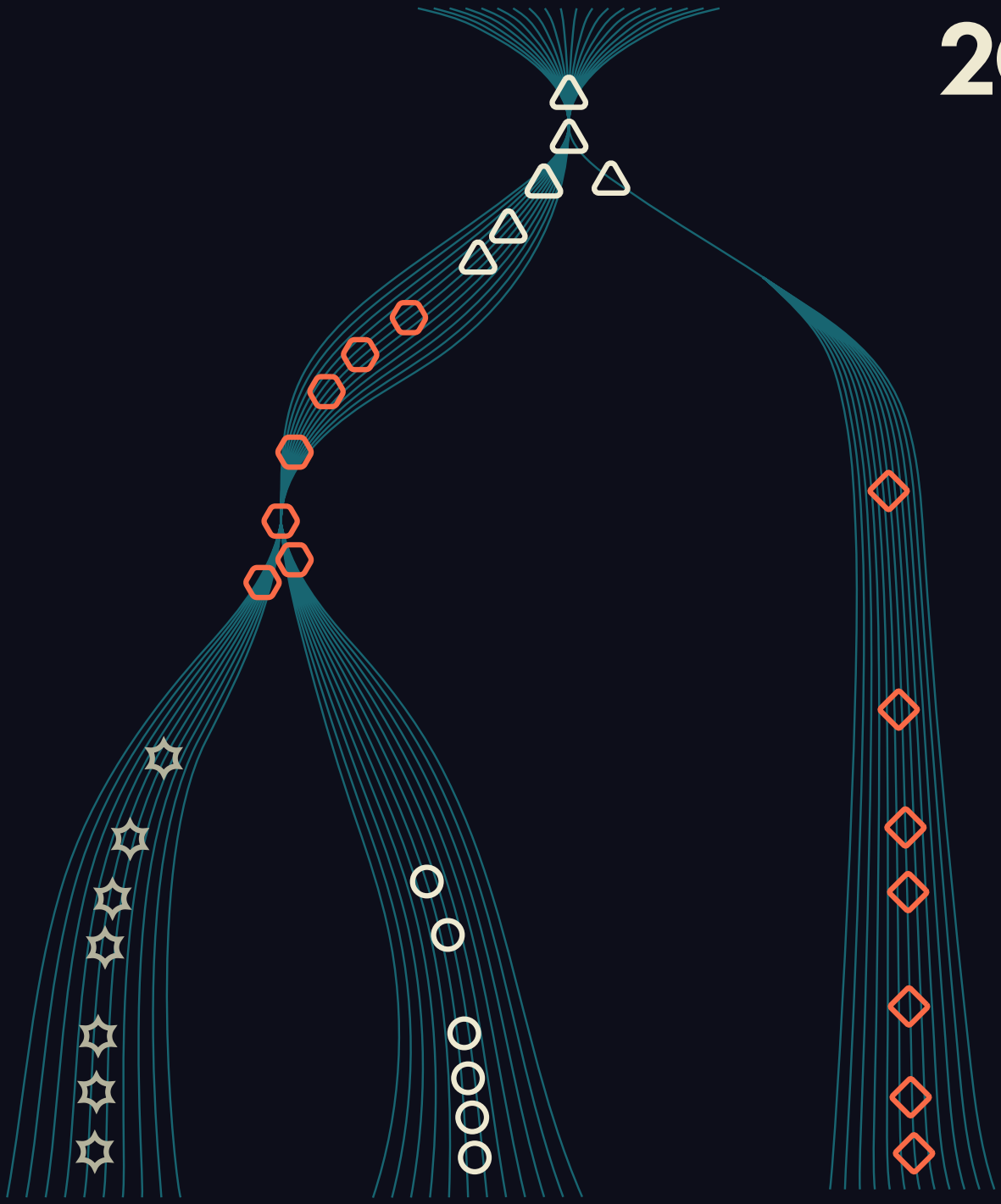

#ISSCR2022

ISSCR ANNUAL MEETING 2022

SAN FRANCISCO

SAVE THE DATE

15–18 JUNE 2022

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ISSCR 2022

Program Schedule

Wednesday, 27 October

09:00 — 09:10

WELCOME REMARKS
Hideyuki Okano, *Keio University School of Medicine, Japan*
Keith Alm, *ISSCR, CEO*

09:15 – 10:45 STEM CELLS AND DEVELOPMENT

Chair: **Joshua Currie**, *Wake Forest University, USA*

09:15 – 09:45 **Magdalena Zernicka-Goetz**, *University of Cambridge, UK and California Technical Institute, USA*
PRINCIPLES OF EMBRYO SELF-ASSEMBLY IN VITRO AND IN VIVO

09:45 – 10:00 **Ritsuko Morita**, *RIKEN Center for Biosystems Dynamics Research, Japan*
TRACING THE ORIGIN OF HAIR FOLLICLE STEM CELLS

10:00 – 10:15 **Atsushi Asakura**, *University of Minnesota Medical School, USA*
ENDOTHELIAL CELL SIGNATURE AND POTENTIAL OF MOUSE SKELETAL MUSCLE STEM CELLS

10:15 – 10:45 **Yukiko Gotoh**, *University of Tokyo, Japan*
ESTABLISHMENT OF EMBRYONIC ORIGIN OF ADULT NEURAL STEM CELLS AT THE SUBVENTRICULAR ZONE

10:45 – 11:15 BREAK

All times are listed in Japanese Time Zone.

Program Schedule

Wednesday, 27 October

11:15 – 12:45 ADVANCED TECHNOLOGIES OF STEM CELL BIOLOGY

Chair: **Yukiko Gotoh**, *University of Tokyo, Japan*

11:15 – 11:45 **Atsushi Miyawaki**, *RIKEN Center for Brain Science & RIKEN Center for Advanced Photonics, Japan*
GENETICALLY ENCODED TOOLS FOR STEM CELL RESEARCH AND REGENERATIVE MEDICINE

11:45 – 12:00 **Yosuke Yoneyama**, *Tokyo Medical and Dental University, Japan*
INTERCELLULAR RNA TRANSFER-DRIVEN REPROGRAMMING OF HUMAN PLURIPOTENT STEM CELLS INTO NAÏVE-LIKE STATE

12:00 – 12:15 **Shintaro Watanuki**, *National Center for Global Health and Medicine, Japan*
AGE-RELATED MITOCHONDRIAL METABOLIC FITNESS ENDORSES CELLULAR COMPETENCE TO OLD HEMATOPEOIETIC STEM CELLS

12:15 – 12:45 **Barbara Treutlein**, *ETH Zürich, Switzerland*
TRACING AND PERTURBING LINEAGES DURING HUMAN BRAIN ORGANOID DEVELOPMENT

12:45 – 13:15 BREAK

13:15 – 14:15 OPENING KEYNOTE SESSION

Chair and Introduction: **Hideyuki Okano**, *Keio University School of Medicine, Japan*

Keynote Address
Shinya Yamanaka, *Gladstone Institutes, USA and Center for iPS Cell Research & Application, Kyoto University, Japan*
RECENT PROGRESS IN IPS CELL RESEARCH AND APPLICATION

14:15 – 15:15 POSTER SESSION I

Odd numbered posters

Thursday, 28 October

09:00 – 11:00 ORGANOIDS: FROM BASIC BIOLOGY TO CLINICAL TRANSLATION

Chair: **Shiro Yui**, *Tokyo Dental and Medical University, Japan*

09:00 – 09:30 **Sergiu Pasca**, *Stanford University, USA*
FROM STEM CELLS TO BRAIN ASSEMBLOIDS: CONSTRUCTING AND DECONSTRUCTING THE HUMAN NERVOUS SYSTEM

09:30 – 09:45 **Kent Imaizumi**, *Keio University, Japan*
SIZE-SCALED PATTERNING OF NEURAL ORGANOIDS

09:45 – 10:00 **Yonatan Lewis Israeli**, *Michigan State University, USA*
GENERATION OF SELF ASSEMBLING HUMAN HEART ORGANOIDS FOR MODELING DEVELOPMENT AND CONGENITAL HEART DISEASE

10:00 – 10:30 **Takanori Takebe**, *Tokyo Medical and Dental University, Japan and Cincinnati Children's Hospital, USA*
HEPATO-BILIARY-PANCREATIC ORGANOGENESIS IN A DISH

10:30 – 11:00 **Misao Fijuta**, *Center for iPS Cell Research and Application, Kyoto University, Japan*
ETHICS OF HUMAN BRAIN ORGANOID RESEARCH

11:00 – 11:30 BREAK

11:30 – 13:00 STEM CELL-BASED DISEASE MODELLING AND DRUG DEVELOPMENT

Chair: **Robert Blelloch**, *University of California, San Francisco, USA*

11:30 – 12:00 **Fred H. Gage**, Salk Institute for Biological Studies, USA
AGE RELATED DYSFUNCTION IN IN-DERIVED NEURONS FROM SPORADIC ALZHEIMER'S PATIENTS

12:00 – 12:15 **Rie Ouchi**, *Tokyo Medical and Dental University, Japan*
MODELING STEATOHEPATITIS WITH MULTICELLULAR ORGANOIDS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

12:15 – 12:30 **Roni Sarel Gallily**, *The Hebrew University, Israel*
ANALYSIS OF HAPLOINSUFFICIENCY DISORDERS IN HUMAN EMBRYONIC STEM CELLS

12:30 – 13:00 **Hideyuki Okano**, *Keio University School of Medicine, Japan*
IPSCS-BASED MODELING, DRUG DEVELOPMENT AND CLINICAL TRIALS FOR ALS

Program Schedule

Thursday, 28 October

13:00 – 13:30 BREAK

13:30 – 15:30 TISSUE ENGINEERING

Chair: **Nissim Benvenisty**, *The Hebrew University of Jerusalem, Israel*

13:30 – 14:00 **Hiromitsu Nakauchi**, *Stanford University, USA*
NOVEL APPROACH FOR IN VIVO ORGANOGENESIS: DEVELOPMENTAL COMPLEMENTATION VS CELL COMPETITION

14:00 – 14:15 **Ge Guo**, *University of Exeter, UK*
MODELLING EARLY HUMAN EMBRYO DEVELOPMENT USING NAÏVE PLURIPOTENT STEM CELLS

14:15 – 14:30 **Svetlan Vassilev**, *Agency for Science, Technology and Research, Singapore*
SELECTION OF O-VE IPSC CLONES FOR HIGH-DENSITY RED BLOOD CELL PRODUCTION IN A SCALABLE PERFUSION BIOREACTOR SYSTEM

14:30 – 15:00 **Yoshimi Yashiro**, *Kanagawa University of Human Services, Japan*
CONSIDERING THE GAP BETWEEN SCIENTISTS' AND THE GENERAL PUBLIC'S PERCEPTIONS OF REGENERATIVE MEDICINE USING HUMAN-ANIMAL CHIMERA RESEARCH AS AN EXAMPLE

15:00 – 15:30 **Matthias Lutolf**, *EPFL, Lausanne, Switzerland*
ENGINEERING EPITHELIAL ORGANOID DEVELOPMENT

15:30 – 16:30 POSTER SESSION II

Even numbered posters

Friday, 29 October

08:00 – 09:00 CLOSING KEYNOTE SESSION

Chair: **Hiromitsu Nakauchi**, *Stanford University, USA*

Closing Keynote
Carl June, *Perelman School of Medicine and Parker Institute for Cancer Immunotherapy at the University of Pennsylvania, USA*
CAR T CELLS: THE CONVERGENCE OF SYNTHETIC BIOLOGY AND CANCER IMMUNOTHERAPY

09:00 – 10:30 GENE THERAPY AND GENOME EDITING

Chair: **Hiromitsu Nakauchi**, *Stanford University, USA*

09:00 – 09:30 **Katherine High**, *AskBio, USA*
GENE THERAPY FOR GENETIC DISEASE-ADVANCES AND HURDLES IN AAV-MEDIATED GENE THERAPY

09:30 – 09:45 **Sho Yoshimatsu**, *Kyushu University, Japan*
A NEW HORIZON OF MARMOSET RESEARCH USING HIGHLY EFFICIENT KNOCK-IN AND PLURIPOTENT STEM CELL-BASED TECHNOLOGIES

09:45 – 10:00 **Miki Ando**, *Juntendo University School of Medicine, Japan*
IPSC-DERVIED OFF-THE-SHELF CTL THERAPY FOR CERVICAL CANCERS

10:00 – 10:30 **Le Cong**, *Standford University, USA*
DCAS9 GENE-EDITING FOR CLEAVAGE-FREE GENOMIC KNOCK-IN OF LONG SEQUENCES IN HUMAN CELLS

10:30 – 11:00 **Erica Jonlin**, *University of Washington, USA*
ETHICAL CONSIDERATIONS FOR CLINICAL APPLICATION OF HUMAN EMBRYO GENOME-EDITING

11:00 – 11:30 BREAK

Program Schedule

Friday, 29 October

11:30 – 13:30 EARLY CAREER INVESTIGATOR EVENT

Chairs: **Rica Tanaka**, *Juntendo University, Japan*
Kiyoshi Okada, *Osaka University, Japan*

The JSRM Diversity Committee is overseeing an Early Career Investigator Event to promote the exchange of information and ideas between young researchers in Japan and abroad. There will also be a breakout room during the meeting where early career researchers can ask questions, exchange ideas, and network. The event of the young researchers, by the young researchers, for all researchers.

11:30 – 11:50 **Aiko Sada**, *Kumamoto University, Japan*
ELUCIDATING THE CELLULAR AND MOLECULAR MECHANISMS OF EPIDERMAN STEM CELL AGING

11:50 – 12:10 **Joshua Currie**, *Wake Forest University, USA*
RECRUITING PROGENITOR CELLS DURING WOUND HEALING, REGENERATION, AND DEVELOPMENT

12:10 – 12:30 **Kyoko Miura**, *Kumamoto University. Japan*
INVESTIGATION OF THE MECHANISMS UNDERLYING DELAYED AGING AND CANCER-RESISTANCE IN THE LONGEST-LIVED RODENT, THE NAKED MOLE-RAT

12:30 – 12:50 **Laura Pellegrini**, *Medical Research Council Laboratory of Molecular Biology, UK*
CHOROID PLEXUS ORGANOIDS WITH CEREBROSPINAL FLUID AS A MODEL FOR BRAIN AND PATHOGEN ENTRY TO THE BRAIN

12:50 – 13:10 **Shiro Yui**, *Tokyo Dental and Medical University, Japan*
THE ART OF INTESTINAL ORGANOIDS IN BASIC RESEARCH AND ITS POTENTIAL FOR CLINICAL APPLICATIONS

13:10 – 13:30 **Simon Mendez-Ferrer**, *Cambridge University, UK*
CHOLINERGIC REGULATION OF BONE MARROW STEM CELLS

Friday, 29 October

13:30 – 15:30 STEM CELL-BASED THERAPIES

Sponsored by SCM Lifescience, Inc.

Chair: **Yoshiki Sawa**, *Osaka University, Japan*

13:30 – 14:00 **Masayo Takahashi**, *Vision Care Inc., Japan*
RETINAL CELL THERAPY USING IPS CELLS

14:00 – 14:15 **Jing Fan**, *Hopstem Bioengineering, China*
PRECLINICAL EFFICACY AND SAFETY STUDY OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVICED FOREBRAIN NEURAL PROGENITOR CELL HNPC01

14:15 – 14:30 **Koji Eto**, *Center for iPS Cell Research and Application, Kyoto University, Japan*
FIRST IN UMAN CLINICAL TRIAL OF AUTOLOGOUS IPSC-DERIVED PLATELETS (IPLAT1)

14:30 – 15:00 **Malin Parmar**, *Lund University, Sweden*
FROM CELL REPLACEMENT TO CIRCUITRY RECONSTRUCTION IN PARKINSON'S DISEASE

15:30 — 16:00 BREAK

16:00 — 17:00 PANEL DISCUSSION: OVERCOMING THE HURDLES OF BRINGING STEM CELL PRODUCTS TO MARKET

Moderator: **Takanori Takebe**, *Tokyo Medical and Dental University, Japan and Cincinnati Children's Hospital, USA*

Panelists:
Hideyuki Okano, *Keio University School of Medicine, Japan*
Malin Parmar, *Lund University, Sweden*
Masayo Takahashi, *Vision Care Inc., Japan*

17:00 — 17:10 CLOSING REMARKS

Closing Remarks
Takanori Takebe, *Tokyo Medical and Dental University, Japan and Cincinnati Children's Hospital, USA*

National Regenerative Medicine Consortium

The Japanese Society for Regenerative Medicine is conducting the following projects with governmental competitive research funds. The Japanese Society for Regenerative Medicine is conducting the following projects, some of which are planned to be prepared for overseas clients. (General inquiries: nc@jsrm.jp)

Clinical Research Support

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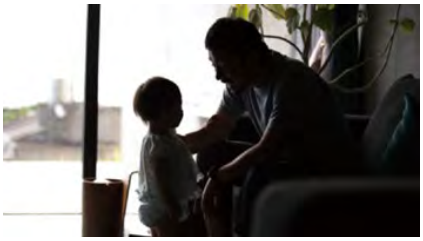

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International Collaborations

Evaluation of the appropriateness of private practices

JSRM

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Discover more about our science at T-CiRA's website.

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LINK-J is a general incorporated association established by Mitsui Fudosan and volunteers from academic circles. With its base in the Nihonbashi area, where several pharmaceutical companies are clustered, LINK-J aims to promote open innovation in the life science realm through industry-government-academia cooperation and to support the creation of new industries. Across the entire life science arena, i.e., the arena where all sciences combine -- ranging from medicine to science and further to engineering and new technologies, such as information and communication technology (ICT) and artificial intelligence (AI) -- it will accelerate interdisciplinary human and technological exchanges.

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PeptiGrowth Inc. develops Growth Factor Alternative Peptides that have the equivalent functions to growth factors in preclinical and GMP quality. Our products are fully chemically synthesized that means not using animal serum or gene recombination technology in order to achieve high purity, no variation among production lots in terms of specification, and cost reducing.

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Sponsored Content: Mini-Symposia

Growth Factor Alternative Peptide: Open the future of regenerative medicine and cell therapy. – Incl. Business Introduction of Mitsubishi Corporation

Organized by PeptiGrowth Inc. and Mitsubishi Corporation

Presenters: Jiro Sugimoto, President and Yosuke Ushimaru, Manager, PeptiGrowth Inc.

PeptiGrowth is a supplier of innovative raw materials for the expanding market of regenerative medicine and cell therapy.

We develop Growth Factor Alternative Peptides that have the equivalent functions to growth factors in preclinical and GMP quality. Our products are fully chemically synthesized that means not using animal serum or gene recombination technology in order to achieve high purity, no variation among production lots in terms of specification, and cost reducing.

Currently, PeptiGrowth is targeting and conducting the development of numerous growth factors. And our first product called "HGF Alternative Peptide" was launched in this August. In this seminar, we will introduce our innovative peptides with the data of HGF Alternative Peptide and upcoming our new product.

Also, we will introduce the business of Mitsubishi Corporation that is the parent company of PeptiGrowth.

Especially, we will explain about the new general incorporated association called "iD4: iPSC Delivery on Demand for Drug Discovery" that aims to promote iPS cells' industrial use and contribute to the development of rare disease therapies.

To Be a Global Leader in Regenerative Medicine / Cell Therapy Field

Organized by Sumitomo Dainippon Pharma Co., Ltd. and S-RACMO Co., Ltd.

Presenters: Tomokazu Nagano, PhD, Sumitomo Dainippon Pharma Co., Ltd. and Atsushi Tsuchida, PhD, S-RACMO Co., Ltd.

Sumitomo Dainippon Pharma is aspiring to establish a position as a “Global Specialized Player” in 2033, and Regenerative Medicine / Cell Therapy is one of our focus R&D area. To establish a position of a front runner aiming for the commercialization of iPS cell-derived cell therapy products, we built and operate a special facility, named SMaRT, for manufacturing of GMP-grade allogenic iPSC-derived cells.

In 2020, we created a new subsidiary, named S-RACMO, for CDMO business in Regenerative Medicine / Cell Therapy field. S-RACMO provides cutting-edge technology and sophisticated manufacturing system as a CDMO in the field.

In this seminar, we will present the activity of Sumitomo Dainippon Pharma and S-RACMO in Regenerative Medicine / Cell Therapy field for possible collaboration with attendees.

ISSCR

STEM CELL REPORTS

THE STEM CELL REPORT

A PODCAST WITH MARTIN PERA

THE STEM CELL REPORT

Stem cell science has catapulted to the forefront of biomedical research over the last decade, bringing with it the potential to transform human health and the treatment of devastating diseases and disorders. Martin Pera, an internationally recognized stem cell pioneer and the dynamic editor-in-chief of *Stem Cell Reports*, explores basic discoveries in stem cell research and its application. Dr. Pera goes "beyond the paper," bringing authors together to draw new insights and explore the questions and creativity that drive new breakthroughs.

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Speaker Abstracts

WEDNESDAY, 27 OCTOBER

Stem Cells and Development

PRINCIPLES OF EMBRYO SELF-ASSEMBLY IN VITRO AND IN VIVO

Zernicka-Goetz, Magdalena

University of Cambridge, UK and California Technical Institute, USA

Natural embryos develop from a single totipotent cell that differentiates into three major lineages. Remarkably, embryonic (ES), trophoblast (TS), and extra-embryonic endoderm (XEN) stem cells, representing these lineages, can self-organize to generate synthetic structures that strongly resemble natural embryos, despite their drastically different starting conditions. I will discuss the principles that we uncover during stem cell-derived embryogenesis and how they apply to embryogenesis in vivo.

TRACING THE ORIGIN OF HAIR FOLLICLE STEM CELLS

Morita, Ritsuko[1], Sanzen, Noriko[1], Sasaki, Hiroko[1], Hayashi, Tetsutaro[2], Umeda, Mana[2], Yoshimura, Mika[2], Yamamoto, Takaki[3,4], Shibata, Tatsuo[4], Abe, Takaya[5], Kiyonari, Hiroshi[5], Furuta, Yasuhide[5,6], Nikaido, Itoshi[7,8], Hironobu, Fujiwara[1]

[1]Laboratory for Tissue Microenvironment, RIKEN Center for Biosystems Dynamics Research, Kobe, Japan, [2]Laboratory for Bioinformatics Research, RIKEN Center for Biosystems Dynamics Research, Kobe, Japan, [3]Nonequilibrium Physics of Living Matter RIKEN Hakubi Research Team, RIKEN Center for Biosystems Dynamics Research, Kobe, Japan, [4]Laboratory for Physical Biology, RIKEN Center for Biosystems Dynamics Research, Kobe, Japan, [5]Laboratory for Animal Resources and Genetic Engineering, RIKEN Center for Biosystems Dynamics Research, Kobe, Japan, [6]Mouse Genetics Core Facility, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY, USA, [7]Degree Programs in Systems and Information Engineering, Graduate School of Science and Technology, University of Tsukuba, Ibaraki, Japan, [8]Functional Genome Informatics, Medical Research Institute, Tokyo Medical and Dental University, Japan

In order for organs to function properly, stem cells and functional cells need to be induced in the appropriate locations during organ morphogenesis. Although hair follicle stem cells are central to the homeostasis and regeneration of adult hair follicles, their developmental process has not been fully elucidated, including specific molecular markers. Here, by combining marker-independent long-term three-dimensional (3D) live imaging and single-cell transcriptomics, we captured cellular dynamics, cell lineages, and transcriptome changes in the entire epithelium of developing mouse hair follicles. We found that different epidermal lineage precursors were aligned in a two-dimensional (2D) concentric manner in the basal layer of the hair placode. Each concentric ring zone acquired unique transcriptomes and telescoped out to form longitudinally aligned 3D cylindrical compartments. Prospective bulge stem cells were derived from the peripheral ring zone of the placode, but not from previously suggested suprabasal cells. We also identified 13 gene clusters in which their ensemble expression dynamics drew the entire transcriptional landscape of epidermal lineage diversification, coinciding with cell lineage data. Combining these findings with insect appendage development, we provide a generalized model termed the "telescope model" supporting signal and cellular compartment formation and stem cell induction in organ development.

Funding Source

This work was supported by a RIKEN intramural grant, the RIKEN Single Cell Project, the Platform Project for Supporting in Drug Discovery and Life Science Research from MEXT and AMED and the JST CREST program (JPMJCR1926) to H.F. and JSPS Grant-in-Aid for Young Scientists (B) (15K19709 and 17K16361), JSPS Grant-in-Aid for Scientific Research (C) (19K08763), the RIKEN BDR-Otsuka Pharmaceutical Collaboration Center (RBOC) founding program and a Shiseido Female Researcher Science Grant to R.M. This work was partially supported by JST CREST grant number JPMJCR16G3 to I.N.

Keywords: Hair follicle stem cells, 4D ex vivo live imaging, Single-cell transcriptomics

ENDOTHELIAL CELL SIGNATURE AND POTENTIAL OF MOUSE SKELETAL MUSCLE STEM CELLS

Asakura, Atsushi

Stem Cell Institute, University of Minnesota Medical School, Minneapolis, MN, USA

Endothelial and skeletal muscle lineages arise from common embryonic progenitors. Despite their shared developmental origin, adult endothelial cells (ECs) and muscle stem cells (MuSCs) (satellite cells) have been thought to possess distinct gene signatures and signaling pathways. Here we shift this paradigm by uncovering how adult MuSC behavior is affected by the expression of a subset of EC transcripts. In particular, using deep single-cell RNA-seq (scRNA-seq) and other computational analyses, we show that MuSCs express low levels of canonical EC markers. We demonstrate that MuSC survival is regulated by one such prototypic endothelial signaling pathway (VEGFA-FLT1). Using

Speaker Abstracts

pharmacological and genetic gain- and loss-of-function studies, we identify the FLT1-AKT1 axis as the key effector underlying VEGFA-mediated regulation of MuSC survival. Altogether, our data support that the VEGFA-FLT1-AKT1 pathway promotes MuSC survival during muscle regeneration, and highlights how the minor expression of select transcripts are sufficient for affecting cell behavior. Finally, ETV2 (an EC-master transcription factor)-mediated EC reprogramming readily occurred when MuSCs were used compared with fibroblasts, indicating that there is a permissive chromatin state allowing for the expression of EC genes in MuSCs. Overexpression of ETV2 actively represses both myogenic gene expression and differentiation and thus restricting these cells to an EC fate. These MuSC-derived induced ECs (iECs) contributed to vessel formation in vitro and in vivo, and thus may have the potential to be developed as an EC source for therapeutic applications.

Funding Source
Regenerative Medicine Minnesota (RMM) NIAMS (NIH R21)
Keywords: Muscle stem cell, myogenesis, endothelial cell

ESTABLISHMENT OF EMBRYONIC ORIGIN OF ADULT NEURAL STEM CELLS AT THE SUBVENTRICULAR ZONE

Gotoh, Yukiko

The University of Tokyo, Japan

Quiescent neural stem cells (NSCs) in the adult mouse brain are the source of neurogenesis that regulates innate and adaptive behaviors. Adult NSCs in the subventricular zone (SVZ) are derived from a subpopulation of embryonic neural stem-progenitor cells (NPCs) that is characterized by a slower cell cycle relative to the more abundant rapid cycling NPCs that build the brain. We have previously shown that slow cell cycle can cause the establishment of adult NSCs at the SVZ, although the underlying mechanism remains unknown. We found that Notch and an effector Hey1 form a module that is upregulated by cell cycle arrest in slowly dividing NPCs. In contrast to the oscillatory expression of the Notch effectors Hes1 and Hes5 in fast cycling progenitors, Hey1 displays a non-oscillatory stationary expression pattern and contributes to the long-term maintenance of NSCs. These findings reveal a novel division of labor in Notch effectors where cell cycle rate biases effector selection and cell fate. I will also discuss the heterogeneity of slowly dividing embryonic NPCs and the lineage relationship between adult NSCs and ependymal cells, which together form the niche for adult neurogenesis at the SVZ.

Keywords: Adult neural stem cells, quiescence, embryonic origin

Advanced Technologies of Stem Cell Biology

GENETICALLY ENCODED TOOLS FOR STEM CELL RESEARCH AND REGENERATIVE MEDICINE

Miyawaki, Atsushi

RIKEN Center of Brain Science, Saitama, Japan

In a signal transduction diagram, arrows are generally used to link molecules to show enzymatic reactions and intermolecular interactions. To obtain an exhaustive understanding of a signal transduction system, however, the diagram must contain three axes in space and the time base, because all events are regulated ingeniously in space and time. The scale over time and space is ignored in biochemical approaches in which electrophoresis is applied to a specimen prepared by grinding millions of cells. A farseeing article entitled “Fluorescence Imaging Creates a Window on the Cell” was written by the late Roger Y. Tsien in 1994, which appeared in Chemical & Engineering News. He advocated employing the so-called real-time and single-cell imaging technique to fully appreciate cell-to-cell heterogeneity. He also had steadfastly pursued the creation of a reliable gate that would enable researchers to better understand the “feelings” of individual cells. Over the past two decades, various genetically encoded probes have been generated principally using fluorescent or bioluminescent proteins and are used to investigate the function of specific signaling mechanisms in synaptic transmission, integration, and plasticity. I will discuss how the probes have advanced our understanding of the spatio-temporal regulation of biological functions inside cells, neurons, embryos, and brains. I will speculate on how these approaches will continue to improve due to the various features of fluorescent/bioluminescent proteins that serve as the interface between light and life. Due to recent remarkable progress in gene transfer techniques, including electroporation, virus-mediated gene transfer, and germline transmission of transgenes, the experimental animals to be studied are not limited to mice but extended to primates. Newly emerging genetically encoded tools will surely stimulate the imagination of many biologists, and this is expected to spark an upsurge in the demand for them.

Keywords: Fluorescence, bioluminescence, bioimaging

INTERCELLULAR RNA TRANSFER-DRIVEN REPROGRAMMING OF HUMAN PLURIPOTENT STEM CELLS INTO NAÏVE-LIKE STATE

Yoneyama, Yosuke[1], Zhang, Ran-ran[2], Takebe, Takanori[1,2,3]

[1]Institute of Research, Tokyo Medical and Dental University, Japan, [2]Division of Gastroenterology, Hepatology and Nutrition, Developmental Biology, Center for Stem Cell and Organoid Medicine (CuSTOM), Cincinnati Children's Hospital Medical Center, OH, USA, [3]Department of Pediatrics, University of Cincinnati College of Medicine, OH, USA

Understanding the molecular basis of intercellular coordination will facilitate our ability to control collective cell behaviors. Here we uncovered a previously unknown mode of intercellular RNA exchanges in the experimental xenogeneic coculture system under naïve and primed pluripotent states. The transfer of RNAs, which accounts for 0.5-3.0% of the transcripts in the cells, occurred between human primed induced pluripotent stem cells (iPSCs) and mouse naïve embryonic stem cells (ESCs), primarily driven by direct cell-cell contact rather than other indirect mechanisms. Remarkably, after coculture with mouse naïve ESCs, human primed iPSCs were converted from flat into domed colony morphology and expressed naïve-specific markers. Furthermore, transcriptome and epigenome analyses revealed that during the coculture mouse ESC-derived, transcription factor coding-mRNAs were detected in human iPSCs accompanied by naïve-specific enhancer openings in human iPSCs. By using RNA interference specifically targeting mouse transcripts, we found that knockdown of mouse Klf4, Tfcp2l1, and Tfap2c mRNAs in human iPSCs reduced the emergence of naïve-like human iPSCs after coculture with mouse ESCs. These results indicate that the direct transfer of transcription factor-coding mRNAs from mouse ESCs into neighboring primed human iPSCs induces the reprogramming of human iPSCs to a naïve-like pluripotent state. Our finding highlights a potential strategy to enable conversion into target cell types in the absence of gene transduction or chemical modification of epigenetic states. More broadly, intercellular RNA transfer-mediated cellular programming phenomenon warrants further investigations in diverse biological contexts.

Keywords: Induced pluripotent stem cell, embryonic stem cell, intercellular communication

AGE-RELATED MITOCHONDRIAL METABOLIC FITNESS ENDORSES CELLULAR COMPETENCE TO OLD HEMATOPOIETIC STEM CELLS

Watanuki, Shintaro, Kobayashi, Hiroshi, Takubo, Keiyo

Department of Stem Cell Biology, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan

Hematopoietic stem cells (HSCs) maintain the lifelong supply of blood cells in cooperation with HSC-derived hematopoietic progenitor cells (HPCs). Proper self-renewal and differentiation of HSCs require adenosine triphosphate (ATP) production through regulation of the glycolytic system and mitochondrial oxidative phosphorylation (OXPHOS). Therefore, a comprehensive understanding of ATP metabolism in HSCs is essential for understanding the regulatory mechanisms of their differentiation and self-renewal. However, current methodology for measuring intracellular ATP levels is technically limited to destructive tests that lyse a large number of cells. In this study, by using a transgenic mouse model harboring a FRET (Fluorescence resonance energy transfer)-based ATP biosensor "GO-ATeam2", we developed a highly-multiplex system to measure real-time ATP concentration in living blood cells at the single-cell level. This system enables a high-resolution analysis of the time course of ATP concentration in young and old HSCs. To clarify the metabolic changes during HSC aging, young and old HSCs were treated with the glycolytic inhibitor 2-Deoxy-D-Glucose (2-DG) or the mitochondrial electron-transfer system (ETC) inhibitor oligomycin. 2-DG-treated old HSCs kept higher ATP level than that in 2-DG-treated young HSCs, suggesting a higher mitochondrial ATP production reserve in old HSCs upon metabolic stress. Treatment of a complex II inhibitor suppressed the ATP production reserve in 2-DG-treated old HSCs. Thus, the mitochondrial respiratory reserve in old HSCs was due to highly-efficient utilization of ETC complex II. In support of this, inducible loss of a glycolytic enzyme phosphoglycerate mutase 1 significantly decreased HSC number in young mice but not in old mice. These results indicate that HSCs acquire mitochondrial fitness against metabolic perturbations during aging.

Funding Source
This work was supported in part by KAKENHI grants from MEXT/JSPS 19K17847 (HK), 18H02845, 20K21621, 21H02957 (KT), AMED grants JP18ck0106444, JP18ae0201014, JP20bm0704042, JP20gm1210011 (KT).
Keywords: Hematopoietic stem cells, adenosine triphosphate, stem cell aging

Speaker Abstracts

TRACING AND PERTURBING LINEAGES DURING HUMAN BRAIN ORGANOID DEVELOPMENT

Treutlein, Barbara

Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland

Induced pluripotent stem cell (iPSC) derived organoids provide models to study human organ development. Organoids are complex, containing numerous cell states and integrative, multi-modal single-cell technologies are needed to understand the mechanisms underlying organoid development. In my talk, I will present two efforts from our lab where we develop novel integrative single-cell methods to understand human brain organoid development. First, I will present iTracer, a lineage recorder that combines reporter barcodes with inducible CRISPR/Cas9 scarring, and is compatible with single-cell and spatial transcriptomics. We apply iTracer to explore clonality and lineage dynamics during cerebral organoid development, and identify a time window of fate restriction as well as variation in neurogenic dynamics between progenitor-neuron families. We also establish long-term 4-D lightsheet microscopy for spatial lineage recording in cerebral organoids and confirm regional clonality in the developing neuroepithelium. We incorporate gene perturbation (iTracer-perturb), and assess the effect of mosaic TSC2 mutations on cerebral organoid development. Second, I will present a data set of paired single-cell transcriptome and accessible chromatin profiling data over a dense time course of human brain organoid development, which we utilize to infer a gene regulatory network of human brain organoid development. To this aim, we have developed Pando, a flexible computational framework that incorporates multi-omic data and transcription binding site predictions to infer a global GRN describing organoid development. We use pooled genetic perturbation with single-cell transcriptome readout to assess transcription factor requirement for cell fate and state regulation in organoid and show interesting alterations of abundance of cell fates. Together, these techniques can be adapted in any iPSC-derived culture system to dissect lineage relationships and regulomes during normal or perturbed development.

Opening Keynote Session

RECENT PROGRESS IN IPS CELL RESEARCH AND APPLICATION

Yamanaka, Shinya[1,2]

[1]Gladstone Institutes, San Francisco, CA, USA, [2]Center for iPS Cell Research & Application, Kyoto University, Japan

Induced pluripotent stem cells (iPSCs) can proliferate almost indefinitely and differentiate into multiple lineages, giving them wide medical application. As a result, they are being used for new cell-based therapies, disease models and drug development around the world. We are proceeding with an iPSC stock project in which clinical-grade iPSC clones are being established from healthy donors with homologous HLA haplotypes to lower the risk of transplant rejection. We started distributing the iPSC stock to organizations in Japan, and related clinical studies have begun for age-related macular degeneration (AMD), Parkinson's disease, corneal epithelial stem cell deficiency and other diseases, giving expectation that iPSC-based regenerative medicine will be widely used in the future. However, donors with HLA homozygous are rare. Genome editing technology could be used to reduce the transplant-rejection risk. Indeed, we reported HLA gene-edited iPSCs that could expand the range of patients who benefit from iPSC therapies faster than the homologous HLA haplotype strategy. This technology also has the potential to prevent or treat genetic diseases and gives great hope to patients. Other applications of iPSCs are drug screening, toxicity studies and disease modeling. In 2017, a new drug screening system using iPS cells for fibrodysplasia ossificans progressiva (FOP) was reported, revealing one drug candidate, Rapamycin, which is now undergoing a clinical trial to treat FOP patients. Additionally, Bosutinib, a drug for leukemia was revealed to be efficacious for amyotrophic lateral sclerosis (ALS) using a disease-specific iPSC model. Accordingly, we initiated a new clinical trial for Bosutinib to treat ALS at Kyoto University Hospital and other centers in 2019. Most recently, we launched a project to study COVID-19 by establishing several lines of iPSCs from recovered COVID-19 patients of different severity. We expect the models made from these cells to improve diagnosis, prevention and treatment for COVID-19. Over the past decade, iPSC research has made great progress, moving toward innovative therapeutics for people with intractable diseases by the application of new findings from basic science and reverse translation from clinics.
Keywords: Induced pluripotent stem cells (iPSCs), cell-based therapies, genome editing technology

THURSDAY, 28 OCTOBER

Organoids: from Basic Biology to Clinical Translation

FROM STEM CELLS TO BRAIN ASSEMBLOIDS: CONSTRUCTING AND DECONSTRUCTING THE HUMAN NERVOUS SYSTEM

Pasca, P. Sergiu[1,2]

[1]Psychiatry and Behavioral Sciences, Stanford University, CA, USA, [2]Stanford Brain Organogenesis Program, Wu Tsai Neuroscience Institute Stanford University, CA, USA

A critical challenge in understanding the molecular rules underlying the development, assembly and dysfunction of the human brain is the lack of direct access to intact, functioning human brain tissue for direct investigation and manipulation. In this talk, I will describe efforts in my laboratory to build functional cellular models and to capture previously inaccessible aspects of human brain development. To achieve this, we have been using instructive signals to derive, from human pluripotent stem cells, self-organizing 3D tissue structures named neural spheroids or region-specific brain organoids that resembles deomains of the delveloping central nervous system. We have shown that these cultures, such as the ones resembling the crebral cortex, recapitulate many features of neural development, can be drived with high reliability across dozens of cell lines and experiments and, when maintained as long-term cultures, recapitulate an intrinsic program of maturation that progresses towards postnatal stages. The formation of specific brain regions is, however, shaped by interactions with other regions through long-distance projections as well as by wiring locally in microcircuits with neurons that have migrated from other niches. To study migration and circuits in vitro and in vivo, we developed a next-generation experimental paradigm that we termed brain assembloids. As devised by us, assembloid generation is modular, relying on producing brain region-specific organoids that are subsequently fused in 3D to allow formation of neural circuits. I will illustrate how these patient-derived models can be applied to study the cellular and molecular consequences of gentic mutations or copy number variants associated with neuropsychiatric disease and to aquire a deeper understanding of human physiology.

Keywords: Assembloids, organoids, brain development

SIZE-SCALED PATTERNING OF NEURAL ORGANOIDS

Imaizumi, Kent, Okano, Hideyuki

Department of Physiology, Keio University, Tokyo, Japan

Proportions of the developing body plan are robustly maintained in spite of size variations. The body plan is established by the morphogen gradient, and this gradient is scaled according to the tissue size to maintain the proportionate patterning. However, the mechanism of the size-scaled morphogen gradient, especially in mammals, is still poorly understood. In this study, we generated pluripotent stem cell-derived neural organoids with varying sizes, and showed that the morphogen Sonic hedgehog (Shh)-driven patterning within these organoids was scaled in size. To investigate the mechanism of this tissue-intrinsic size scaling, we focused on expanders that enhance Shh diffusion. Expanders expanded the Shh morphogen gradient, and in turn, Shh inhibited the expression of expanders. This feedback system realized the spontaneous establishment of size-scaled morphogen gradient. Our study highlighted a novel application of oragnoids to study the developmental biology, and also will contribute to generating more in vivo-like organoids with precise regional patterning.

Keywords: Neual orgnaoid, Sonic hedgehog, size scaling

GENERATION OF SELF ASSEMBLING HUMAN HEART ORGANOIDS FOR MODELING DEVELOPMENT AND CONGENITAL HEART DISEASE

Lewis Israeli, Yonatan R.[1], Wasserman, Aaron H.[1], Gabalski, Mitchell A.[1], Volmert, Brett D.[1], Ming, Yixuan[2], Ball, Kristen A.[1], Yang, Weiyang[3], Zou, Jinyun[2], Ni, Guangming[2], Pajares, Natalia[4], Chatzistavrou, Xanthippi[4], Li, Wen[3], Zhou, Chao[2], Aguirre, Aitor[1]

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Congenital heart defects (CHD) are the most common birth defect in humans, affecting approximately 1% of all live births. The accessibility to study the developing human heart is greatly limited by our ability to model the complexity of the human heart in vitro. There is a pressing need to develop more representative organ-like platforms recapitulating complex in vivo phenotypes to study human development and disease in vitro. There is a pressing necessity to develop efficient organ-like platforms recapitulating complex in vivo phenotypes to study human

Speaker Abstracts

development and disease in vitro. Here we report a novel method to generate physiologically relevant human heart organoids by self-assembly using pluripotent stem cells. Our protocol is fully defined, highly efficient, exhibits high reproducibility, is cost-effective, scalable, and is compatible with screening and high-content approaches. Human heart organoids (hHOs) are generated through a three-step Wnt signaling modulation approach using a combination of chemical inhibitors and growth factors under completely defined culture conditions. hHOs recapitulate aspects of human cardiac development and are comparable to age-matched fetal cardiac tissues at the transcriptomic, structural, and cellular levels. hHOs develop sophisticated internal chambers with well-organized multi-lineage cell type regional identities reminiscent of the heart fields and atrioventricular specificity, as well as the epicardium, endocardium, and an endothelial vascular network. hHOs exhibit robust functional activity measured by both microelectrode arrays and calcium transients. We also demonstrated that hHOs can model complex metabolic disorders associated with CHD by establishing an in vitro human heart organoid model of pregestational diabetes (PGD) to study embryonic CHD. Our heart organoid model constitutes a powerful novel tool for translational studies in human cardiac development and disease and facilitates access to human heart developmental stages hitherto inaccessible.

Funding Source
National Heart, Lung, and Blood Institute of the National Institutes of Health. American Heart Association. Spectrum-MSU. National Institutes of Health. National Science Foundation. Michigan State University Graduate Excellence Fellowship.
Keywords: Heart organoid, cardiovascular disease modeling, self-organization

HEPATO-BILIARY-PANCREATIC ORGANOGENESIS IN A DISH

Takanori, Takebe[1,2]

[1]Division of Gastroenterology, Hepatology and Nutrition and Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, OH, USA, [2]Tokyo Medical and Dental University, Japan

Organoids are three-dimensional structures that self-organize from human pluripotent stem cells or primary tissue, potentially serving as a traceable and manipulatable platform to facilitate our understanding of organogenesis. Despite the ongoing advancement in generating organoids of diverse systems, biological applications of in vitro generated organoids remain as a major challenge in part due to a substantial lack of intricate complexity. The studies of development and regeneration enumerate the essential roles of highly diversified non-epithelial populations such as mesenchyme and endothelium in directing fate specification, morphogenesis, and maturation. Such human organoids allow for the study of direct and indirect inter-organ crosstalk recapitulating what is seen in health and disease. For example, we show in vitro modeling of the inter-coordinated specification and invagination of the human hepato-biliary-pancreatic (multi-organ) system in 3D stem cell culture, paving a way for the study of inter-organ connectivity failure such as biliary atresia. I herein summarize the evolving organoid technology at the cell-, tissue-, organ- and system-level complexity with a main emphasis on endoderm derivatives and discuss its promise and impact to elucidate personalized disease mechanisms and understand drug reactions in humans.

Funding Source
NIH DP2, UH3\r\nAMED CREST
Keywords: Organoid, iPSC, organogensis

ETHICS OF HUMAN BRAIN ORGANOID RESEARCH

Fujita, Misao[1,2]

[1]Center for iPS Research and Application (CiRA), Kyoto University, Japan, [2]Institute for the Advanced Study of Human Biology (WPI-ASHBi), Kyoto University, Japan

The ISSCR released the amended guidelines this May, including new research areas such as human brain organoid. The guidelines clarify whether a particular type of brain organoid research should go through a specialized oversight process because it is considered to involve ethical issues in some extent. These issues include, but are not limited to, informed consent of the donors, biobanking, consciousness and moral status, chimera research, translational research, regulations and oversight. In my talk, I mainly focus on consciousness and moral status, as well as transplantation into animal brains, and report the results of social science surveys conducted by my lab. I introduce exploratory research that summarizes trends in newspaper coverage on how brain organoids and consciousness are reported in society. I also report the results of questionnaire surveys which investigate the concerns of the general public about chimeric animals with brains derived from human-induced pluripotent stem cells. The results are not intended to directly lead to the development of research regulations or guidelines. However, social reactions and public concerns cannot be ignored, as societal trust is essential for the development of science and technology. Therefore, it is important to monitor them on a continuing basis and share concerns with the research community.

Keywords: Ethics, human brain organoid, social science

Stem Cell-Based Disease Modelling and Drug Development

AGE RELATED DYSFUNCTION IN IN-DERIVED NEURONS FROM SPORADIC ALZHEIMER'S PATIENTS

Gage, H. Fred, Jeffrey, Jones, Joseph, Herdy, Jerome, Mertens

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Alzheimer's Disease (AD) is a highly prevalent neurodegenerative disease that exclusively affects elderly people. I will discuss how we used direct conversion of primarily sporadic AD patient fibroblasts into induced neurons (iNs) to generate an age-equivalent neuronal model. Patient-derived iNs exhibit strong AD-specific transcriptome neuronal signatures characterized by down-regulation of mature functional and morphological properties and up-regulation of immature neuronal and neural stem cell-associated pathways. Mapping AD and control iNs to longitudinal transcriptome data from maturing human neurons demonstrated that AD iNs are fully converted into iNs, but reflect a de-differentiated neuronal identity. Epigenetic landscape profiling revealed an aberrant cellular program underlying their immature neuronal state, which shares similarities with malignant transformation and age-dependent epigenetic erosion. To probe for the involvement of aging, we generated iPSC neurons from the small cohort, which, indeed, showed non-significant disease-related transcriptome signatures. This is consistent with epigenetic aging clock and brain oncogenesis mapping, which indicated that unlike iPSC neurons, iNs more closely reflect adult and old brain stages, rendering them a valuable tool for studying adult-specific, age-related neurodegeneration. In this model, AD-related neuronal changes appear less as a mere accumulation of damaging events, but more an age-dependent cellular program that impairs neuronal identity.

Funding Source
FHG: AHA-Allen Initiative in Brain Health and Cognitive Impairment award made jointly through the American Heart Association and The Paul G. Allen Frontiers Group: 19PABH134610000, JPB Foundation, Dolby Family Foundation, R01 AG056306. JM: K99 AG056679
Keywords: Alzheimer's disease, neurodegeneration, induced neurons

MODELING STEATOHEPATITIS WITH MULTICELLULAR ORGANOIDS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Nonalcoholic steatohepatitis (NASH) is a liver disease caused by chronic and excessive fat accumulation, which leads to highly fatal conditions such as cirrhosis and liver cancer. The global prevalence of NASH is rapidly growing, but there is no effective pharmacotherapy for NASH to date. A significant cause for the delay in pharmaceutical development involves the lack of a patient-based culture system that mirrors the clinical progression of human steatohepatitis, including fat accumulation, inflammation, and fibrosis. This study established a culture method to generate human liver organoids (HLO) from induced pluripotent stem cells of more than 30 healthy donors and diseased patients. Transient addition of retinoic acid signals at the hepatic endoderm stage generates HLO including cells that resemble hepatic resident macrophages, Kupffer cells, and hepatic stellate cells, which play significant roles in inflammation and fibrosis. Treatment of the generated HLO with oleic acid, a type of free fatty acid, resulted in a marked accumulation of triglycerides, increased expression and secretion of inflammatory cytokines such as TNF-alpha and IL-8, and collagen production. In addition, Kupffer-like and hepatic stellate-like cells in HLO were responsible for establishing the inflammation and fibrosis phenotype. Furthermore, stiffness measurements by atomic force microscopy showed that oleic acid-treated HLO were stiffer compared with untreated HLO, and this increased stiffness likely correlated with the severity of organoid fibrosis. These results indicate that liver organoids recapitulate a series of pathological events in steatohepatitis through pathogenic cell-cell crosstalk among hepatocytes, Kupffer cells, and hepatic stellate cells. Collectively, HLO based steatohepatitis modeling approach serves as a precision hepatology platform for drug discovery and identification of novel therapies against NASH.

Speaker Abstracts

Funding Source
This work was supported by Cincinnati Children's Research Foundation grant, NIH Director's New Innovator award DP2DK128799-01, NIH grant UG3 DK119982, CREST grant from Japan Agency for Medical Research and Development (AMED) JP20gm120012h0001, Cincinnati Center for Autoimmune Liver Disease Fellowship award, PHS grant P30DK078392 of the Digestive Disease Research Core Center in Cincinnati, the Falk Catalyst Research Awards Program, JST Moonshot R&D Grant JPMJMS2033 and JPMJM2022, Takeda Science Foundation award, Mitsubishi Foundation award, AMED JP19fk0210037, JP19bm0704025, JP19fk0210060, JP19bm0404045, and JSPS JP18H02800, JP19K22416, and JP21K15370, Astellas Foundation for Research on Metabolic Disorders, and Naito Grant for Female Scientist after Maternity Leave. RO is a researcher in the Next Generation Researcher Training Unit at Tokyo Medical and Dental University. TT is a New York Stem Cell Foundation - Robertson Investigator.
Keywords: Nonalcoholic steatohepatitis, Liver organoid, Induced pluripotent stem cell

ANALYSIS OF HAPLOINSUFFICIENCY DISORDERS IN HUMAN EMBRYONIC STEM CELLS

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The Hebrew University, Jerusalem, Israel

Haploinsufficiency describes a phenomenon where one functioning allele of a gene in a diploid cell or organism is insufficient for a normal phenotype. Although haploinsufficiency underlies several human diseases, the effect of haploinsufficiency on human embryonic stem cell (hESC) growth and proliferation has not been thoroughly studied. Here, we aimed to identify genes affecting the normal growth of hESCs when one of their two alleles is lost. To establish a genome-wide loss-of-function screening for heterozygous mutations, we fused normal haploid cells with a library of mutant haploid hESCs. We have identified over 600 genes with a negative effect on hESC growth in a haploinsufficient manner and characterized them as genes that show less tolerance to mutations, more conservation during evolution and depletion from telomeres and X chromosome. Interestingly, a large fraction of these haploinsufficiency genes is associated with the extra-cellular matrix and the plasma membrane. We have revealed an enrichment of genes causing haploinsufficiency disorders within WNT and TGF-BETA signal transduction pathways. We could thus identify haploinsufficiency-related genes and pathways that show growth retardation in early embryonic cells, suggesting dosage-dependent phenotypes in hESCs. Overall, we have constructed a novel model system for studying haploinsufficiency and identified important dosage-dependent pathways involved in hESC growth and survival.
Keywords: Haploinsufficiency, Disease Modeling, Human Embryonic Stem Cells

IPSCS-BASED MODELING, DRUG DEVELOPMENT AND CLINICAL TRIALS FOR ALS

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The application of iPSC technology to cell therapy, including regenerative medicine, as well as disease research and drug discovery, is becoming increasingly active. We have been working on the iPSCs-based modeling, drug development, and clinical trials for ALS. Ropinirole hydrochloride (ROPI) was identified as a drug that suppressed ALS-related symptoms (FUS/TDP-43 mislocalization, stress granule formation, LMN death/damage, and neurite retraction) after screening a library of 1,232 existing drugs using LMNs-derived from FUS and TDP-43 ALS iPSCs. ROPI is known as an anti-parkinsonian drug with D2R/D3R agonist activity. Anti-ALS action of ROPI is likely to be mediated by D2R-dependent and independent mechanisms. The D2R-independent action of ROPI is putatively mediated via suppression of hyperexcitability of LMNs and induction of Beclin1-dependent autophagy. D2R is coupled with Gi and reduces cAMP level upon its activation, which would lead to suppressing the neuronal hyperexcitability and eventually exerts a neuroprotective role. On the other hand, ROPI's D2R-independent action includes improvement of mitochondrial function and suppression of oxidative stress, abnormal protein aggregation, and LMN deaths putatively mediated its oxindole structure. Using ROPI, a randomized, double-blind, placebo-controlled, single-center, and open-label continuation Phase I/IIa clinical trial for ALS ((ROPALS trial) was performed at Keio University Hospital (UMIN000034954). As a result, we confirmed the safety and tolerability of ROPI for ALS patients. Over the entire 1-year treatment period, ROPI significantly reduced the decline in ALSFRS-R and disease progression of ALS. In fact, time to death or respiratory failure was extended in RPOI-treated ALS patients. Thus, it was concluded that ROPI is possibly a safe and effective therapy for ALS. This trial is the first time in the world that iPSC drug discovery has been shown to be useful as a new drug discovery modality.

Funding Source
This work was supported by MEXT Grant-in-Aid, Japan, grant no. JP20H00485 to H.O. and the Center Network for Realization Research Centers/Projects of Regenerative Medicine (the Program for Intractable Disease Research Utilizing Disease-specific iPS Cells and the Acceleration Program for Intractable Diseases Research Utilizing Disease-specific iPS Cells) from AMED (Grant No. JP 19bm0804003, JP 20bm0804003, JP 21bm0804003) to H.O.
Keywords: Induced pluripotent stem cells (iPSCs), ALS, drug developmen

Tissue Engineering

NOVEL APPROACH FOR IN VIVO ORGANOGENESIS: DEVELOPMENTAL COMPLEMENTATION VS CELL COMPETITION

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Development of iPS cell technology has enabled the generation and potential use of patient- derived pluripotent stem cells (PSCs) in regenerative medicine. However, despite numerous attempts, in vitro derivation of truly functional organs from PSCs remains difficult. To address this issue, we first postulated that “organ niche” exists in a developing animal and that this niche was empty when development of an organ is genetically disabled. This organ niche, we reasoned, should be compensated developmentally by blastocyst complementation using wild-type pluripotent stem cells. We generated functionally normal mouse-sized rat pancreases in mice and in the reverse experiment, we generated functional rat-sized mouse pancreases in rats. Islets prepared from these mouse pancreata generated in rats were transplanted into mice with streptozotocin-induced diabetes. The transplanted islets successfully maintained normal host blood glucose levels for over 370 days without immunosuppression. These data provide proof-of-principle evidence for the therapeutic potential of PSC-derived organs generated by interspecies organogenesis. However, interspecies chimeras with high donor chimerism also display embryonic lethality and malformation during early embryogenesis, hindering high chimeric fetus formation. To circumvent this problem, we used Insulin-like growth factor 1 receptor (Igf1r) deficient embryos as a host. Since the Igf1r deletion increases donor chimerism from the mid to late developmental stages, highly chimeric fetuses can evade the early developmental arrest observed in interspecies chimera formation. Indeed, Igf1r KO hosts creates what we have termed “cell competitive niche”, which significantly increases donor chimerism in both intra and inter-species chimera chimeras. The enhanced donor chimerism continuously increased and even took over the whole organs in some intra-species chimeras. Among them, donor chimerism in kidney, brain, and lung approached 100. This approach, now being tested in large animals, should facilitate donor cell contribution to host tissues, resulting in whole-organ generation via blastocyst complementation across wide evolutionary distances.

Funding Source
CIRM (LA1_C12-06917; DISC1-10555) and the Ludwig Foundation, and Leading Advanced Projects for Medical Innovation, Japan Agency for Medical Research and Development, JSPS KAKENHI grant, number (JP18K14602 and JP18J00499).
Keywords: Organ generation, blastocyst complementation, cell competition

MODELLING EARLY HUMAN EMBRYO DEVELOPMENT USING NAÏVE PLURIPOTENT STEM CELLS

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The blastocyst is the hallmark structure formed in the early development of mammals. It consists of three tissues; trophectoderm, hypoblast and epiblast. Trophectoderm forms first. Morphological segregation of trophectoderm and inner cell mass (ICM) marks the initiation of blastocyst development. Within the ICM the hypoblast and naïve epiblast are then specified. Naïve pluripotent stem cells in vitro resemble the naïve epiblast in the fully expanded blastocyst. We recently discovered that, unlike in mouse, human naïve epiblast cells can regenerate authentic trophectoderm. This plasticity is maintained in human naïve pluripotent stem cells, which are also able to produce hypoblast. We established a 3D human blastocyst model, blastoid, by harnessing the tri-lineage differentiation potential. Induction of trophectoderm in naïve cell aggregates leads to formation of blastocyst-like structures within three days. Blastoids are composed of three tissue layers displaying exclusive lineage markers, mimicking the natural blastocyst. Single cell transcriptome analyses confirm segregation of trophectoderm, hypoblast and epiblast with fidelity to the human embryo. This versatile and scalable system provides a robust experimental model for human embryo research. I will present new findings from molecular genetic dissection of a key signalling pathway controlling human embryo morphogenesis.

Funding Source
Medical Research Council, UK
Keywords: Human naive pluripotent stem cell, stem cell derived blastocyst model, self-organization

Speaker Abstracts

SELECTION OF O-VE IPSC CLONES FOR HIGH-DENSITY RED BLOOD CELL PRODUCTION IN A SCALABLE PERFUSION BIOREACTOR SYSTEM

Vassilev, Svetlan[1], Sue, Yu[1], Lim, Zhong Ri[1], Sivalingham, Jaichandran[1], Lam, Alan[1], Ho, Valerie[1], Hang, Jing Wen[2], Leong, Yew Wai[2,3], Renia, Laurent[3], Malleret, Benoit[2,3], Reuveny, Shaul[1], Oh, Steve[1]

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Blood is one of the most vital resources in modern medicine. Blood transfusions have become an essential and lifesaving procedure for accidents, surgery, and chronic disorders such as anemia, sickle cell disease, and cancer. However, despite the rapidly growing world population, the availability of healthy blood donors is declining with aging populations. Furthermore, natural, and man-made calamities often produce sudden and concentrated shocks in demand, which strains global supply chains. The COVID-19 pandemic has demonstrated this issue on a global scale by reducing the number of blood drives and donations, resulting in 39% of blood centers in the United States being left with only one- to two-day supplies, and a 50% drop of blood units collected in countries such as Zambia. Additionally, storage limitations of 42 days for donor blood limit stock availability during peak demand. Large-scale generation of universal red blood cells (RBCs) from O-ve human induced pluripotent stem cells (hiPSCs) offers the potential to alleviate blood shortages and provide a secure year-round supply. Mature iPSC-derived RBCs and reticulocytes could also find important applications in research in malaria and COVID-19 studies. In this study, we have reprogrammed hiPSC from CD34+ O-ve cells and demonstrated the small-scale generation of high-density cultures of erythroblasts in a stirred perfusion bioreactor system. Twenty O-ve iPSC lines were derived, screened, and characterized for their ability to differentiate towards the erythroid lineage, showing high expression of mesoderm (KDR+, 64.9%), hematopoietic (CD34+/CD45+, 68.4%; CD34+/CD43+, 84.9%), and erythroid markers (CD235a+, 83,5%), and were able to undergo enucleation in vitro. Using the best clones, we were able to achieve erythroblast peak cell density of 34.7 million cells/mL with 92.2% viability in an Applikon perfusion bioreactor using an ultrasound system (Sonosep) to concentrate cells while removing waste media. This resulted in a cumulative-fold expansion of over 1,500 after 29 days of culture. Cells carried O2 effectively as demonstrated by haemoglobin dissociation curves. This perfusion platform paves the way for controlled high-density bioreactor culture for the generation of functional RBCs.

Funding Source
A*STAR Graduate Academy, Agency for Science, Technology and Research, Singapore
Keywords: hiPSC, RBC, Bioreactor

CONSIDERING THE GAP BETWEEN SCIENTISTS' AND THE GENERAL PUBLIC'S PERCEPTIONS OF REGENERATIVE MEDICINE USING HUMAN-ANIMAL CHIMERA RESEARCH AS AN EXAMPLE

Yashiro, Yoshimi

Kanagawa University of Human Services, Japan

The 21st century is said to be a knowledge-based society in which new knowledge, information, and technology will dramatically increase in importance as the basis for activities in all areas of society, including politics, economics, and culture. Therefore, it is necessary for academic research and technological applications to establish the trust of various stakeholders, including the general public. In other words, it is a social challenge for science policy makers to promote effective communication between experts and the general public and to foster trust.Although regenerative medicine is highly anticipated by the general public, the supporters of regenerative medicine do not always have accurate knowledge of basic research and clinical applications. In fact, there is a possibility that insufficient proof of efficacy and unclear assurance of safety may be created by hype that takes advantage of expectations.In this presentation, I will discuss the difference in consciousness between regenerative medicine scientists and the general public, and think about the necessary communication, using opinion surveys and fiction about human-animal chimera.

Keywords: Human-animal chimera, ELSI, Responsible Research & Innovation

ENGINEERING EPITHELIAL ORGANOID DEVELOPMENT

Lutolf, Matthias P.

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Organoids form through poorly understood morphogenetic processes in which initially homogeneous ensembles of stem cells spontaneously self-organize in suspension or within permissive three-dimensional extracellular matrices. Yet, the absence of virtually any predefined patterning influences such as morphogen gradients or mechanical cues results in an extensive heterogeneity. Moreover, the current mismatch in shape, size and lifespan between native organs and their in vitro counterparts hinders their even wider applicability. In this seminar I will discuss some of our ongoing efforts in developing next-generation organoids that are assembled by guiding cell-intrinsic self-patterning through engineered stem cell microenvironments.

Keywords: Organoids, self-organisation, tissue engineering

FRIDAY, 29 OCTOBER

Closing Keynote Session

CAR T CELLS: THE CONVERGENCE OF SYNTHETIC BIOLOGY AND CANCER IMMUNOTHERAPY

June, Carl[1,2]

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Advances in the understanding of basic immunology have ushered in two major approaches for cancer therapy over the past 10 years. The first is checkpoint therapy to augment the function of the natural immune system. The second uses the emerging discipline of synthetic biology and the tools of molecular biology and genome engineering to create new forms of engineered cells with enhanced functionalities. The emergence of synthetic biology approaches for cellular engineering provides a broadly expanded set of tools for programming immune cells for enhanced function. Advances in T cell engineering, genetic editing, the selection of optimal lymphocytes, and cell manufacturing have the potential to broaden T cell-based therapies and foster new applications beyond oncology, in infectious diseases, organ transplantation, autoimmunity and fibrosis.

Funding Source
National Cancer Institute
Keywords: Cancer immunotherapy, human genome editing, CAR T cell

Gene Therapy and Genome Editing

GENE THERAPY FOR GENETIC DISEASE-ADVANCES AND HURDLES IN AAV-MEDIATED GENE THERAPY

High, A. Katherine

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Currently approved gene therapies include multiple CAR T cells for hematologic malignancies, and multiple products for single gene disorders. In addition to these approved therapies, there has been a dramatic increase in clinical research activity in gene therapy over the past 5 years, and there are multiple programs in late-stage clinical investigation. This presentation will discuss the current state of the field, with a focus on AAV products and investigational agents. Hurdles that were addressed early on in clinical development programs, including human immune responses to AAV that were not well predicted by studies in animals, will be reviewed, as will problems arising from the absence of accepted clinical endpoints in the setting of diseases that have not previously been treated. More recently described toxicities, revealed in the setting of clinical investigation using higher doses of systemically infused AAV, will also be reviewed, along with successful mitigation strategies.

Keywords: AAV, Gene Therapy, Genetic Disease-Advances

A NEW HORIZON OF MARMOSET RESEARCH USING HIGHLY EFFICIENT KNOCK-IN AND PLURIPOTENT STEM CELL-BASED TECHNOLOGIES

Yoshimatsu, Sho[1,2,3,4], Okahara, Junko[4,5], Nemoto, Akisa[3], Sato, Tsukika[2,3], Kishi, Noriyuki[4], Noce, Toshiaki[4], Hayashi, Katsuhiko[1], Okano, Hideyuki[3,4]

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Genome editing technology has been utilized in many organisms, including non-human primates (NHPs) which harbor physiological and anatomical features that are in common with humans. We previously reported the efficient genome editing in the common marmoset (Callithrix jacchus), a small NHP species . Knock-in (KI) gene targeting is a homologous recombination (HR)-based versatile and flexible strategy for genome editing. KI enables precise introduction of reporter genes (e.g.

Speaker Abstracts

fluorescent protein genes) and mutations into targeted loci. Owing to the low in vivo chimeric potential of pluripotent stem cells derived from non-rodent mammalian species including NHPs, KI in early-stage embryos is indispensable for production of KI animals in these species. However, especially for NHPs, the limitation of bioresource (oocytes and surrogate mothers) hinders the robust production of KI animals. In this context, technological development of highly efficient KI in early-stage embryos is crucial.In this study, we aimed to establish an HR-biased genome editing technology for efficient KI in marmoset early-stage embryos based on the CRISPR-Cas9 system. In addition, with the usage of recently established reprogramming technologies in marmoset cells, we introduce our new approaches for the rapid in vitro validation of KI alleles in the NHP model.Moreover, we also show our recent attempts for in vitro derivation of functional gametes from marmoset pluripotent stem cells, by using stepwise differentiation methods of germ cells and gonadal somatic cells.

Funding Source
This study was funded by the Construction of System for Spread of Primate Model Animals project under the Strategic Research Program for Brain Sciences and Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) from the Japan Agency for Medical Research and Development (AMED; grant ID: JP20dm0207001 to H.O.) and KAKENHI Grant Number 19J12871, 20K22660, 21J00381 and 21K15112 (to S.Y) from Japan Society for the Promotion of Science (JSPS).
Keywords: Genome editing, Pluripotent stem cell, Non-human primate

IPSC-DERIVED OFF-THE-SHELF CTL THERAPY FOR CERVICAL CANCERS

Ando, Miki[1,2], Ando, Jun[1,3], Ishii, Midori[1], Honda, Tadahiro[1], Kinoshita, Shintaro[1], Furukawa, Yoshiki[1], Masuda, Ayako[4], Kitade, Mari[4], Terao, Yasuhisa[4], Nakauchi, Hiromitsu[2,5]

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Cervical cancers harbor human papilloma virus (HPV) oncoproteins. Although HPV vaccines effectively prevent HPV infections, they have no effect on established cancers. As metastatic cervical cancer is highly refractory to chemotherapy and prognosis is extremely poor, especially in young patients, development of an effective cervical cancer treatment is an urgent issue. Antigen-specific cytotoxic T lymphocytes (CTL) therapy can induce durable remission in selected tumors such as melanoma. As E6 and E7 are the key oncoproteins of cervical cancer, these would be ideal targets of T cell therapy, unfortunately, CTL continuously exposed to their target antigen become exhausted. CTL generated from induced pluripotent stem cells (iPSC) are functionally rejuvenated (rejT) and survive better in vivo than original CTL . We have successfully demonstrated that HPV type16 (HPV16) E6-rejT sustainedly suppress HPV16-expressing cervical cancer . However, generating rejT from individual patients is time-consuming and costly. Although use of allogenic rejT can obviate this problem, HLA-mismatched immunorejection then becomes a major obstacle. A realistic solution for this problem is the development of an HLA-edited rejT exploiting CRISPR/Cas9 technology. We could demonstrate that the HLA class I-edited HPV-rejT are not rejected by recipient CD8+ T cells and can evade NK cell attack. Furthermore, these rejT can maintain robust tumor suppressive effect against cervical cancer. We are now starting a Phase I study of HPV-rejT therapy for patients with cervical cancer to confirm its safety. To supply clinical-grade HPV-rejT as quickly as possible, we are generating a master cell bank of HLA class I-edited iPSC originated from HPV16 E6-CTL. We believe that HLA-engineered HPV-rejT would provide a sustainable and promising approach to "off-the-shelf" T cell therapy.

Funding Source
AMED (JP20bm0404032 and JP21bk0104117)
Keywords: Rejuvenated CTL, gene editing, cervical cancer

DCAS9 GENE-EDITING FOR CLEAVAGE-FREE GENOMIC KNOCK-IN OF LONG SEQUENCES IN HUMAN CELLS

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Exemplified by CRISPR-Cas9 systems, gene-editing technology is a powerful collection of tools for probing the mechanisms of human health and diseases. Recently, limitations of the initial CRISPR methods have stimulated new modalities of editing. However, existing tools that use Cas9 cutting or nicking causes DNA damage at on- and off-target sites, leading to potential cellular stress, unwanted mutations, and enrichment of p53-mutant cells. Additionally, most gene-editing tools rely on endogenous DNA repair mechanisms, particularly for knock-in of large transgenes. This results in significant levels of indels and

non-homologous events. Here, we developed a cleavage-free knock-in gene-editing tool using catalytically-dead dCas9 and microbial single-strand annealing proteins (SSAPs), termed dCas9-SSAP editor. dCas9-SSAP editor generated near-zero indels at target loci while achieving comparable knock-in efficiencies as wild-type Cas9 in human cell line and stem cells. Using chemical perturbations, we showed that dCas9-SSAP editor was less dependent on endogenous mammalian repair pathways, and our data supported the proposed model that SSAPs may promote DNA strand exchange when genomic DNA becomes transiently accessible via dCas9 unwinding and R-loop formation. We demonstrate that this editing tool is compatible with human stem cell engineering and also could be delivered via AAV-based vector. Overall, this tool could open new opportunities towards safer genome engineering in mammalian cells with minimal DNA damage.

Funding Source
National Institutes of Health grant 1R35HG011316 (LC)
Keywords: CRISPR-Cas, SSAP Knock-in, Stem Cell

ETHICAL CONSIDERATIONS FOR CLINICAL APPLICATION OF HUMAN EMBRYO GENOME-EDITING

Jonlin, Erica

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Long-term goals of basic research in gene therapy and genome-editing include providing the foundational knowledge for development of therapies that will benefit human health. In addition to its value as a research tool to uncover mechanisms of disease in in vitro systems and in animal models, germline genome-editing may someday be applied to human embryos and used clinically, to create a pregnancy. While the use of germline genome-editing for enhancement is widely regarded by most societies, and by most people (scientists and non-scientists alike) as unacceptable, the use of this technology for the prevention of serious disease may one day be deemed acceptable to many. In this presentation I will discuss some of the ethical considerations that remain to be addressed in assessing the benefits and risks of germline genome-editing. Benefits and risks will be informed not only by the state of the science, but also by the choice of diseases to which the technology might be applied, the realistic alternatives that the parents may have to have a biological offspring, and what safety bar must be met in light of the seriousness of the disease to be prevented. Design of the clinical protocol itself poses profound ethical questions: for example, once a pregnancy is achieved, difficult decisions may have to be made regarding what monitoring should be conducted and whether circumstances might arise that would suggest that the pregnancy not be allowed to be carried to term. The rights of the parents must be protected and honored; however, scenarios may arise in which decisions of the parents conflict with those of the research team. Achieving meaningful informed consent poses major challenges. Not insignificantly, questions as to the rights of the genome-edited individual – during gestation, childhood, and ultimately adulthood – must be addressed. Finally, a balance must be found between respecting personal autonomy versus avoiding negative societal impact.

Early Career Investigator Event

The JSRM Diversity Committee is overseeing an Early Career Investigator Event to promote the exchange of information and ideas between young researchers in Japan and abroad. There will also be a breakout room during the meeting where early career researchers can ask questions, exchange ideas, and network. The event of the young researchers, by the young researchers, for all researchers.

ELUCIDATING THE CELLULAR AND MOLECULAR MECHANISMS OF EPIDERMAL STEM CELL AGING

Sada, Aiko, Raja, Erna, Changarathil, Gopakumar, Oinam, Lalhaba, Yanagisawa, Hiromi

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Adult stem cells maintain tissue integrity during homeostasis and show remarkable plasticity in response to various stress or tissue damage. Dysfunction or misregulation of stem cells leads to tissue dysfunction, including impaired wound healing, tumorigenesis, and aging. Emerging evidence suggests the presence of heterogeneous stem cell populations within adult tissues with specific roles in physiological and pathological conditions. Tissue stem cells divide infrequently to minimize risks from replication stress and accumulation of DNA damage which have been discussed as contributors to stem cell aging. However, it is unclear whether “slow-cycling” nature confers protection to stem cells and delays their aging. Taking advantage of a study model using the slow- and fast-cycling stem cell populations that we previously identified in the mouse skin, we demonstrated that during aging, fast-cycling stem cells were gradually depleted, and the unique lineage identities of distinct stem cell populations were compromised. We found that loss of the extracellular matrix fibulin-7

Speaker Abstracts

accelerates epidermal stem cell clone loss, delays wound healing, and increases inflammation-related genes. Fibulin-7 overexpression, on the other hand, kept primary cultured epidermal stem cells in a slower cycling speed and undifferentiated state. Molecularly, fibulin-7 binds to other ECM proteins that are important for basement membrane integrity and ECM remodeling after injury. Together, our results suggest that fibulin-7 maintains long-term stem cell heterogeneity, thereby protecting skin from the detrimental effects of aging and maintaining long-term tissue resilience. Our work opens a new avenue for understanding stem cell dynamics through all life stages, from development to aging, with implications for applications in regenerative therapy and future treatments of age-related disorders, including cancer.

Funding Source
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Keywords: Stem cell aging, Skin stem cells, Extracellular matri

RECRUITING PROGENITOR CELLS DURING WOUND HEALING, REGENERATION, AND DEVELOPMENT

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The ability to construct, restore, and regenerate tissues relies on access to a competent source of cellular building materials. Regeneration of the salamander limb is an extreme example where tissue-resident cells are recruited to the amputation plane to form a mass of regenerative progenitors, termed the blastema, which will fully recapitulate lost limb elements. Using in vivo single-cell imaging and multicolor “Limbow” transgenic axolotls, we could track and identify the contributing mesenchymal cell types to the blastema as well as the spatiotemporal rules that influence their migration and differentiation. In conjunction, using an ex vivo culture system and in vivo validation, we uncovered a role for PDGF signaling in recruiting cells for the limb blastema. To better understand whether blastema-like progenitors exist in mammals, we used a Prrx1 enhancer-inducible Cre transgenic mouse to identify and trace a previously uncharacterized subpopulation of dermal limb fibroblasts. Prrx1 enhancer-positive cells are injury-responsive and become enriched in full thickness skin wounds relative to other fibroblast populations. After wounding, enhancer-positive cells also emigrate out of the dermis and display increased differentiation potential in the subcutaneous space beneath the wound bed. We aim to further elucidate mechanisms of full limb regeneration and determine the similarities and deficits that prevent full regeneration in mammals.

INVESTIGATION OF THE MECHANISMS UNDERLYING DELAYED AGING AND CANCER-RESISTANCE IN THE LONGEST-LIVED RODENT, THE NAKED MOLE-RAT

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Naked mole-rats (NMRs) are the longest-lived rodent species and live underground in northeastern Africa. The maximum lifespan of NMRs exceeds 37 years despite their body size being similar to that of mice. Moreover, NMRs have remarkable resistance to cancer; spontaneous carcinogenesis has rarely been observed in more than 2,000 necropsies of captive NMR colonies. Since 2011, we have studied the characteristics of NMRs . Previously, we generated iPS cells from NMRs and showed that NMR-iPS cells exhibit marked resistance to tumorigenesis when transplanted into immunodeficient mice . We recently found that activation of the INK4a?RB pathway, which plays an important role in the induction of cellular senescence, leads to cell death in NMR fibroblasts via species-specific activation of a metabolic pathway leading to ROS production. This phenomenon may contribute to suppressing senescent cell accumulation in NMR individuals. Next, we successfully isolated and cultured neural stem/progenitor cells from the SVZ of neonatal NMRs (NMR-NS/PCs) and found that NMR-NS/PCs were resistant to γ-irradiation . In this symposium, I would like to introduce NMRs and talk about the recent progress in our research.

Funding Source
AMED under Grant Numbers JP21bm0704040, JP-21gm5010001
Keywords: Naked mole-rat, neural stem cell, cellular senescence

CHOROID PLEXUS ORGANOIDS WITH CEREBROSPINAL FLUID AS A MODEL FOR BRAIN AND PATHOGEN ENTRY TO THE BRAIN

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The choroid plexus (ChP) is a highly conserved and surprisingly understudied secretory tissue in the brain. This tissue displays a number of important functions in the brain such as forming a protective epithelial barrier and secreting the cerebrospinal fluid (CSF). The CSF is important for the maintenance of physiological levels of nutrients in the brain and for the transport of signalling molecules and growth factors. To explore the role of the ChP-CSF system, we established a protocol to generate ChP organoids using a combination of signalling molecules that are physiologically present during the stages of development of this tissue. Not only do these organoids develop the ChP but they also recapitulate fundamental functions of this tissue, namely secretion and formation of a tight epithelial barrier. Combining single-cell RNA-sequencing with immunohistochemical and EM validation, we detected the presence of ChP specific channels and transporters localised on the apical border of the epithelium. By testing different compounds, we were able to demonstrate the selective permeability of the ChP barrier in vitro, using NMR. In addition, we noticed the formation of large fluid-filled cysts protruding from the organoids, the contents of which, analysed by mass spectrometry, highly resembles human CSF. Finally, we used this model to test pathogen entry in the brain and we infected the organoids with live SARS-CoV-2. We found that SARS-CoV-2 infects ChP epithelial cells causing damage of this key brain barrier. In conclusion, we believe this system represents an excellent tool to study pathogen and drug entry in the brain.

Funding Source
MRC-LMB (UKRI), NC3Rs
Keywords: Choroid plexus, Organoid, SARS-CoV-2

THE ART OF INTESTINAL ORGANOIDS IN BASIC RESEARCH AND ITS POTENTIAL FOR CLINICAL APPLICATIONS

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Stem cells in gut reside at the bottom of the crypts and play a pivotal role to keep the homeostasis of intestinal epithelium in our healthy state. Recent advances of biological research provide the system to culture these stem cells in vitro as protruding organoids. I not only developed our original organoids using Type I collagen gel as scaffolding matrices, but also firstly provide an experimental proof that the organoids can be used in regenerative medicine in murine colitis model. Since its first publication, the culture system as well as organoids transplantation system were successfully adapted for various different types of normal primary organoids derived from lower intestinal tube. In our multifaceted study to characterize organoids cultured in collagen type I, unique characteristic were identified in its nature of regenerative phase of epithelial cells upon tissue damage/inflammation. Key term to understand this process is YAP-dependent fetalization, and I now try to adapt our organoids to control the cell fate based on 'back-switch' concept. As the organoids recapitulate features of intestinal epithelial in inflammation, this would be another application to use our organoids to understand inflammatory-specific epithelial signature in vitro. Since 2018, I adapted the method for human intestinal cells, and basically same fate conversion were identified in human intestinal organoids. Organoids transplantation system was also improved since 2012 as a versatile experimental tool. I would like to present details of these in the session. I also would like to introduce our recent proceeding in the development of organoids therapy for intractable cased of human ulcerative colitis, which are proceeded in Japan. I believe that organoids study held in Tokyo Medical and Dental University should have a wide impact on a basic stem cell research as well as on a development of stem cell therapy.

Funding Source
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Keywords: Intestinal organoids, organoids therapy, fetalization

Speaker Abstracts

CHOLINERGIC REGULATION OF BONE MARROW STEM CELLS

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The autonomic nervous system is a master regulator of homeostatic processes and stress responses. Although sympathetic noradrenergic innervation decreases bone mass, the role of cholinergic signaling in bone remains largely unknown. We found that early postnatally, a subset of sympathetic nerve fibers undergoes an IL-6-induced cholinergic switch upon contacting the bone. A neurotrophic dependency between GFRα2 and its ligand, NRTN, is established between sympathetic cholinergic fibers and bone-embedded osteocytes, which require this cholinergic innervation for their survival and connectivity. Bone-lining osteoprogenitors amplify and propagate cholinergic signals in the bone marrow (BM). Moderate exercise augments trabecular bone partly through an IL-6-dependent expansion of sympathetic cholinergic nerve fibers. Consequently, loss of cholinergic skeletal innervation reduces osteocyte survival and function, causing osteopenia and impaired skeletal adaptation to moderate exercise. These results uncover a cholinergic neuro-osteocyte interface that regulates skeletogenesis and skeletal turnover through bone-anabolic effects. The sympathetic nervous system (SNS) has been evolutionary selected to respond to stress and activates haematopoietic stem cells (HSCs) via noradrenergic signals. However, the pathways preserving HSC quiescence and maintenance under proliferative stress remain largely unknown. We found that cholinergic signals preserve HSC quiescence in endosteal BM niches. BM cholinergic neural signals increase during stress haematopoiesis and are amplified through cholinergic osteoprogenitors. Lack of cholinergic innervation impairs balanced responses to chemotherapy or irradiation and reduces HSC quiescence and self-renewal. Cholinergic signals activate α7 nicotinic receptor in BM MSCs, leading to increased CXCL12 expression and HSC quiescence. Consequently, nicotine exposure increases endosteal HSC quiescence in vivo and impairs hematopoietic regeneration after HSC transplantation in mice. In humans, smoking history is associated with delayed normalisation of platelet counts after allogeneic HSC transplantation. These results suggest that cholinergic signals preserve stem cell quiescence under proliferative stress.

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Keywords: Cholinergic, HSC, MSC

Stem Cell-Based Therapies

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RETINAL CELL THERAPY USING IPS CELLS

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Our aim is to develop outer retinal cell therapy using iPS cells. The first in man application of iPS cells started in 2013 for age-related macular degeneration. At first autologous iPS-derived retinal pigment epithelial (RPE) cell transplantation was done and then we proceeded to clinical research using HLA matched allogeneic iPSC-derived RPE cells from 2017. Immune responses to transplanted allogeneic cells could be controlled with topical steroids without systemic immunosuppressants. These clinical studies have confirmed the safety of iPS-derived RPE.The next challenge is photoreceptor replacement. iPSC-retinal organoid transplantation is a promising treatment to restore visual function in degenerated retinas.

We proved that (1) grafted immature retinal sheets matured after transplantation in the eye, and (2) grafted photoreceptor cells could form synapses with host secondary neurons in adult mice with photoreceptor degeneration. (3) MEA (multi-electrode array) recordings showed that the grafted cells could elicit light responses in host ganglion cells. (4) The blinded mice could response to light stimuli in the behavior test after transplantation. With those findings as POC, we conducted a clinical study using retinal organoids for retinitis pigmentosa in two patients. Based on these experiences, we are developing therapies for each case of outer retinal diseases. Our company regards regenerative medicine not only as the manufacture of final products but also as therapies. I will talk about the current status and future vision of retinal cell therapy.
Keywords: Retina, photoreceptor, iPSC

PRECLINICAL EFFICACY AND SAFETY STUDY OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED FOREBRAIN NEURAL PROGENITOR CELL HNPC01

Fan, Jing, Ren, Fang, Wang, Anxin, Yan, Yinggang, Zou, Tan, Liu, Qianyun, Zhu, Xinni, Ma, Hong

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Stroke is the leading cause of death and disability worldwide. At chronic stage, behavioral deficits tend to be permanent and no current therapy bring significant benefit to patients. Human fatal tissue stem cells (so called 'fatal neural stem cells') or immortalized cell lines from these cells have been used to develop clinical cell products, such as ReN001 and NSI-566, and reached phase II with FDA. These cell products have exhibited more than 5 years of clinical safety and some chronic stroke patients with treatment show impressive neurological score improvement. However, all these fatal neural stem cells or cell line products have not yet shown significant benefit in random controlled clinic studies. Directed differentiation of human induced pluripotent stem cells (hiPSCs) into function neurons and glia cells is a promising new way to treat brain injury, so called cell replacement therapy. We have manufactured Foxg1 positive forebrain neural progenitor cells (NPCs) product hNPC01 from GMP hiPSC line working cell bank we established. These progenitor cells have shown ability to differentiated into six cortical layer functional neurons and glia cells both in vitro and in vivo. Moreover, these differentiated human neurons form significant synaptic connection to distal brain regions in previous mechanism studies. Besides an extensive set of quality control and stability tests, hNPC01, was subjected to biodistribution, toxicity, and tumorigenicity assessments in rats or nude mice under GLP conditions by third party. There were no adverse effects attributable to the grafted cells, no distribution detected outside the brain, and no cell overgrowth or tumor formation. An efficacy study was also performed and demonstrated long term survival of the grafted cells and behavioral amelioration in permanent MCAO Macaca stroke model. Thus, these preclinical studies indicate that human iPSCs-derived NPCs are clinically applicable for the treatment of patients with stroke.
Keywords: Neural progenitor cells, stroke, iPSC

FIRST IN HUMAN CLINICAL TRIAL OF AUTOLOGOUS IPSC-DERIVED PLATELETS (IPLAT1)

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Platelet transfusion have saved lives of patients with thrombocytopenia through preventing or treating bleeding complications. Our ageing society bears the risk of supply in the future. Furthermore, alloimmune platelet transfusion refractoriness (allo-PTR) is still found in 5% of platelet transfusion patients. Gestation and previous platelet transfusion cause sensitization to produce alloantibodies mostly against class I human leukocyte antigens (HLA-I) and less frequently against human platelet antigens (HPA), resulting in allo-PTR. In these cases, platelets from compatible donors are transfused, but for patients with rare HLA or HPA, donors are difficult to find. As a possible solution, we have succeeded in the ex vivo production of platelets from induced pluripotent stem cells (iPS-platelets) at clinical scale. iPS-platelets are produced from megakaryocyte cell lines (imMKCLs) as master cells and using a "turbulent flow" bioreactor and various new drugs. To evaluate the safety of autologous iPS-platelets administered to an aplastic anemia patient with allo-PTR of rare platelet type. Preclinical studies showed that iPS-platelets were competent in the in vitro assays and mouse and rabbit models for circulation and hemostasis, and

Speaker Abstracts

pathogen and tumorigenicity. The clinical study was performed from March 2019 to January 2020, by dose escalation. Three doses of 1x10^10, 3x10^10 and 1x10^11 were administered in a dose escalation single-center open-label uncontrolled study. The primary endpoint was safety, which were evaluated by the outside Efficacy Safety Assessment Committee for each dose cohort. Three doses of the administration of autologous iPS-platelets have been completed. The insights gained from the current study should contribute to development of allo iPS-platelet products that can be administered to wide range of patients, so that anyone can have transfusion of safe and compatible platelet products whenever in need.

Funding Source
Japan Agency for Medical Research and Development (Japan AMED), Kakenhi (Grant-in aid for scientific research) from Japan Society for the Promotion of Science (JSPS)
Keywords: iPS, Blood, Platelet

FROM CELL REPLACEMENT TO CIRCUITRY RECONSTRUCTION IN PARKINSON'S DISEASE

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Cell based transplantation aimed at the replacement of lost dopamine (DA) neurons holds great potential for the treatment of Parkinson's disease (PD). We have developed robust and efficient differentiation protocols resulting in the formation of authentic and functional DA neurons from human embryonic stem cells (hESCs). Here, I will summarize the translational trajectory and discuss the future potential to move from cell replacement to repair and reconstruction of the DAergic circuitry.

Poster Abstracts

Stem Cells and Development

101

REGULATION OF SENESCENCE AND UNDIFFERENTIATED STATE IN THE MESENCHYMAL STEM/STROMAL CELL

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Mesenchymal stem/stromal cells (MSCs) are present in various body tissues and help in maintaining homeostasis. MSCs have garnered enormous interest as a potential resource for cell-based therapies. However, the molecular mechanisms regulating senescence and undifferentiated state in MSCs remain unclear. To elucidate these mechanisms, we performed gene expression profiling to compare clonal immature MSCs exhibiting multipotency with less potent MSCs. We found that the transcription factor Frizzled 5 (FZD5) is expressed specifically in immature hMSCs. The FZD5 cell surface antigen was also highly expressed in the primary MSC fraction (LNGFR+ THY-1+) and cultured MSCs. Upon FZD5 knockdown, hMSCs exhibited markedly attenuated proliferation and differentiation ability. The observed increase in the levels of senescence markers suggested that FZD5 knockdown promotes cellular senescence. Conversely, FZD5 overexpression delayed cell cycle arrest during the continued culture of hMSCs. Furthermore, we improved the culture method and made it possible to control the undifferentiated and differentiated state in vitro. These results indicated that controlling stemness offers the potential to regulate hMSC quality and improve the efficacy of cell-replacement therapies using MSCs.

Funding Source

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Keywords: Mesenchymal stem/stromal cells, Cellular senescence, Undifferentiated state

103

CROSSTALK OF PARP1 AND ZSCAN4 IN DNA REPAIR AND TELOMERE LENGTH REGULATION

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Zinc finger and scan domain containing 4 (ZSCAN4) elongates telomeres through telomerase-independent alternative lengthening of telomere (ALT) pathway in mouse embryonic stem cells (mESCs). The Poly-ADP ribose polymerase 1 (PARP1), a DNA break repair factor, has been shown to modulate telomere sister chromatid exchange and telomere length homeostasis. Here we are interested to know if PARP1 interacts with ZSCAN4 in DNA repair and in telomere length regulation. We treated telomerase deficient Terc-/- mESCs with a PARP inhibitor 3-aminobenzamide (3-AB) at 5 mM. This treatment led to effective declined the expression of PARP1 level evidenced by both quantitative RT-PCR (qPCR) and Western blot assays, but much higher telomere content as measured by the T/S ratio, demonstrating that PARP inhibition promotes telomere elongation through telomerase independent pathway in mESCs. Interestingly, a reciprocal relationship of ZSCAN4 and PARP1 was revealed. To further understand this relationship, we next overexpressing Flag tagged ZSCAN4 (Flag-ZSCAN4) and HA tagged PARP1 (HA-PARP1) in human 293 cells followed by immunoprecipitation assay and show that these two factors bind each other. To determine which ZSCAN4 motif(s) binds with PARP1, we generated Flag-ZSCAN4 mutants either with a missing domain of the five alpha-helix motifs, or with a missing domain of the four zinc finger (ZF) motifs, and show that the deletion of the 2nd alpha-helix motif or the 1st, 2nd, or the 4th ZF motif diminished the binding. Further, deletion of the 2nd alpha-helix and the 4th ZF (delta-a2ZF4) motifs at the same time completed abolished the binding between Flag-ZSCAN4 and HA-PARP1. We next overexpressed this mutant ZSCAN4 (delta-a2ZF4) that is incapable to bind PARP1, in comparison

Poster Abstracts

with the intact Flag-ZSCAN4, in Terc-/- mESCs. We show that WT Zscan4, when overexpressed, led to substantially reduced signals of rH2AX, a marker of DNA double stranded breaks (DSBs); whereas the mutant Zscan4 almost completely lost this beneficial effect, indicating that the PARP1-ZSCAN4 interaction is essential in ZSCAN4 mediated DSB repair. Together, the present work reveals the Parp1 and Zscan4 interaction and show that such interaction is important in DNA repair and potentially telomere length regulation in mESCs.

Funding Source
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Keywords: Zscan4, Telomere length, DNA repair

104

INVESTIGATION OF MOLECULAR MECHANISMS UNDERLYING THE EXPANSION AND GYRIFICATION OF THE CEREBRAL CORTEX USING FERRETS

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The cerebral cortex has changed during evolution, and there are many differences between the cerebral cortices of humans and mice. The cerebral cortex has become larger, and gyri were formed on the surface of the cerebral cortex. Although these changes in the cerebral cortex are considered to be important for the development of the brain functions, the mechanisms underlying the expansion and folding of the cerebral cortex remain unclear. One of the reasons for this would be that it is difficult to analyze these mechanisms using mice. Therefore, we utilized ferrets, which have relatively large and gyrencephalic cerebral cortex and established in utero electroporation techniques for ferrets in order to manipulate gene expressions in the ferret cerebral cortex. Furthermore, we found that gene knockout in the ferret cerebral cortex can be achieved by combining in utero electroporation and the CRISPR/Cas9 system. Using our techniques, we recently uncovered that FGF signaling and sonic hedgehog (Shh) signaling are involved in the expansion and folding of the cerebral cortex. Here we show the downstream mechanisms triggered by FGF signaling and Shh signaling leading to the expansion and folding of the cerebral cortex. Our genetic manipulation techniques for ferrets should be useful for uncovering the molecular mechanisms underlying the development, evolution and diseases of the complex brains in vivo.

Funding Source
KAKENHI
Keywords: expansion, gyrification, ferret

105

ALTERNATIVE MRNA SPLICING DURING HUMAN NEPHRON PROGENITOR DIFFERENTIATION

Pode Shakked, Naomi P.[1,3,4], Wineberg, Yishay[2], Ben-haim, Nissim[2], Bucris, Efrat[2], Bar-lev, Tali Hana[2], Oriel, Sarit[2], Reinus, Harel[2], Yehuda, Yishai[2], Gershon, Rotem[2], Shukrun, Rachel[3,4], Bar-lev, Dekel Dov[3,6], Urbach, Achia[5], Dekel, Benjamin[3,4,6], Kalisky, Tomer[2]

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Nephrons are the functional units of the kidney. During kidney development, cells from the cap mesenchyme - a transient kidney-specific progenitor state - undergo a mesenchymal to epithelial transition (MET) and subsequently differentiate into the various epithelial cell types that create the tubular structures of the nephron. Faults in this transition can lead to a pediatric malignancy of the kidney called Wilms' tumor that mimics normal kidney development. While human kidney development has been characterized at the gene expression level, a comprehensive characterization of alternative splicing is lacking. Therefore, in this study, we performed RNA sequencing on cell populations representing early, intermediate, and late developmental stages of the human fetal kidney, as well as three blastemal-predominant Wilms' tumor patient- derived xenografts. Using this newly generated RNAseq data, we identified a set of transcripts that are alternatively spliced between the different developmental stages. Moreover, we found that cells from the earliest developmental stage have a mesenchymal splice-isoform profile that is similar to that of blastemal-predominant Wilms' tumors. RNA binding motif enrichment analysis suggests that the mRNA binding proteins ESRP1, ESRP2, RBFOX2, and QKI regulate alternative mRNA splicing during human kidney development.These findings illuminate new molecular mechanisms involved in human kidney development and pediatric kidney cancer.

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Keywords: Alternative splicing, Human embryonic kidney development, Wilms' tumor

106

IDENTIFICATION OF PLURIPOTENT PERIODONTAL TISSUE STEM CELLS AND NICHE FACTORS

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Periodontal tissue is a tooth supporting complex composed of hard and soft tissues. How periodontal tissues are maintained is our central question in order to conserve healthy tooth function. Although it has been speculated that stem cells are involved in periodontal tissue homeostasis and regeneration, and abnormal regulation of stem cells contributes to disease pathogenesis, in vivo analysis of periodontal tissue stem cells has not been thoroughly performed yet. Periodontal cervical area, which we call the crescent region, is a unique place for maintaining the periodontal tissue integrity. Here in, we identified Gli1 (+) stem cell populations in periodontal crescent region and their regulating mechanisms by using genetic lineage tracing technology. Gli1 (+) cells have proliferation ability and differentiation ability to give rise to gingiva, alveolar bone and periodontal ligament tissues. Injury promotes expansion of Gli1 (+) stem cells supported by niche factors, Shh and CXCL12. The Gnas gene, which encodes GALPHAs that transduces GPCR signaling, has an essential role to keep balance of ossification and fibrosis in the periodontal crescent region. We revealed that Gnas gene mutations alter periodontal tissue cell lineage determination and cellular plasticity. Loss of Gnas promotes osteogenic cell fate determination of mesenchymal cells by activating the CXCL12 and Hedgehog signaling, while gain of Gnas function led to fibrosis and caused periodontitis. Thus, our work shows that a novel Gli1(+) stem cell population in the crescent region is regulated by unique niches, which provide new insights into selective lineage determination of periodontal stem cells.

Keywords: Periodontal tissue, Lineage tracing, GPCR signaling

108

THE ROLE OF MURINE ENDOGENOUS RETROVIRUS-L (MERVL) IN PREIMPLANTATION DEVELOPMENT

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After fertilization, the embryonic genome is activated through a process called zygotic gene activation (ZGA) which is critical event for embryos to acquire totipotency. In mice, associated with ZGA, the expression of MERVL, an endogenous retrovirus-derived retrotransposon is transiently upregulated in a 2-cell (2C) stage specific manner. However, the role of MERVL in preimplantation development is still elusive because of few conflicting reports, although the expression of MERVL can be used as a maker for cells in the totipotent state. Here, we show that MERVL is essential for accurate transcriptional regulation and preimplantation development. At early-to-mid 2C stage, MERVL RNA is mainly detected in the euchromatic region of nuclei, but few in the cytoplasm. To decipher the functionalities of MERVL in preimplantation development, we developed anti-sense oligos to knockdown (KD) the expression of MERVL and monitored the effects on preimplantation development. The majority of MERVL-KD embryos suffered developmental delay and halted the development at morula-to-blastocyst transition. The blastomeres from MERVL-KD embryos also displayed the signatures of genomic instability such as increased micronuclei formation and polyploidy. Besides, the expression levels of Oct4 and Cdx2, associated with lineage-specific differentiation are significantly compromised in MERVL-KD embryos. RNA-sequencing analysis revealed that the expression profile of MERVL-KD embryos was different from that of control embryos. Among the genes that were dysregulated in MERVL-KD embryos, the so-called 2C-specific genes including Zscan4c and Tdpoz2 were presented. Furthermore, the genes adjacent to transcriptionally active MERVL loci were significantly downregulated in MERVL-KD embryos. Since we have confirmed that expressed MERVL loci exhibited active epigenetic features such as a loose chromatin structure and H3K27ac/H3K4me3 modifications, suggesting its role in defects of accurate transcriptional regulation. Taken together, our results propose that the expression of MERVL fine-tunes 2C transcriptome in the mouse preimplantation development.

Funding Source
Grant-in-Aid for Scientific Research on Innovative Areas (Research in a proposed research area) to H.S.

Keywords: Preimplantation development, Totipotency, Retrotransposon

Poster Abstracts

109

REGULATION OF THE EPIPHYSEAL STEM CELLS IN THE GROWTH PLATE BY GROWTH HORMONE

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Longitudinal bone growth takes place via the epiphyseal growth plates, positioned at the end of long bones and continuously supplies chondrocytes. Recent findings identified that continuous generation of chondrocytes in the growth plate is facilitated by novel stem cells (epiphyseal stem cells, epSCs). The epSCs continuously self-renew, give rise to chondrogenic and osteogenic lineages and can be identified based on parathyroid hormone-related protein (PTHrP) expression. Growth hormone (GH) is a key regulator of longitudinal bone growth used clinically to promote growth among undergrown children. It remains unknown whether GH can regulate these novel epSCs. First, we pulsed PTHrP-mCherry reporter mice with single injection of either GH or vehicle and harvested them 2 hours later. Activation of JAK2-STAT5 pathway was observed in PTHrP+ epSCs of GH-treated mice, indicating direct action of GH. Consecutive GH treatment of PTHrP-mCherry mice for 10 days (from postnatal day 28 (P28) to P37) decreased the number of PTHrP+ epSCs ($p<0.0001$). To assess proliferation of these slow-dividing cells, EdU was injected consecutively for 4 days (from P34-37). The percentage of EdU+/mCherry+ cells showed no difference between GH- and vehicle-treated mice, suggesting that the division of epSCs was not affected by GH treatment. To further explore epSCs response, PTHrP-creER:R26tdTomato mice were pulsed with tamoxifen at P25 and traced for 13 days in vehicle- and GH-treated mice (treatment from P28 to P37). In line with the observation on PTHrP-mCherry mice, the percentage of single Tomato+ cells decreased in GH-treated group (38.4±6.4% versus 51.3±2.6%, $p<0.005$). Simultaneously, the percentage of clones with more than 3 Tomato+ cells increases from 9.5±2.7% to 19.3±6.1% ($p<0.05$). Importantly, the clones were arranged in longitudinally oriented columns indicating their recruitment toward chondrogenesis. Conclusively, GH stimulates lineage-committed division of epiphyseal stem cells.

Funding Source

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Keywords: growth hormone, epiphyseal stem cells, growth plate chondrocytes

110

ROLE OF GFP-POSITIVE SUBODONTOBLASTIC LAYER IN THE PROCESS OF ODONTOBLAST-LIKE CELL DIFFERENTIATION AFTER TOOTH DRILLING IN NESTIN-ENHANCED GFP TRANSGENIC MICE

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Recently, we demonstrated that endogenous Nestin protein and Nestin mRNA were intensely expressed in differentiated odontoblasts while green fluorescent protein (GFP) immunoreactivity, which reflects the activity of Nestin second intron enhancer-mediated transcription, was mainly observed in the subodontoblastic layer, suggesting that the original odontoblasts and regenerated odontoblast-like cells may differently regulate Nestin expression. This study aimed to investigate the role of GFP-positive subodontoblastic layer in the process of odontoblast-like cell differentiation after tooth drilling in Nestin-enhanced GFP (EGFP) transgenic mice. We prepared the groove-shaped cavity on the mesial surface of maxillary first molars of 5- or 6-week-old mice under deep anesthesia. The animals were perfusion-fixed 1 day to 2 weeks after the operation, and their maxillae were dessected, decalcified, dehydrated, and embedded in paraffin. mmunohistochemistry for Nestin and GFP and Nestin in situ hybridization were conducted. Degenerated odontoblasts lost Nestin immunoreaction 1 day after the injury, and subsequently Nestin-positive newly differentiated odontoblast-like cells arranged along the pulp-dentin border during days 3-5 to form reparative dentin. The commitment of GFP-positive cells into the odontoblast-like cells suggests that the restriction of endogenous Nestin expression in the subodontoblstic layer at the static state was removed by exogenous stimuli to differentiate into Nestin-positive odontoblast-like cells.

Funding Source

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Keywords: Cell Differentiation, Nestin, Odontoblasts

111

DYNAMIC CHANGES IN THE ULTRASTRUCUTURE OF THE PRIMARY CILIUM IN MIGRATING NEUROBLASTS IN THE ADULT BRAIN

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In the adult rodent brain, neural stem cells still reside in the ventricular-subventricular zone and continuously produce immature neurons (neuroblasts). These neuroblasts migrate through the rostral migratory stream (RMS) toward the olfactory bulb, where they differentiate into mature interneurons. Primary cilium is a microtubule-based tiny protrusion that contains signaling receptors on its membrane, and involved in the processing of extracellular signals for the regulations of neural stem cell proliferation and neuronal maturation. The dynamic changes of ultrastructural features in neuroblasts during migration are not yet fully understood. Here we report the presence of a primary cilium, and its ultrastructural morphology and spatiotemporal dynamics, in migrating neuroblasts in the adult RMS. We found that migrating neuroblasts express Arl13b, a ciliary small GTPase, in the close vicinity of centrioles. Serial section transmission electron microscopy revealed that each migrating neuroblast possesses either a pair of centrioles, or a basal body with an immature or mature primary cilium. Furthermore, we investigated the localization of the primary cilium in migrating neuroblasts using live imaging and serial block-face scanning electron microscopy. We demonstrated that the localization and orientation of the primary cilium are altered depending on the saltatory migration. Together, our results highlight a close mutual relationship between spatiotemporal regulation of the primary cilium and efficient migration of neuroblasts in the adult brain. Our results provide the basis to understand the regulation of primary cilium dynamics in neuroblast migration in the adult brain.

Funding Source
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Keywords: neuronal migration, primary cilium, electron microscopy

112

L1CAM IS INVOLVED IN THE CELL MOTILITY OF GLIOMA STEM CELLS AND CAN BE A POTENTIAL THERAPEUTIC TARGET TO CONTROL INVASION OF GLIOMA STEM CELLS

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Gliomas are malignant intracranial tumors and are one of the rare cancers. They show highly invasive and migratory potential, and thereby are difficult to cure with current standard therapies. The analysis of the mechanism of glioma cell migration may lead to the prevention of their invasion and contribute to the improvement of therapeutic outcomes. Glioma stem cells (GSCs) play a central role for glioma formation and maintenance. Although there are many studies suggest biological properties of GSCs, their motility has not been fully revealed. In order to characterize the mechanobiological mechanism of GSCs migration and its relationship to the acquisition of tumor invasiveness, we focused on the mechanobiological roles of cell adhesion molecule L1CAM in GSCs. L1CAM is expressed during

Poster Abstracts

nervous system development, and also well-known as a tumor marker and the association of prognosis. We established GSCs from patient-derived glioblastoma tissues using neutrosphere method, and evaluated their motility by the single-cell tracking method. Migration distance of L1CAM-positive GSCs on laminin matrix was greater than that of L1CAM-negative GSCs. Inhibition of L1CAM function by antibody addition impaired GSCs motility, and conversely, forced expression of L1CAM resulted in promotion of GSCs migration. These results indicate that L1CAM plays an important role in the cell motility of GSCs providing some insight into the mechanobiological mechanisms of malignant gliomas, and suggest L1CAM can be a potential therapeutic target to control invasion of GSCs.

Funding Source
AMED-CREST
Keywords: Glioma, Stem cell, L1CAM

113

MYCL PROMOTES IPSC-LIKE COLONY FORMATION VIA MYC BOX DOMAINS

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iPSCs, induced pluripotent stem cells, theoretically have the potential to differentiate any tissue cells in our body and can be applied not only for regenerative medicine but also for studying embryology. For clinical applications using iPSCs, the problem is that there is the low efficiency of iPSC generation and the quality differences of iPSCs among clones. The problem of quality differences influences the differentiation ability and the reproducibility into several tissue cells. Our previous study reported that MYC proteins (c-MYC, MYCN, and MYCL) are important for reprogramming efficiency and germline transmission of generated iPSCs and that MYCL can generate iPSC colonies more efficiently than c-MYC. However, it remains unknown what makes the difference between c-MYC and MYCL during reprogramming. In addition, there are still many unknowns about the molecular mechanism to promote reprogramming. In this study, we found that several MYC Box domains, which are one of the functional domains conserved in MYC family protein, could bring the phenotypic difference, and promote iPSC generation in MYCL-induced reprogramming. Comprehensive proteomic analysis suggested that MYCL contributed iPSC-like colony formation undergo increasing cell adhesion-related cytoskeletal proteins via MYC Box domains. In this meeting, we would like to talk about the functional analysis of MYCL, including the latest findings.

Keywords: Reprogramming, iPS cell, MYC

114

EFFECT OF 3D CULTURE ON THE EPIGENETIC MODIFICATION AND PLURIPOTENCY REGULATION IN HUMAN INDUCED PLURIPOTENT STEM CELLS

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Expansion of human induced pluripotent stem cells (hiPSCs) under three-dimensional (3D) suspension culture conditions has emerged as a scalable and robust approach for practical cell manufacturing, however, intracellular mechanism governing the regulation of pluripotency maintenance in the 3D culture system is still not well understood. In present study, we elucidated the effect of 3D aggregate culture on cell behavioural changes and epigenetic modification in association with the pluripotent state of hiPSCs. Compared with cells in two-dimensional (2D) monolayers, cells in 3D culture spontaneously aggregated with each other to form a ball-like structure. In response to the 3D environment, during growth, the cells exhibited distinct modulation of cell adhesion and Rho/Rac proteins, which are considered as key cytoskeletal effectors. Rho was upregulated, while Rac was constantly maintained at low level in 3D cultured cells. The cells also significantly downregulated the phosphorylation of myosin light chain, a motor protein that possesses actin cytoskeletal contraction. Compared with 2D cultured cells, quantitative analysis of global histone methylation revealed that 3D cultured cells earlier increased the active H3K4me3 mark and notably persisted the repressive H3K27me3 mark throughout the culture period; furthermore, gene expression analysis demonstrated the substantial expression of naïve pluripotency-associated markers in 3D cultured cells. These extended understandings of the interplay between mechanotransduction mechanism and epigenetic and pluripotency regulation may help design culture environments for stable and efficient production of hiPSCs.

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Keywords: Epigenetic Modification, Human Induced Pluripotent Stem Cells, Three-Dimensional Culture

116

RAPID AND EFFICIENT REGION-SPECIFIC ASTROCYTE INDUCTION FROM HUMAN PLURIPOTENT STEM CELLS WITHOUT EXOGENOUS TRANSCRIPTION FACTORS

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In recent years, astrocytes, which exist around neurons, are thought also to be involved in functional abnormalities in neurological diseases. Therefore, efficient astrocyte induction methods from human induced pluripotent stem cells (iPSCs) are needed to make more appropriate disease models. Although forced induction of astrocytes using transcription factors is robust and highly efficient, it does not completely guarantee the identity of astrocytes with naturally differentiated astrocytes without the use of transcription factors. However, the previously reported methods of astrocyte induction without transcription factors are not sufficiently efficient and require a longer culture period. In this study, we established a protocol to induce astrocyte differentiation in a region-specific manner with high efficiency without using transcription factors. Using the neurosphere method with growth factor cocktails, we differentiated iPSCs into astrocytes via neural stem cells and glial progenitor cells. Both neural stem cells and glial progenitor cells are highly proliferative and we were able to increase the number of cells by passaging. We analyzed the induced cells by immunostaining and qPCR. In several iPSC lines, including control and disease lines, we succeeded in differentiating astrocytes from neurospheres in the spinal cord and midbrain regions with a purity of up to about 70% by 60 days with relatively small numbers of neurons and undifferentiated cells in contamination. Our method has greatly shortened the number of culture days to induce astrocytes in a region-specific manner, but the purity of astrocytes needs to be further improved. We are currently analyzing the detailed characteristics of region-specific astrocytes and analyzing their functions under normal and diseased conditions.

Keywords: Astrocyte, Human iPS Cells, Neurosphere

117

ASSESSMENT OF IPS CELL-DERIVED DOPAMINERGIC PROGENITOR CELLS PROPERTIES WITH LONG-TERM PASSAGING AND AMPLIFICATION

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Human-induced pluripotent stem cells (iPSCs) derived dopaminergic (DA) progenitor cells have the potential to be a resource of cells for regenerative medicine for Parkinson's disease, and mature DA neurons derived from the DA progenitors are used as in vitro disease models for exploring pathomechanisms or drug screening. For broader use of iPSC-derived DA progenitors, it is necessary to supply many DA progenitor cells with consistent quality. We have previously shown the induction method using neurospheres with the regional identity of the ventral midbrain can efficiently induce human iPSC-derived DA neurons. The neurospheres in this method containing many DA progenitor cells can be passaged and amplified; however, it has been unclear how stable the quality of neurospheres is over long-term passaging. The present study tried to show whether ventral midbrain-specific neurospheres could maintain their quality after long-term culture. We induced neurospheres from healthy control-derived human iPSCs and added purmorphamine (a sonic hedgehog agonist) and CHIR99021 (a GSK3 inhibitor) to provide the regional identity of the ventral midbrain and passaged the neurospheres 9 times weekly. The expression levels of midbrain markers (EN1, FOXA2, LMX1A, and TH) analyzed by qPCR were not changed after secondary neurospheres. Microarray analysis of the genome structure revealed that neurospheres have a stable karyotype and no detectable genomic abnormalities. Our results suggest that ventral midbrain-specific neurospheres induced by our method can be maintained with stable quality for at least 70 days.

Keywords: Human-induced pluripotent stem cells, Dopaminergic Neurons, Neural differentiation

Poster Abstracts

119

DENTAL PULP STEM CELL PROPERTIES WITH OSTEOGENIC DIFFERENTIATION CAPABILITY

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Dental pulp stem cells (DPSC) and stem cells from exfoliating deciduous teeth (SHED) were identified as a novel and promising cell source in regenerative medicine. Apparently, SHED, which can differentiate into osteoblasts and odontoblasts have higher plasticity than DPSCs. However, it is realistic to use DPSC as a cell source to reconstruct bone formation in adult case. Therefore, we focused on the osteogenic-specific differentiation ability of DPSCs. The aim of this study is to clarify a characteristic of the DPSCs having high bone differentiation ability.DPSCs from human permanent teeth were isolated by enzymatic treatment, which pulp tissue were digested in DMEM containing 0.3% collagenase for 45min at 37°C. Cells and small piece of pulp tissue were cultured with DMEM supplemented with 20% fetal calf serum (FBS) and antibiotics. And expand cells were cultured with DMEM with 10% FBS and antibiotics.DPSCs passage 3 and 8 were initially plated in monolayers at 1x105cells/cm2 onto fibronectin coated dish in mesenchymal stem cell conditioning medium. After confluency, these cells were induced by osteogenic differentiation medium for 2 weeks.Induced cells were stained with Alizarin red at room temperature for 10 min on a shaker to detect the level of calcium mineral deposition.Single cell suspensions were obtained and incubated with a anti-human stem cell lineage surface antibody (CD105, CD73, CD90, CD29, CD44, CD146, CD106, CD140a, STRO-1, CD271, CD49e, CD117, SSEA-3, SSEA-4) and hematopoietic lineage marker antibody (CD31, CD45, CD34, CD11b, CD19, HLA-DR). The expression profile of cell surface marker was examined using flow cytometry.5 case dental pulp stem cells at passage 3 and 8 were induced by osteogenic differentiation medium. At passage 3, 3 to 5 dental pulp cells case could be stained with Alizarin red. At passage 8, no case could be stained with Alizarin red. CD146 expression of cell surface marker was significant higher at cells with osteogenic differentiation inducibility than without inducibility cells($p<0.05$). AndCD34 expression was significant lower cells with osteogenic differentiation inducibility than without inducibility cells ($p<0.05$).CD146 expression of cell surface marker might be high capability to osteogenic differentiation. and CD34 might be low inducibility marker.

Funding Source

Research funding with Nippon Dental University

Keywords: Dental pulp stem cell, osteogenic differentiation, cell surface marker

120

REFEE, A NUCLEAR RNA BINDING PROTEIN, REGULATES EARLY DEVELOPMENT IN MICE

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Cell totipotency is crucial for mammalian development. However, this developmental ability is limited only for a short period. For instance, 2 to 8 cell stage-embryos have totipotency in mice. To understand how the totipotency is operated, we have investigated a function of one of the genes highly and specifically expressed in mouse 2-cell embryos (2C genes). This gene product has >67% similarity with Aly-RNA Export Factor (Alyref) in amino acid sequences. Thus, we named the gene RNA Export Factor in Early Embryos, Refee. Alyref, which is well-conserved beyond species such as flies to humans, has a central role in RNA export from the nucleus to the cytoplasm. Based on Alyref's known function, we hypothesized that Refee might selectively bind totipotency factor transcripts to enhance their export, thereby regulating totipotency.To confirm its expression, we immunostained the Refee protein of 2C embryos with a specific antibody we produced and found that this protein expectedly located in the nucleus. We then conducted iCLIP with the anti-Refee antibody and observed interactions between RNAs and Refee. In addition, RNA-seq of the iCLIP products revealed that Refee mainly bound with Mouse endogenous retrovirus-L (MERVL) transcripts, as well as Zscan4 and Refee mRNAs: Those are all known 2C genes. Interestingly, when we induced Refee expression exogenously in mES cells, the mES cells showed increased MERVL Gag, Zscan4 and endogenous Refee proteins. These results suggest that Refee can enhance either the export or the translation of its targeting transcripts or both. Finally, to know the impact on Refee in early embryogenesis, we knocked down (KD) Refee in mice early embryos with antisense oligos and found that loss of Refee leads to arrest in early embryogenesis on 2C to 4C. Further, these KD embryos showed less MERVL Gag protein level, supporting our observation in the mES cells. These totally indicate that Refee is essential for early embryogenesis.In conclusion, we revealed; 1) one of the gene products in totipotent embryos, Refee, could contribute to mouse early development to operate totipotency, 2) this Refee-mediated operation enhances expressions of its bound RNAs. How Refee boosts the post-transcriptional events and how the loss of Refee hampers early embryogenesis will be discussed in the meeting.

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Keywords: Refee, Totipotency, 2C genes

121

ETHICS OF EARLY DEVELOPMENTAL RESEARCH

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Early developmental research aims to elucidate the mechanism of the early development of human embryos. Albeit the recent revision in the ISSCR guideline, this field has been regulated by a de facto international regulation called the 14-day rule that stipulates the experiments with embryos must be terminated before 14 days or the appearance of the primitive streak. Hence, there is a limit to the extent to which developing human embryos can be observed, and this field has evolved in an effort to circumvent this regulation. As such, there is an ever-increasing need for research that explores and incorporates proximal domains of the field. Today, the kinds of entities studied in early developmental research are no longer limited to human embryos. They include embryo models, organoids (e.g., brain organoids), in-vitro derived (IVD) gametes, aborted human embryos, the embryos of non-human animals, and the embryos of human-nonhuman chimeras. Nonetheless, the experiments on these entities have not been regulated in a consistent manner. Their regulation has been taken up rather on an ad hoc basis by different nations, regulatory bodies, and institutions. Such differentiated practice of regulation reflects that ethical and policy discussions have been reactive to advances and closed within each area of study. As a result, some concerns over inconsistencies have arisen between regulations governing different entities and the moral reasonings behind them. Thus, our research aims to broadly scope all of the relevant terrains of early developmental research concerning its recent scientific advances and the status of regulations and ethical arguments to identify some regulatory inconsistencies from the perspective of moral status. Moral status is a philosophical concept with a practical implication that is thought to oblige us to treat an entity with that feature in a certain respect. From this perspective, we will suggest options towards a possible solution in conclusion.

Funding Source
ASHBi Fusion Research Grant, Institute for the Advanced Study of Human Biology (WPI-ASHBi), Kyoto University
Keywords: embryo, 14-day rule, moral status

122

ELUCIDATING HUMAN SKELETAL DEVELOPMENT BY INTEGRATING SINGLE-CELL ANALYSIS AND BONE TISSUE INDUCTION USING HUMAN PLURIPOTENT STEM CELLS

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Modeling human skeletal development is an essential step in elucidating the detailed mechanisms underlying this process. Although human pluripotent stem cells (hPSCs) have the ability to differentiate into any cell type, it is challenging to recapitulate 3D bone tissues, which are composed of multiple cell types. To generate human bone tissues, we performed a stepwise induction of the sclerotome from hPSCs in vitro and implanted the induced cells beneath the renal capsules of immunodeficient mice. RNA-seq analysis demonstrated cell type-specific gene expression, indicating a sequential differentiation of hPSCs into the sclerotome via the paraxial mesoderm. In vivo micro-CT images obtained after implantation showed the growth of bone tissues over the time course. Histological and immunohistochemical analyses revealed endochondral bone-like structures with specific patterns of marker expression: columnar structure of chondrocytes, bone collars, and bone marrow. The induced bone tissues were then analyzed by single-cell RNA-seq, and the obtained data were integrated with the publicly available gene expression profiles of human embryonic long bones at 8 weeks post conception. Clustering analysis identified multiple skeletal cell types with distinct gene expression signatures; the gene expression profiles of the hPSC-derived bone tissues overlapped with those of the embryonic long bones. Pseudotime analysis predicted a bifurcating trajectory from embryonic skeletal progenitors to osteoblasts or chondrocytes. By integrating differentially expressed genes, gene regulatory network analysis, and ligand-receptor analysis, we extracted novel transcriptional regulators that may play important roles in osteogenesis. In situ hybridization of the hPSC-derived bone tissues showed a partial co-expression of the identified regulators with RUNX2 and SP7 in the bone collar and bone marrow.

Poster Abstracts

Knockdown of these regulators with short hairpin RNA downregulated osteoblast marker genes in a human osteosarcoma cell line (Saos2), indicating the involvement of these regulators in osteogenesis. In conclusion, our bone induction method recapitulates human endochondral bone formation and will be a valuable tool to investigate hard-to-access human skeletal development.

Funding Source
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Keywords: human pluripotent stem cells, skeletal development, single-cell analysis

123

ELUCIDATION OF NEURITE OUTGROWTH MECHANISM USING GAMMA-SECRETASE INHIBITOR-TREATED HIPSCS-DERIVED NEURAL STEM/PROGENITOR CELLS AS SCREENING SAMPLES

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Neurons extend neurites and form synapses with other neurons to exchange signal transduction substances, thereby they collectively can execute brain functions such as learning and memory. During neural network formation, active neurite extension is required for cerebrum development, especially during human fetal development. Although neurites can be elongated by the polymerization of microtubules, few signaling cascades have been discovered that control this mechanism in human neurons, and few compounds have been found that can extend neurites by promoting the polymerization of microtubules. Here, we found that the new signaling pathway was a key to enhance the neurite outgrowth in human neurons by promoting the polymerization of microtubules. In this study, we induced the expression of neural lineage genes by treating human induced pluripotent stem cell-derived neural stem/progenitor cells with GAMMA-secretase inhibitors and used them as screening samples to identify pathways that elongate neurites. Additionally, Compound Y from Streptomyces sp. was shown to enhance microtubule polymerizations and promote neurite outgrowth by reinforcing this new signaling. Neurites regress and brain dysfunctions increase as time goes by, due to aging and neurodegenerative diseases. Therefore, clarifying the mechanism underlying neurite outgrowth might help to find a cure for neurodegenerative diseases and senescence.

Keywords: hiPSC, hiPSC-NS/PC, neurite

124

NEUROGENIC POTENTIAL IN COMMON MARMOSET VENTRICULAR-SUBVENTRICULAR ZONE DURING POSTNATAL BRAIN DEVELOPMENT

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In many mammals, neuronal production continues in the ventricular-subventricular zone (V-SVZ) located at the lateral walls of lateral ventricles and hippocampal dentate gyrus even after birth. In rodents, most new V-SVZ-derived neurons migrate along the rostral migratory stream towards the olfactory bulb, where they differentiate into interneurons throughout life. In human brain, neuroblast production and migration toward the neocortex and the olfactory bulb occur actively only for a few months after birth, and then sharply decline with age. Due to methodological limitations in human study, the precise spatiotemporal profiles and fates of postnatally born neurons remain unclear. Here, using common marmosets, small non-human primates, injected with BrdU and/or EdU during various post-natal periods, we studied dynamic spatiotemporal changes in neurogenesis during development. Cell-proliferation in the V-SVZ and neuroblast migration toward the olfactory bulb and neocortex sharply decreased by 4 months. The new neurons matured within a few months in the olfactory bulb, but remained to express immature neuron markers until 6 months in the neocortex. Although activity of progenitor proliferation was sustained for a month after birth, the distribution and/or differentiation diversity was more restricted in 1-month-born cells than in the neonatal-born population. These findings suggest primate-specific mechanisms of neurogenesis in the postnatal V-SVZ.

Funding Source
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Keywords: ventricular-subventricular zone, neuroblast, common marmoset

125

THE CRADLE OF CEREBROSPINAL FLUIDS NURTURING EMBRYONIC NEURAL STEM CELLS

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Embryonic neural stem cells that reside in the luminal side of the neural tube are exposed to the cerebrospinal fluids (CSF) on their apical surface. CSF contains various secreting molecules, such as Shh, IGF and Wnt, each of which has been shown to be required for the proper growth of neural stem cells. In fact, slice culture of the cerebral cortex of mouse embryos can be maintained with 100% embryonic CSF but not with artificial CSF, indicating that CSF contains factors essential for the maintenance and proliferation of neural stem cells.Primates including human have markedly expanded cerebral cortex. Expansion of the cerebral cortex is one of the important factors that archives higher cognitive abilities and sensorimotor functions. However, it remains unclear how a species-specific expansion period of neural stem cells is determined and mechanisms underlying massive enlargement of human cerebral cortex.We have identified several secreting factors in the embryonic CSF which are preferentially enriched in macaque monkey (Macaca fascicularis) compared to mouse. We would found that these factors promoted proliferation of human neural stem cells, as well as murine ones. Furthermore, antibodies against one of those cancelled the pro-proliferative effect of macaque CSF on neural stem cells. This factor could indeed induce cortical expansion in vivo, upon injection into the lateral ventricle of mouse embryos in utero. Interestingly, it is not expressed in the embryonic choroid plexus (ChP) which produce CSF but adult ChP in mice, suggesting that interspecific heterochrony of this gene may contribute to brain evolution. We would like to propose that these primate specific CSF factors play important roles in the expansion of cerebral cortex. CSF provides an optimal species-specific environment for nurturing neural stem cells.

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Keywords: neural stem cells, cerebrospinal fluids, primates

126

THE ESSENTIALOME OF HUMAN PLURIPOTENT STEM CELLS AND THEIR DIFFERENTIATED DERIVATIVES

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Maintenance of pluripotency requires coordinated expression of a set of essential genes. A thorough identification of these essential genes is vital for gaining a better understanding of the pluripotency state, as well as of the differences between this unique cellular state and differentiated states. To this end, we have generated a genome-wide loss-of-function library in haploid human pluripotent stem cells (hPSCs) that we recently isolated. For this screening platform, we utilized CRISPR/Cas9 technology targeting more than 18,000 coding genes, using over 180,000 sgRNAs. This library enabled us to define the genes essential for the normal growth and survival of undifferentiated hPSCs. By defining the essential genes for hPSCs, we could allude to an intrinsic bias of essentiality across cellular compartments, uncover two opposing roles for tumour suppressor genes and link autosomal-recessive disorders with growth retardation phenotypes to early embryogenesis. We then set out to map the essential genes for the differentiation of hPSCs into the three embryonic germ layers by using our loss-of-function library. Through the analysis of essential genes for the differentiation of hPSCs into ectoderm, mesoderm and endoderm, we defined the essentialome of each germ layer separately and also identified commonly essential genes for the transition from pluripotency stage into differentiated cells. More recently, we utilized our screening platform to identify genes regulating the differentiation into more advanced stages of neural differentiation, revealing essentiality phenotypes for a group of HOX genes among other transcription factors. Overall, our work sheds light on the gene networks regulating pluripotency, specifications of all germ layers and early neural differentiation in human.

Keywords: Human embryonic stem cells, Differentiation, Genome-wide CRISPR screens

127

LARGE-SCALE ANALYSIS OF LOSS OF IMPRINTING IN NAIVE HUMAN PLURIPOTENT STEM CELLS

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Naive human pluripotent stem cells (hPSCs) hold great potential for studying molecular processes and cell fatedecisions that occur in human pre-implantation embryos. Genomic imprinting is a parent-of-origin

Poster Abstracts

dependentmonoallelic expression of a subset of genes and is required for normal growth and development. Previous studiesshowed that the conversion of primed hPSCs into naive pluripotency is accompanied by genome-wide loss ofmethylation that includes imprinted loci. However, the extent to which the loss of methylation at imprinted locicauses aberrant bi-allelic expression of imprinted genes is still unknown. Here we analyze loss of imprinting (LOI)in a large cohort of both bulk and single cell RNA sequencing samples of naive and primed hPSCs. We show thatnaive hPSCs exhibit much higher levels of LOI and biallelic expression than their primed counterparts. This imprint loss is not random, but rather shows a clear bias towards certain imprinted regions. Specifically, genes under the control of paternally methylated imprinting control regions (ICR) are more prone to lose imprinting than genes under the control of maternally methylated ICR. Importantly, we show that different protocols used for the primed-to-naive conversion led to different extents of LOI, a difference which is tightly correlated to FGF signaling. This analysis sheds light on the process of LOI that occurs during the conversion to naive pluripotency and highlights the importance of taking these events into consideration when modeling disease and development or when utilizing the cells for therapy.
Keywords: Early embryo, Naive human pluripotent stem cells, Imprinting

128

GENERATION AND CHARACTERIZATION OF TRIPLOID HUMAN EMBRYONIC STEM CELLS

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Humans are diploid organisms, but many tissues in our body harbor different number of genomic copies. Triploidy in human embryos is responsible for ~10% of all spontaneous miscarriages but, surprisingly, some of these pregnancies proceed to term. These complete or mosaic triploid infants suffer from many physical and mental deficiencies, leading to their early death. To investigate the impact of triploidy on human development, we generated triploid human embryonic stem cells (hESCs) by fusing haploid and diploid hESCs. This allowed us to study the behavior of triploid compared to diploid hESCs carrying the same genetic background. The triploid hESCs showed a typical morphology of hESCs, with a larger volume than the diploid cells. We analyzed the genome-wide transcription, methylation, and replication timing profiles, as well as the pluripotency of these cells. Both diploid and triploid hESCs express all pluripotent stem cell markers, although we identified differential expression in genes linked to several developmental processes, cellular response, and metabolism. Interestingly, we have observed a very significant decrease in the ability of triploid hESCs to differentiate in vivo, mostly into central and peripheral nervous system, when compared to their diploid counterparts. Our research established a platform to study triploidy in humans and points to their pathology as observed in triploid embryos.
Keywords: Triploidy, Human pluripotent stem cells, Human development

129

AMNION FORMATION IS THE FIRST EMT PROGRAM IN HUMAN DEVELOPMENT

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Mouse biology has served as a general template for the understanding of other mammals' physiology. However, amnion development is challenging to translate from mouse development to human, as in the mouse, it occurs after gastrulation, whereas primate epiblast (EPI) gives rise to amniotic epithelial (AME) cells before gastrulation. We here aim to elucidate the mechanism of AME induction during human development. We used our in-house developed iPSCs, where reprogramming using the Yamanaka factors was aided by DNA demethylase TET1 (T-iPSCs) to model human development. T-iPSCs were treated with Activin A and WNT3A; the latter factor secreted from the AME-adjacent trophoblast in primates. BMP4 expression, a critical factor currently considered to induce AME, was gradually upregulated after WNT3A addition. We observed concomitant cell shape changes from columnar epithelium, representative of EPI, to AME-like squamous epithelium/mesenchymal cells. We then examined the expression of epithelial-to-mesenchymal transition (EMT) transcription factors (TFs) (TWIST1, SNAI1/2, and ZEB1/2) and found that all EMT TFs increased their expression in response to WNT3A stimulation. Surprisingly, the upregulation of these EMT TFs was not affected by BMP inhibitor. Together, we concluded that the contribution of BMP4 on the cell shape changes caused by these EMT TFs is indirect, downstream of WNT signaling. By analyzing human early embryo single-cell RNA-seq data, we found that upregulation of all the EMT TFs occurred during human amnion development. These results suggest that previous differentiation studies where BMP4 was the sole inducer would result in aberrant AME differentiation. We conclude that WNT3A secreted by trophoblast would be a crucial inducer for the appropriate AME induction in humans. EMT, which is observed in almost all malignant transformations, is considered to mimic the processes of gastrulation or neural crest formation during

development. However, these processes activate only a small subset of the EMT TFs. Therefore, the EMT in AME differentiation revealed in this study can be considered the first and appropriate prototype of the EMT program in human development.

Funding Source
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Keywords: Human pluripotent stem cells, Amnion, EMT

130

HYDROGEL ENVIRONMENT INDUCES IBLASTOID GENERATION DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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A blastocyst will not only help study the early development of a cell but can also be used in drug discovery for pregnancy failure and birth deficiency prevention. However, especially in human beings, it is very difficult to obtain blastocysts from fertilized eggs for fundamental research as well as industrial usage due to limited cell sources / donors and ethical concerns. Recent studies have reported methods of creating blastocysts using human pluripotent stem cells (hPSCs). However, most of those studies use cells that have been transformed into naïve cells from primed hPSCs. Here, we develop a method to generate iBlastoids from primed hPSCs in a three-dimensional (3D) hydrogel environment. We name it as the HG-iBlastoid. This method uses a thermo-responsive hydrogel [a copolymer of poly (N-isopropylacrylamide) and poly (ethylene glycol) (PNIPAAm-PEG)], which provides cultured hPSC aggregates with mild physical stimuli. Importantly, the hydrogel needs not to adhere the cells to prevent migration into the gel. Additionally, because it can perform a sol-gel transition via temperature, the hydrogel allows indroducing and harvesting of HG-iBlastoids from the solution at a low temperature < 20°C), and culture them at 37°C. To visualize the expression of octamer-binding transcription factor 4 (OCT4) ICM marker, we used OCT4 promoter-driven KhES1 hESCs with an enhanced green fluorescent protein (K1-OCT4-EGFP). Using K1-OCT-EGFP, we cultured with DMEM supplemented with 10% FBS with the hydrogel for four days, and with iBlastoid medium with the hydrogel for four days. To confirm the cellular distribution of trophoblasts and ICM cells in HG-iBlastoids, we observed the expression patterns of OCT4 and CDX2 in HG-iBlastoids via whole-mount immunocytochemistry. In conclusion, we established a simple method to generate human HG-iBlastoids from only hPSCs. The HG enables better environments for the formation of HG-iBlastoids, which suggests that a certain physical property of the gel plays an important role in the blastocyst formation and not the fluid property.

Funding Source
The Japan Society for the Promotion of Science (JSPS;17H02083).
Keywords: Blastoid, Hydrogel, hESC

131

A CALCINEURIN-MEDIATED MECHANISM THAT CONTROLS A K+-LEAK CHANNEL TO REGULATE SCALING OF AN ENTIRE VERTEBRATE APPENDAGE

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Generation and regeneration of all organs require the precise proportional contribution of stem and progenitor cells; otherwise, disproportional growth or cancer will result. In this regard, all anatomical structures form to the specific dimensions to the body. The proportional growth of stem and progenitor cells is a fundamental yet poorly understood phenomenon that involves tissue scaling mechanisms. Previous findings show that the continued activity of the two-pore potassium-leak channel Kcnk5b produces allometric growth of zebrafish appendages. However, it remains unknown how the bioelectric activity of this channel is integrated into the scaling mechanisms of these anatomical structures. We show the activation of Kcnk5b is sufficient to activate several development programs (Shh, Retinoic acid, Fgf) in adult, larva and embryonic structures. In vivo mosaic analyses indicate that the activation of these developmental programs by Kcnk5b is cell autonomous. However, the electrophysiological changes induced by Kcnk5b are not restricted to specific developmental signaling cascades, since this bioelectric signal induces the expression of different combinations of developmental genes in different mammalian cell lines. We also provide evidence that the post-translational modification of serine 345 in Kcnk5b by calcineurin regulates channel activity to scale the entire fin anatomical structure by controlling these developmental programs. Thus, we show how an endogenous electrophysiological mechanism can be regulated to coordinate the activity of different developmental cascades to scale the proportions of a vertebrate appendage.

Funding Source
Deutsche Forschungsgemeinschaft Shanghaitech University
Keywords: Tissue Scaling, Electrophysiology, zebrafish fin

Poster Abstracts

132

THE ESTABLISHMENT AND PROGRESS OF NATIONAL CONSORTIUM FOR REGENERATIVE MEDICINE IN JAPAN

Okada, Kiyoshi[1], Umezawa, Akihiro[3], Susumu, Eguchi[4], Kinooka, Masahiro[5], Sato, Yoji[6], Takahashi, Jun[13], Takahashi, Masayo[7], Tanaka, Rica[8], Terai, Shuji[9], Hata, Kenichiro[10], Yashiro, Yoshimi[11], Nakamura, Masaya[2], Morio, Tomohiro[12], Nishida, Koji[1], Okano, Hideyuki[2]

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Since 2014, when the current laws and regulations related to regenerative medicine came into effect in Japan, regenerative medicine has received more attention as a medical field that has the potential to treat diseases that were previously difficult to cure. The Japanese Society for Regenerative Medicine has been establishing the National Consortium for Regenerative Medicine to serve as a research support platform for five years with the support of the Japan Agency for Medical Research and Development. The National Consortium for Regenerative Medicine is developing the infrastructure under the five themes of clinical research support, human resource development for regenerative medicine, database construction, industry-academia collaboration, and company-academia collaboration. In 2021, the number of consultations related to clinical research exceeded 70, and the sale of textbooks for certified doctors and clinical incubators and the E-Learning system were completed and are already in operation. In addition, the National Regenerative Medicine Database, which is a regenerative medicine patient registration system that has been constructed in collaboration with the Pharmaceuticals and Medical Devices Agency, has already started operation in 2017. We hope that the continuous promotion of this activity will lead to the universalization of a new era of regenerative medicine.

Funding Source
This work was supported by the Project for Improvement of Infrastructure to Promote Clinical Research for Regenerative Medicine (Japan Agency for Medical Research and Development).
Keywords: Regenerative Medicine, Clinical trial, Database

133

SARCOMERE SHORTENING OF PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES USING FLUORESCENT-TAGGED SARCOMERE PROTEINS DELIVERED VIA ADENO-ASSOCIATED VIRUS

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Pluripotent stem cell-derived cardiomyocytes (PSC-CMs) are a promising tool for cardiac disease modeling and drug screening. For cardiomyopathies, Sarcomere shortening is a standard functional test that is used to study the disease phenotypes of adult cardiomyocytes. However, the available conventional methods are not suitable to assess the contractility of PSC-CMs, as these cells have underdeveloped sarcomeres that are invisible under phase-contrast microscopy. To address this issue we designed plasmids containing fluorescent-tagged sarcomere protein sequences. Then we transduced them into PSC-CMs via Adeno-Associated viral vectors thus enabling endogenous expression of these fluorescent-tagged proteins without the need for knock-in. Two Z-line fluorescent-tagged proteins: actin-associated LIM protein (PDLIM3-GFP) and Telethonin (TCAP-GFP) successfully localized to the Z-line of the sarcomere. No extra sarcomeric localization was observed. Further truncation of Telethonin was done in which the first 80 amino acids were sufficient to localize to the Z-line (TCAP1-80-GFP). These fluorescent sarcomere protein tags are novel, flexible, and easy-to-use tools for fluorescent live-imaging of PSC-CMs.

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Keywords: Pluripotent stem cell-derived cardiomyocytes, fluorescent-tagged proteins, fluorescent live-imaging

134

FUNCTION OF SCHWANN CELL PRECURSORS AS THE AUTONOMIC NEURAL STEM CELLS

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Schwann cells are classically defined as supporting cells in the peripheral nervous system that play an essential role in axon myelination. Recent genetic cell labeling studies discovered that Schwann cell precursors (SCPs) are capable of differentiating into parasympathetic and enteric neurons in vivo. Although these data identify SCPs as the autonomic neural stem cells, overall distribution and potential involvement in human diseases of the SCP-derived autonomic neurogenesis remains unclear. In this presentation, we show that SCP-derived neurogenesis acts to compensate for a neuronal loss in mouse models of Hirschsprung disease, a congenital disorder characterized by the absence of the enteric ganglia. We also show that SCP-derived neurogenesis is abundant throughout the body in developing mouse embryos, can be enhanced by a genetic manipulation, and responds to an oncogenic driver mutation. SCP-derived neurogenesis therefore displays positive and negative impacts on human pathogenesis.

Funding Source

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Keywords: Schwann cells, autonomic neurons, neural stem cells

135

TRANSIENT RECEPTOR POTENTIAL ANKYRIN 1 CHANNELS REGULATE THE MATURATION OF MOUSE EMBRYONIC STEM CELL-DERIVED CARDIOMYOCYTES

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Embryonic stem cells (ESCs) can self-renew and maintain pluripotency to differentiate into all cell lineages including cardiomyocytes. These cardiomyocytes have been widely accepted as a promising cell source for drug screening and cell replacement therapy. However, the immature properties of ESC-derived cardiomyocytes (ESC-CMs) hinder their usage. Previous studies showed that transient receptor potentialankyrin 1 (TPRA1) channels are expressed in adult cardiomyocytes; however, whether TRPA1 channels are expressed in ESC-CMs and their function in ESC-CMs are unexplored. This project aimed to study the roles of TRPA1 in the regulation of functions and maturation of mouse ESC-CMs. Western blot and immunocytochemistry revealed the expression of TRPA1 in ESC-CMs. Using confocal microscopy, TRPA1 blocker or knockdown of TRPA1 was found to increase the time-to-peak and decrease the decay rate of calcium transients. By patch-clamping, TRPA1 blocker or knockdown of TRPA1 was found to decrease the maximum upstroke velocity of action potentials. On the other hand, activation or overexpression of TRPA1 caused the opposite effect on ESC-CMs. Interestingly, overexpression of TRPA1 increased the size of ESC-CMs, percentage of multi-nucleate cells, percentage of cells with developing t-tubules, and decreased the percentage of cells with disorganized sarcomeres, suggesting a more mature status of the ESC-CMs. Conversely, knockdown of TRPA1 hindered the maturation of ESC-CMs. Altogether, this study reveals that TRPA1 is a positive regulator of the maturation of ESC-CMs. The results of this study provide important insights for the potential strategy to improve the maturation of ESC-CMs for future use in drug screening and/or regenerative medicine.

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Keywords: transient receptor potential ankyrin 1 channel, embryonic stem cell-derived cardiomyocytes, maturation

137

DONOR CELLS PREDOMINANTLY PROLIFERATE IN IGF1R KNOCKOUT RODENT EMBRYOS

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Although successful interspecies organ generation has been demonstrated in rodents using blastocyst complementation, similar success is yet to be observed in other species due to the early developmental arrest hindering high chimeric fetus formation. Here, we demonstrate that the deletion of Insulin-like growth factor 1 receptor (Igf1r) in mouse embryos creates what we have termed the "cell competitive niche", which significantly increases donor chimerism in both intra (mouse→mouse) and inter-species (rat→mouse and vice versa) rodent chimeras. We have

Poster Abstracts

found that Igf1r deletion augments donor chimerism starting from embryonic day 11 (E11) onward in several organs except blood. Remarkably, donor chimerism in Igf1r null chimera increased approximately 3 times higher than wild-type chimera at E18.5. The enhanced donor chimerism continuously increased and even took over the whole organs in some intra-species chimeras. Among them, donor chimerism in kidney, brain, and lung approached 100%, not the case in other organs or tissues. We further investigated the structure and function of these almost entirely donor-derived organs. The donor-derived kidneys were morphologically normal, with unremarkable tissue architecture, and the level of serum blood urea nitrogen and creatinine in these chimeras were in normal range indicating these kidneys functioned. Immunohistochemistry revealed all renal component of the kidneys were derived from donor cells. Donor chimersm significantly increased in rat→mouse and mouse→rat interspecies chimeric embryos. Since the Igf1r deletion increases donor chimerism from the mid to late developmental stages, highly chimeric fetuses can also evade the early developmental arrest observed in interspecies chimera formation. This observation should facilitate donor cell contribution to host tissues, resulting in whole-organ generation via blastocyst complementation across wide evolutionary distances.

Funding Source
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Keywords: Pluripotent stem cells, Organ regeneration, Chimera

139

ROLES OF CELL CYCLE INHIBITION IN THE GENESIS OF ADULT NEURAL STEM CELLS

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Adult neural stem cells (aNSCs) generate neurons that modify cognitive functions such as learning and memory. Our previous studies demonstrate that most aNSCs in the lateral wall of the SVZ are derived from slowly dividing subpopulation of embryonic neural progenitor cells (NPCs), while the rapidly dividing subpopulation give rise to neurons and glial cells that populate the brain during development. High expression of the cyclin-dependent kinase inhibitor (CKI) p57 in the slowly dividing subpopulation inhibits proliferation and neural differentiation and is required for genesis of adult NSCs. However, the molecular and cellular mechanisms by which embryonic cell cycle inhibition maintains the undifferentiated state of embryonic NPCs resulting in the emergence of a stable population of postnatal adult NSCs remains unknown. In this study, we found that Notch and an effector Hey1 form a module that is upregulated by cell cycle arrest in slowly dividing NPCs. In contrast to the oscillatory expression of the Notch effectors Hes1 and Hes5 in fast cycling progenitors, Hey1 displays a non-oscillatory stationary expression pattern and contributes to the long-term maintenance of NSCs. These findings reveal a novel division of labor in Notch effectors where cell cycle rate biases effector selection and cell fate.

Funding Source
Grant-in-Aid for Young Scientists, Japan Society for the Promotion of Science, Japan 21K15180
Keywords: Neural stem cell, Quiescent, Notch signaling

140

CHARACTERIZATION OF NEURAL STEM/PROGENITOR CELLS IN THE SUBVENTRICULAR ZONE OF THE NAKED MOLE-RAT

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The naked mole-rat (NMR) is the longest-lived rodent with a maximum lifespan of over 37 years and shows a negligible senescence phenotype, suggesting that their somatic stem cells are highly capable of maintaining homeostasis. However, the properties of NMR somatic stem cells are still largely unknown. Here, we isolate and characterize neural stem/progenitor cells (NS/PCs) from the subventricular zone (SVZ) of neonate NMRs. NMR-NS/PCs express several neural stem cell marker genes and can differentiate into neurons, astrocytes, and oligodendrocytes. NMR-NS/PCs show markedly slower proliferation and a higher percentage of cells in the G0/G1 phase than those of mice (MS-NS/PCs). Notably, upon gamma-irradiation, NMR-NS/PCs showed fast DNA damage responses and less prone to cell death than MS-NS/PCs. The slow proliferative potential and resistance to DNA damage in NMR-NS/PCs may cooperatively contribute to the prevention of stem cell exhaustion in the NMR brain during their long lifespan. Our findings provide novel insights into the mechanism of delayed aging in the NMR brain.

Keywords: Naked mole-rat, Neural stem cell, Longevity

141

PROSTAGLANDIN E2 RECEPTOR EP2 IS IMPORTANT FOR THE SELF-RENEWAL AND MAINTENANCE OF SKELETAL MUSCLE STEM CELLS

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Muscle satellite cells are skeletal muscle-specific stem cells indispensable for regeneration and homeostasis of skeletal muscle. Therefore, their self-renewal and maintenance are important for skeletal muscle integrity, and elucidation of the mechanisms is expected to contribute to the development of therapies for muscle diseases, such as Duchenne muscular dystrophy. Many research groups reported the role of Notch signaling for self-renewal and maintenance of muscle satellite cells, but the downstream events are not clear. Previously, we reported that EP2, one of the four prostaglandin E2 receptors, suppresses myogenic differentiation of human skeletal muscle progenitor cells downstream of Notch signaling (Sakai-Takemura et al., 2020). In this study, we analyzed the role of EP2 in muscle satellite cells using EP2 conditional knockout mice (Pax-7CreERT2/+; EP2flox/flox) and a control group (EP2flox/flox). Four weeks after intraperitoneal administration of tamoxifen for 5 consecutive days to both groups, muscle satellite cells were isolated using a cell sorter and their functions were analyzed in vitro. Inactivation of the EP2 gene (Ptger2) resulted in a significant decrease in the number of muscle satellite cells. The isolated satellite cells from EP2 conditional knockout mice showed proliferative capacity similar to that of the control satellite cells, but their differentiation capacity (fusion index) was increased and self-renewal capacity (frequency of reserved cells) was decreased, compared with control satellite cells. Together, our results suggest that EP2 regulates both self-renewal and maintenance of muscle stem cells downstream of Notch signaling.

Keywords: EP2 receptor, Muscle stem cells, Muscular dystrophy

142

THE PARACRINE EFFECT OF ADIPOSE-DERIVED STROMAL VASCULAR FRACTION ON HUMAN CHONDROCYTES

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Adipose-derived stromal vascular fraction (SVF) is composed of heterogeneous cells including adipose-derived stem cells (ADSC), macrophages, and more. The paracrine effect of heterogeneous cells in SVF could support therapeutic effect, but their roles for osteoarthritis treatment remain unclear. This study aimed to investigate the therapeutic effect of heterogeneous cells in SVF on chondrocytes. In our preliminary study, based on the CFU-F assay, the ADSC ratio in SVF was defined as 10%. Chondrocytes (1 × 105 cells) were seeded in culture plates, and co-cultured with SVF (1× 105 cells) or ADSC (1 × 104 cells) on cell culture inserts. The control group was established with chondrocytes only. After 48h co-culture, the mRNA expressions of collagen II, TIMP-3, and MMP-13 of chondrocytes were evaluated with RT-PCR, and TGF-BETA level in the supernatant was measured with ELISA. To confirm the macrophages in SVF, F4/80, CD86, and CD163 were detected by immunohistochemical staining. To evaluate whether the Smad2/3 signaling pathways were involved in the paracrine effect of SVF, chondrocytes were pretreated with the inhibitor of phosphorylation of Smad2/3, and then stimulated with SVF. After 48 h, the phosphorylation of Smad2/3 was examined by western blotting, and mRNA expressions of collagen II, TIMP-3, and MMP-13 of chondrocytes were evaluated with RT-PCR. Additionally, pellets of chondrocytes (1 × 105 cells) were cultured in the same condition, and the pellet size was measured after 3 weeks. In the SVF group, mRNA expression of collagen II and TIMP-3 were higher, and expression of MMP-13 was lower than the other groups. The TGF-BETA level in the supernatant of the SVF group was higher than the other groups. In the immunohistochemical staining, the majority of macrophages in SVF were anti-inflammatory macrophages defined as double-stained cells with F4/80 and CD 163. In western blotting, smad2/3 phosphorylation

Poster Abstracts

levels were increased by the stimulation of the SVF and suppressed by specific inhibitors. Additionally, the inhibitors suppressed the stimulation of SVF for RT-PCR of chondrocytes and pellet size. These findings suggested that the paracrine effect of heterogeneous cells including anti-inflammatory macrophages in SVF partly supports the regeneration of chondrocytes through TGF-BETA-induced Smad2/3 phosphorylation.

Funding Source
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Keywords: stromal vascular fraction, paracrine effect, Smad2/3 signaling pathways

143

IDENTIFYING THE MINIMAL SET OF PRINCIPLES FOR COMPUTATIONAL RECONSTRUCTION OF EMBRYOGENESIS WITH SPATIO-TEMPORAL TRANSCRIPTOME ANALYSIS

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Biological processes are tightly controlled by dynamic regulatory networks to ensure the correct functions. During embryogenesis and development processes, cells are formed into complex 3D tissues and organs accompanied with highly organized events in time and space. Different spatio-temporal modulation can regulate cell fate decision and body plan. Therefore, analysis approaches with considering of the dynamic nature of biological processes are required to provide a deeper insight into the space and time-varying events. In this study, we proposed an integrated analysis strategy to decipher the underlying mechanism of embryogenesis with temporal and spatial analysis methods. We incorporated the temporal analysis with developmental trajectories to investigate the dynamic gene expression patterns of each germ layers and to find out the key developmental genes that play a vital role in the developmental stage transformation. For the investigation of spatial information, we designed an enhanced model based on the previous developed self-organizing map (SOM) clustering approach with interaction models for more complicated 3D structures. This in silico method provide better insights into the spatial relationship of cells that can help us to identify spatial discriminator genes and reconstruct the complex 3D structures. Combining with temporal and spatial information, we can provide a more comprehensive view into the molecular basis of dynamic biological processes and identify the key set of principles that can be utilized for the design of 3D tissue organoid and its clinical application.

Keywords: embryogenesis, spatio-temporal analysis, developmental trajectory

144

CULTURING NON-HUMAN PRIMATE EMBRYOS BEYOND POST-IMPLANTATION

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Blastocyst implantation is a landmark event in mammalian embryonic development. Mammalian embryos develop inside the uterus, making it challenging to directly observe the significant early developmental processes and perform experimental analysis. Therefore, the molecular mechanisms that govern early mammalian development are poorly understood. Recently established in vitro implantation platforms in mammalian model animals, including mouse, human, and cynomolgus monkeys, have expanded our knowledge of post-implantation development. These studies demonstrated that the mechanism of early development in primates differs from that of the mouse. However, researchers' access to and use of human embryos have always been limited due to ethical considerations. Therefore, we need to analyze mammalian embryonic development using non-human primate model animals. As the only model which allows access to both in vitro fertilized embryos (IVF) and naturally conceived embryos (NAT), the common marmoset (Callithrix jacchus) could prove to be instrumental in studying primate embryogenesis.In this study, we established the in vitro post-implantation culture using marmoset embryos obtained from IVF and NAT. Our results show that 16 of the 33 (48.5%) embryos successfully developed and expressed Oct4 in the epiblast and Gata6 in the hypoblast. Although the development of these cultured marmoset embryos was slower than that in humans and cynomolgus monkeys, they recapitulated the structure observed in other primates. Furthermore, gene expression analysis shows that the BURACHURY gene, a primitive streak marker expressed during the developmental stage, was clearly expressed in cultured marmoset embryos. These results have paved the way for advanced analysis including single-cell RNA seq analysis.In the future, the in vitro marmoset post-implantation embryo culture system would be invaluable for improving our understanding of the molecular mechanisms driving early mammalian development.

Funding Source
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Keywords: common marmoset, embryo culture, implantation

145

ENHANCED CARDIOMYOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS THROUGH MIGRATION-DRIVEN EPIGENETIC REGULATION ON A DENDRIMER-IMMOBILIZED SURFACE

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The dynamic migratory behavior of human mesenchymal stem cells (hMSCs) has a significant impact on epigenetic profiles that contribute to fate choice and lineage commitment; however, the alteration in cell behavior are associated with the formation and maintenance of epigenetic memory remain to be unclear. In this study, we report a novel approach to enhance repeated migration-driven epigenetic memory which induces cardiomyogenic differentiation on a dendrimer surface with fifth generation (G5). Cell aggregates grown on G5 surface showed dynamic changes in morphology associated with repetitive stretching and contracting during migration. They exhibited the altered cytoskeletal formation and nuclear lamina, leading to the epigenetic modifications and these aggregates showed strong expression of the cardiac-specific marker cardiac troponin T (cTnT) at 10 days. When cell aggregates were passaged onto a fresh G5 surface over three passages of 40 days, the expression levels of the multiple cardiac-specific markers including GATA4, NKX2.5, MYH7, and TNNT2 were higher compared to those passaged as single cells. To investigate whether cardiomyogenic differentiation of hMSCs was enhanced by repeated aggregate migration-driven epigenetic memory, cells on the G5 surface were reseeded onto a fresh G5 surface during three passages using aggregate-based and single cell-based passage methods. Analyses of global changes in H3 histone modifications exhibited pattern of increased H3K9ac and H3K27me3, and decreased H3K9me3 in aggregate-based passage cultures during three passages of 40 days. However, the pattern of their histone modification on the plain surface was repeated after the initialization and reformation during three passages in single cell-based passage cultures. Our findings demonstrate that culturing on the G5 surface is an efficient method for the regulation of migratory behavior-driven epigenetic mechanisms under aggregate-based passage of hMSCs and is an adequate strategy for ensuring cardiomyogenic differentiation in stem cell culture systems.

Funding Source

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Keywords: Human mesenchymal stem cells, Dendrimer surface, Cardiomyogenic differentiation

146

IMPROVEMENT IN DETECTION SENSITIVITY FOR PLURIPOTENT STEM CELLS IN HUMAN PLURIPOTENT STEM CELL-DERIVED NEURAL PROGENITOR CELLS USING THE SELECTIVE CYTOTOXIC ADENOVIRAL VECTOR

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Cell-processed therapeutic products (CTPs) derived from human induced pluripotent stem cells (hiPSCs) have innovative applications in the regenerative therapy. However, since undifferentiated hiPSCs possess tumorigenic potential, there is a potential risk of tumor formation if CTPs contain residual undifferentiated hiPSCs. Flow cytometry analysis can detect residual undifferentiated hiPSCs in differentiated cells, by using pluripotent stem cell markers, while its detection limit is reported to be 1/103 (0.1%, undifferentiated hiPSCs/differentiated cells). To improve detection sensitivity for undifferentiated hiPSCs, we have previously constructed adenovirus (Ad) vectors expressing a suicide gene, iCaspase9, regulated by the CMV promoter, which is dormant in hiPSCs, for the selective expression of iCaspase9 in differentiated cells. The Ad vector possessed cytotoxicity to human neural progenitor cells (hNPCs) but not to hiPSCs. In this study, we investigated whether the Ad vector concentrates undifferentiated hiPSCs in preparations of hiPSC-derived NPCs (hiPSC-NPC preparations) and makes possible their sensitive detection. The levels of TRA-1-60 and rBC2LCN, which are markers of hiPSCs, were determined by flow cytometry to detect undifferentiated hiPSCs in hiPSC-NPC preparations. Flow cytometry analysis showed that most of the hiPSC-NPC preparations were both TRA-1-60 and rBC2LCN negative. When hiPSC-NPC preparations were supplemented with hiPSCs at a ratio of 1% to evaluate the capability of the Ad vectors to concentrate hiPSCs, the percentage of cells positive for hiPSC markers was higher in cells treated with the Ad vector than in mock-treated cells. We also found that this concentration effect was dependent on culture media used during the Ad vector treatment. Under optimal conditions, approximately 20-fold enrichment of cells positive for hiPSC markers was obtained by the Ad vector treatment. Furthermore, the Ad vector treatment enabled sensitive detection of cells positive for the hiPSC markers in

Poster Abstracts

hiPSC-NPC preparations spiked with hiPSCs at a concentration (0.002%) lower than reported previously (0.1%), by selectively eliminating hiPSC-derived NPCs. This cytotoxic adenoviral vector could contribute to ensuring the quality and safety of hiPSC-
derived NPCs for therapeutic use.
Keywords: neural progenitor cell, induced pluripotent stem cell, tumorigenicity

147

EXTRACELLULAR VESICLE (EV)-MEDIATED PHENOTYPIC SYNCHRONY AND MIMICKING EV FUNCTIONS WITH MICRORNA-CONTAINING PLGA NANOPARTICLES

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We previously established a mouse embryonic stem cell (ESC) line harboring an inducible constitutively active protein kinase A (CA-PKA) gene and found that the ESCs rapidly differentiated into mesoderm after PKA activation. In this study, we performed a co-culture of Control-ESCs and PKA-ESCs, finding that both ESC types rapidly differentiated in synchrony even when PKA was activated only in PKA-ESCs. We named the phenomenon "Phenotypic Synchrony of Cells (PSyC)". We further demonstrated PSyC was mediated by extracellular vesicles (EVs). It was suggested that the PSyC is mediated by the exchange of EVs between cells in close proximity, which is different from the distant signaling, a well-known function of EVs. When mouse embryos were cultured with this EVs, beating cardiomyocytes were induced in a wide range of embryos, suggesting that EVs have a potent cardiomyocyte inducing effect. Small RNA-seq was performed to analyze the functional molecules contained in the PKA-ESC-derived EVs, and six miRNAs were identified. Among them, miRNA-132, which showed strong mesoderm induction effect by itself, was encapsulated in polymer poly (DL-lactide-co-glycolide) (PLGA) nanoparticles to mimic the EV function. MiR-132-containing PLGA nanoparticles enhanced mesoderm and cardiomyocyte differentiation in ESCs and ex vivo embryos, respectively.In summary, we have demonstrated the biological mechanism of EV-mediated synchronization of differentiation between neighboring cells and the potential of artificial nanoparticles to mimic EV function for regenerative medicine applications.

Funding Source
This work was supported by JST CREST Grant Number JPMJCR17H5, Japan.
Keywords: Extracellular vesicle (EV), Exosome, PLGA, artificial nanoparticle, cell-cell communication

148

TGFB-REGULATED COLLAGEN SWITCH DURING OSTEOCYTOGENESIS IN 3D MODEL FROM HUMAN IPSC

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Bone remodeling involves alternatively repeated two processes; bone resorption by osteoclasts and bone formation by osteoblasts and osteocytes, which are on a same cell lineage but have quite different characters. Osteoblasts are known to differentiate into osteocytes by being buried in newly formed collagen matrices produced by themselves. The precise molecular event of this transition, however, has not been elucidated yet.We here established the experimental system to observe this transition during bone-like nodule formation on type I collagen gel by the previously reported method using human iPSC. Serial histological sections during the induction demonstrated that osteoblast-like cuboidal cells spreaded over the surface of the collagen gel and then osteocyte-like dendritic cells were found inside the gel. Confocal imaging with immunostaining showed that cells on the surface expressed osteoblast-marker and produced type I collagen beneath the cell-layer and cells in the gel showed osteocyte-markers.We focused on the role of type I collagen in this transition. Using the same system, we observed that MC3T3-E1 (osteoblastic cell line) produced type I collagen and rarely migrated into the gel, whereas MLO-Y4 (osteocytic cell line) actively migrated into gel without the production of type I collagen. Based on the localization of smad2/3, the TGFB signal was ON in osteoblasts and OFF in osteocytes, and the inhibition of TGFB signal clearly decreased collagen production. Treatment of cells recovered from the gel with TGFB induced round osteoblast-like shape, whereas the TGFB inhibitor induced them with polygonal osteocyte-like shape. From these data, we proposed that the transition is regulated by TGFB via the production of extracellular matrix, which plays a role of switch turning ON and OFF of TGFB signal. Our system contributes to understanding the bone metabolism and new therapeutic approach for the bone disease.

Funding Source
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Keywords: iPSC, osteogenesis, collagen

149

EFFICIENT DIFFERENTIATION OF HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS TO CD19+ B CELLS IN A FEEDER-FREE AND SERUM-FREE CULTURE SYSTEM

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B cells play an essential role in the adaptive immune response to clear infection and provide long-term protection from previously recognized antigens. If they could be generated in sufficient quantities in vitro, transplantation of culture-expanded B cells could revolutionize vaccine development, immunotherapy, and facilitate disease modeling and immunology research. B cells arise from hematopoietic stem and progenitor cells (HSPCs) in vivo. Because of their multi-phase and complex development, it has been difficult to develop protocols that support the production of large quantities of B cells without the conventional use of serum or feeder cells. We have developed a culture method for generating CD19+ B cells from CD34+ HSPCs purified from cord blood (CB). CB-derived CD34+ progenitors were cultured for two weeks, producing 5% CD10+ cells (range: 2 - 8%, n = 19) with a yield of 7 CD10+ cells per input CD34+ cell (range: 2 - 14, n = 19). Cells were then cultured for another two weeks, producing 30% CD19+ cells (range: 18 - 53%, n = 19) with a yield of 216 CD19+ cells per CD34+ cell (range: 30 - 558, n = 19). One percent (1%) of CD19+ cells at this stage also express IgM (range: 0 - 6%, n = 19). After a final week of culture, we were able to produce an average of 73% CD19+ cells (range: 65 - 83%, n = 12) with an average yield of 1851 CD19+ cells per CD34+ cell (range: 172 - 5988, n = 12). Three percent (3%) of CD19+ cells also co-expressed IgM (range: 1 - 11%, n = 12). V(D)J gene recombination in the CD19+ cells was confirmed using quantitative PCR to detect kappa-deleting recombination excision circles (KRECs). Additionally, using an enzyme-linked immunospot (ELISpot) assay, we demonstrated that a fraction of the in vitro-produced CD19+ cells secrete either IgM or IgG antibodies. These findings show that HSPCs can be efficiently expanded and differentiated into CD19+ B cells under feeder- and serum-free conditions, and demonstrate the potential of this system as a tool for advancing basic and translational immunology research.

Keywords: B cells, Differentiation, Feeder-free

150

IDENTIFICATION AND APPLICATION OF P75 NEUROTROPHIN RECEPTOR-EXPRESSING HUMAN TRABECULAR MESHWORK PROGENITOR CELLS

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The trabecular meshwork is a tissue that originates from the neural crest via the periocular mesenchyme and plays a role in draining water and maintaining intraocular pressure. Damage to the trabecular meshwork is associated with pathologically elevated intraocular pressure, and cell-based therapy is expected to restore the functions of the trabecular meshwork in the future. We aimed to isolate and characterize trabecular meshwork progenitor cells (TMPs) from human trabecular tissue and focused on the p75 neurotrophin receptor (p75NTR), a stem cell marker of the neural crest. Approximately 32 percent of p75NTR-expressing cells were present in the trabecular meshwork by flow cytometry. Furthermore, laminin-511 was expressed in the trabecular meshwork as basement membrane components. p75NTR-expressing TMPs could proliferate in serum-free culture with laminin-511E8 fragment. The colony formation efficiency of the TMPs was 1.1 percent. TMPs expressed neural crest markers (p75NTR, SOX9, SOX10, and TFAP2B), periocular mesenchymal markers (FOXC1, FOXC2, and PITX2), and stem cell markers (CD73, CD90 and CD166), but not trabecular meshwork differentiation markers by immunostaining or flow cytometry. The TMPs differentiated into mature trabecular meshwork cells (dTMCs) and keratocan-expressing keratocytes. The dTMCs from the TMPs expressed high levels of trabecular meshwork markers (AQP1, MGP, PTGDS, and AnkG). Furthermore, the TMPs showed enhanced expression of myocilin, a glaucoma susceptibility gene, following induction of differentiation with dexamethasone. The TMPs also differentiated into adipocytes, osteocytes, and chondrocytes. These data suggest that p75NTR-expressing TMPs may be a useful cell source in cell-based therapy and pathological models of glaucoma.

Funding Source
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Keywords: trabecular meshwork, trabecular meshwork progenitor cells, p75NTR

Poster Abstracts

151

VASCULAR ENDOTHELIAL STEM CELLS RESIDE IN THE MURINE RETINA: POSSIBLE REMODELING MECHANISMS OF RETINAL BLOOD VESSELS

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The eye is a unique organ that contains multiple component of tissue such as vascular, nervous and epithelial system. We hypothesize that physiological remodeling, i.e. cells are replaced by newly emerging cells originated from somatic stem cells, takes place even in vascular cells which were thought to be quiescent. Because diabetic retinopathy is the leading cause of vision loss due to neovascularization originated from vascular obliteration, we aim to elucidate whether time-dependent remodeling of vascular endothelial cells are involved in the retina. Here, using vascular endothelial stem cell (VESC) marker such as Procr1 or Bst1, we show the possible presence of VSEC in murine retina. Moreover, endothelial single-cell transcriptomics (scRNAseq) for mouse retinal vessels provides molecular definitions for the tissue resident VESCs in retina. We uncover the transcriptional basis of the presence of an endothelial hierarchy within retinal blood vessels and successfully deconstructed the stem cell population in the retinal vascular systems.

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Keywords: Retina, Vascular endothelial cells, Vascular remodeling

152

A NOVEL PROTOCOL FOR NAIVE CONVERSION OF HUMAN PLURIPOTENT STEM CELLS

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Pluripotent stem cells have been reported to exhibit two stages of pluripotency, named the "primed" and "naive" states.Mouse embryonic stem cells (ESCs) are pluripotent stem cells that can give rise to all somatic cells except those of the placenta and also have a chimera-forming ability characteristic of the "naive" state.On the other hand, human embryonic stem cells (hESCs) and human induced pluripotent stem cells (iPSCs) lack the aforementioned chimera formation ability, making these cells more similar to mouse Epi stem cells (mEpiSCs), which are in a "primed" state.Here, we report that the short-term expression of gene constitutively active form of p38 (CA-p38) or treating gene CA-p38 activator AMPK activator can convert both hESCs and hiPSCs to the "naive" state.In this study, we used H1 hESCs carrying the EOS-C(3+)-GFP-IRES-Puro (EOS-GFP) reporter(H1-EOS), which monitors the activity of a multimerized CR4 element derived from the Oct3/4 distal enhancer driven by an early transposon promoter for detecting naive state.In order to test the effect of gene CA-p38, we generated H1-EOS carrying doxycycline (DOX)-inducible CA-p38 (H1-EOS- CA-p38).After treating with DOX for 5 days, H1-EOS-X expressed GFP, and these GFP+ cells also expressed SUSD2 and CD75, which are markers of the naive state.Then, we cultured H1-EOS with AMPK (an activator of gene CA-p38) under PXGL condition (containing PD03, LIF, the protein kinase inhibitor Go6983, and the tankyrase inhibitor XAV939), which was recently reported as a more efficient protocol for naive induction.After 2 weeks of PXGL+AMPK activator treatment, we obtained GFP+ cells which also expressed both SUSD2 and CD75.These results reveal new mechanisms to convert primed human pluripotent stem cells to the naive state and provide a better understanding of the mechanisms of naive conversion.
Keywords: human naive pluripotent, AMPK, human iPS cells

153

DECIPHERING REGULATORY MECHANISM OF HUMAN IPS-NKT DIFFERENTIATION BY SINGLE-CELL TRANSCRIPTOME ANALYSIS

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Natural killer T (NKT) cells, one of lymphocytes, exert strong anti-tumor effects by killing tumor cells directly and activating other immune system including NK cells and killer T cells, and thus NKT cells have been expected to be utilized for cancer immunotherapy. However, due to very limited number of NKT cells in human blood, it is extremely difficult to grow the collected NKT cells from blood in vitro to the required number for immunotherapy. To overcome this difficulty, we succeeded to establish human iPSCs

derived from NKT cells that were provided by volunteers and re-differentiated iPSCs into functional NKT cells. We previously reported that NKT cells derived from iPSCs possess significant anti-tumor activity in tumor-bearing mice. This has provided a first proof of concept for the clinical application of iPSC-NKT cells and we have started doing clinical trial. The current induction protocol is a stable, however, it still remains unclear what kinds of cell populations are proliferating and eventually differentiating into NKT cell in our protocol and what is the regulatory mechanism that controls iPS-NKT cell determination.To tackle these issues, we first performed time-course single cell RNA-sequencing and found that potential NKT progenitors and premature NKT cells appeared up to day 7 and after day 23, respectively. Pseudo-time analysis also suggested some cell surface markers as novel landmark of potential NKT progenitors, and we validated that the sorted cells using the markers could differentiate into NKT cells. We are further performing chromatin profiling by using the sorted cells to determine what transcriptional factors may regulate the cell fate decision during iPS-NKT differentiation. Taken together, these results will contribute to establish the next-generation protocol for NKT induction with shorter culture duration and higher induction efficiency, bringing us the success for future cancer immunotherapy.

Funding Source
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Keywords: NKT cells, single-cell RNA sequencing, chromatin profiling

154

LABEL-FREE EVALUATION OF HIPS-DERIVED HEPATOCYTES BY RAMAN MICROSCOPY

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A major obstacle hindering the clinical application of hiPS-derived cells is the safety concern. Traditional biological approaches generally require the destruction of cell products, making transplantation following evaluation difficult. A method that allows the assessment of differentiation efficiency without external labeling and cell damage has great potential to facilitate the clinical translation of hiPS-derived cells. Raman microscopy has become a powerful tool for label-free observation and characterization of biological samples because it can detect the vibrational frequencies given by the intrinsic biochemical components. By simply shining a laser onto a living specimen, the specific biochemical information given by individual cells can be collected and analyzed without additional processing, enabling non-destructive evaluation of cellular products for subsequent clinical use. Here, we demonstrated the potential of using label-free Raman microscopy to assess hiPS differentiation into hepatocyte lineage. Several functional molecules of hepatocytes are observed simultaneously, which has not been achieved in other methods. With this label-free and high-content feature, Raman microscopy will be a promising analytical tool for hiPS products.

Funding Source
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Keywords: iPS-derived hepatocytes, Raman microscopy, Label-free evaluation

155

THE MOUSE SRY LOCUS HARBORS A CRYPTIC SECOND EXON THAT IS ESSENTIAL FOR MALE SEX DETERMINATION

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Sexual differentiation is essential for the survival and evolution of a species. Expression of the Y chromosomal gene Sry is required for male development in mammals. Since its discovery 30 years ago, Sry has been believed to be a single-exon gene. Here, we identified a cryptic second exon of mouse Sry and a corresponding novel transcript, two-exon Sry (Sry-T). XY mice lacking Sry-T were sex-reversed, and ectopic expression of Sry-T in XX mice induced male development. Sry-T mRNA is expressed similarly to that of canonical single-exon Sry (Sry-S), but SRY-T protein is expressed predominantly, due to the absence of a degron which we identified in the C-terminus of SRY-S. Sry exon2 appears to have evolved recently in mice, through acquisition of retrotransposon-derived coding sequence to replace the degron. Our findings suggest that in nature, SRY-T, not SRY-S, is the bona fide testis-determining factor.

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Keywords: Sex development, Sry, gene evolution

Poster Abstracts

156

THE HSA-MIR-302 CLUSTER CONTROLS ECTODERMAL DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELL VIA REPRESSION OF DAZAP2

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Recent studies have revealed that microRNAs (miRNAs, miRs) are important for self-renewal, differentiation, and cellular reprogramming of somatic cells into induced pluripotent stem cells (iPSC); however, their functional roles and target genes that are regulated by human PSC-specific miRs including hsa-miR-302 clusters remain largely unknown. Analysis of their target gene will give us the opportunity to understand the functional roles of such miRs.We analyzed the expression profiles of miRs in 4 somatic cell lines, 8 human iPSC lines derived from 4 different cell types, 3 human ESC lines, and embryoid bodies differentiated from the human ESCs to identify human PSC-specific miRs. We also analyzed the simultaneous expression profiles of miRs and mRNAs to identify candidate targets of human PSC-specific miRs. Then, we constructed a vector for overexpressing one of the target genes to dissect the functions of human PSC-specific miR in maintenance of self-renew and differentiation.We focused on hsa-miR-302 cluster as a human PSC-specific miR and identified 22 candidate targets of hsa-miR-302 cluster that were moderately expressed in undifferentiated human PSCs and up-regulated in differentiated cells. Deleted in azoospermia-associated protein 2 (DAZAP2), one such target, was directly repressed by hsa-miR-302a, -302b, -302c, and -302d, but not by hsa-miR-367. Overexpression of DAZAP2 caused a decrease in cell proliferation of undifferentiated human iPSCs, although morphology and undifferentiated marker gene expression were not affected. In addition, neural differentiation was suppressed in DAZAP2-overexpressing human iPSCs.Our study revealed that hsa-miR-302 cluster controls the cell proliferation of human PSCs and the neural differentiation of human PSCs by repression of DAZAP2, thereby highlighting an additional function of human PSC-specific miRs in maintaining pluripotency.

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Keywords: MIR302 cluster, Human PSCs, DAZAP2

157

MAINTENANCE OF NEURAL STEM-PROGENITOR CELLS BY THE LYSOSOMAL BIOSYNTHESIS REGULATORS TFEB AND TFE3

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Lysosomes have recently been implicated in regulation of quiescence in adult neural stem cells (NSCs). Whether lysosomes regulate the differentiation of neural stem-progenitor cells (NPCs) in the embryonic brain has remained unknown, however. We here show that lysosomes are more abundant in rapidly dividing NPCs than in differentiating neurons in the embryonic mouse neocortex and ganglionic eminence. The genes for TFEB and TFE3, master regulators of lysosomal biosynthesis, as well as other lysosome-related genes were also expressed at higher levels in NPCs than in differentiating neurons. Anatomic analysis revealed accumulation of lysosomes at the apical and basal endfeet of NPCs. Knockdown of TFEB and TFE3, or that of the lysosomal transporter Slc15a4, resulted in premature differentiation of neocortical NPCs. Conversely, forced expression of an active form of TFEB (TFEB-AA) suppressed neuronal differentiation of NPCs in association with upregulation of NPC-related genes. These results together point to a previously unappreciated role for TFEB and TFE3, and possibly for lysosomes, in maintenance of the undifferentiated state of embryonic NPCs.

Funding Source

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Keywords: neural stem-progenitor cell, lysosome, neurogenesis

158

BMP SIGNALING SUPPRESSES GEMC1 EXPRESSION AND EPENDYMAL DIFFERENTIATION OF MOUSE TELENCEPHALIC PROGENITORS

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The lateral ventricles of the adult mammalian brain are lined by a single layer of multiciliated ependymal cells, which generate a flow of cerebrospinal fluid through directional beating of their cilia as well as regulate neurogenesis through interaction with adult neural stem cells. Ependymal cells are derived from a subset of embryonic neural stem-progenitor cells (NPCs, also known as radial glial cells) that becomes postmitotic during the late embryonic stage of development. Members of the Geminin family of transcriptional regulators including GemC1 and Mcidas play key roles in the differentiation of ependymal cells, but it remains largely unclear what extracellular signals regulate these factors and ependymal differentiation during embryonic and early-postnatal development. We now show that the levels of Smad1/5/8 phosphorylation and Id1/4 protein expression-both of which are downstream events of bone morphogenetic protein (BMP) signaling-decline in cells of the ventricular-subventricular zone in the mouse lateral ganglionic eminence in association with ependymal differentiation. Exposure of postnatal NPC cultures to BMP ligands or to a BMP receptor inhibitor suppressed and promoted the emergence of multiciliated ependymal cells, respectively. Moreover, treatment of embryonic NPC cultures with BMP ligands reduced the expression level of the ependymal marker Foxj1 and suppressed the emergence of ependymal-like cells. Finally, BMP ligands reduced the expression levels of Gemc1 and Mcidas in postnatal NPC cultures, whereas the BMP receptor inhibitor increased them. Our results thus implicate BMP signaling in suppression of ependymal differentiation from NPCs through regulation of Gemc1 and Mcidas expression during embryonic and early-postnatal stages of mouse telencephalic development.

Funding Source

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Keywords: Neural Stem Cell, Ependymal Cell, BMP

159

RETINOIC ACID REGULATES ERYTHROPOIETIN PRODUCTION COOPERATIVELY WITH HYPOXIA-INDUCIBLE FACTORS IN HUMAN IPSC-DERIVED ERYTHROPOIETIN-PRODUCING CELLS

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Erythropoietin (EPO) is an essential hormone for erythropoiesis and produced by adult kidneys and fetal liver. Insufficient EPO production in chronic kidney disease (CKD) can cause renal anemia. Although renal anemia has been treated with human recombinant EPO (rhEPO) agents, the fluctuation of red blood cell concentration by intermittent treatment can increase the risk of cardiovascular diseases. Hypoxia-inducible factors (HIFs) are known as a main regulator of EPO production. Nevertheless, the responsible mechanisms for this regulation have not been fully elucidated. Furthermore, mechanistic analyses of EPO production in vitro are difficult to perform, because isolating and maintaining human EPO-producing cells are difficult. Therefore, the development of more physiological therapies based on the mechanistic elucidation of EPO production in human is required for renal anemia.In this study, we developed a method to generate EPO-producing cells from human induced pluripotent stem cells (hiPSC-EPO cells) by modifying previously reported hepatic differentiation protocols. The EPO protein secreted by hiPSC-EPO cells induced the erythropoietic differentiation of human umbilical cord blood progenitor cells in vitro. hiPSC-EPO cells increased EPO expression and protein secretion in response to low oxygen conditions and by treatment with prolyl hydroxylase domain-containing protein (PHD) inhibitors that upregulate HIF signals. We also found that retinoic acid (RA) regulated the EPO production by hiPSC-EPO cells under hypoxic conditions and increased EPO production additively with PHD inhibitors. The combination treatment with RA and a PHD inhibitor, FG4592, increased EPO production by the kidney tissues of adult mice ex vivo and ameliorated renal anemia in adenine-induced CKD model mice fed a vitamin A-free diet. Thus, our hiPSC-EPO cells can be used to clarify EPO-production mechanisms and develop novel therapies for renal anemia.

Poster Abstracts

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Keywords: erythropoietin, human pluripotent stem cell, renal anemia

160

POSTSYNAPTIC STRUCTURE FORMATION OF HUMAN IPS CELL-DERIVED NEURONS TAKES LONGER THAN PRESYNAPTIC FORMATION DURING NEURAL DIFFERENTIATION IN VITRO

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The generation of mature synaptic structures using neurons differentiated from human-induced pluripotent stem cells (hiPSC-neurons) is expected to be applied to physiological studies of synapses in human cells and to pathological studies of diseases that cause abnormal synaptic function. Although it has been reported that synapses themselves change from an immature to a mature state as neurons mature, there are few reports that clearly show when and how human stem cell-derived neurons change to mature synaptic structures. This study was designed to elucidate the synapse formation process of hiPSC-neurons. We propagated hiPSC-derived neural progenitor cells as neurospheres by dual SMAD inhibition and then differentiated them into hiPSC-neurons in vitro. After 49 days of in vitro differentiation, hiPSC-neurons significantly expressed pre- and postsynaptic markers at both the transcript and protein levels. However, the expression of postsynaptic markers was lower than in normal human or normal rat brain tissues, and immunostaining analysis showed that it was relatively modest and was lower than that of presynaptic markers and that its localization in synaptic structures was insufficient. Neurophysiological analysis using a microelectrode array also revealed that no synaptic activity was generated on hiPSC-neurons at 49 days of differentiation. Analysis of subtype markers by immunostaining revealed that most hiPSC-neurons expressed vesicular glutamate transporter 2 (VGLUT2). These results suggest that during the synaptogenesis of hiPSC-neurons, the formation of presynaptic structures is not the only requirement for the formation of postsynaptic structures and that the mRNA expression of postsynaptic markers does not correlate with the formation of their mature structures. Technically, we also confirmed a certain level of robustness and reproducibility of our neuronal differentiation method in a multicenter setting, which will be helpful for future research.

Funding Source
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Keywords: synapse formation, human neuron, human-induced pluripotent stem cells

161

ENHANCED MATURATION OF HUMAN STEM CELL DERIVED INTERNEURONS BY MTOR ACTIVATION

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The use of stem cell derived neurons for cell-based therapies is limited by a protracted maturation. We present a novel approach for accelerating the post-mitotic maturation of human stem cell derived interneurons via the constitutive or transient activation of mTOR signaling. For constitutive activation, Lox sites were placed within PTEN, a key mTOR inhibitor, in a cortical interneuron (CIn) reporter line in which GFP is expressed by the Lhx6 locus. Following directed differentiation and purification by FACS, the CIns were exposed to Cre-expressing lentivirus, then transplanted into mouse neocortex or plated onto cultured rat neocortex. Input synaptogenesis and dendritogenesis was greatly enhanced in the PTEN-deleted CIns. Whole-cell recording of the PTEN-deleted CIns in slices of transplanted neocortex revealed multiple indices of enhanced maturation. Finally, we observed similar effects using transient, doxycycline-i nducible activation of AKT. Enhanced maturation of neurons is currently being further characterized by single nuclei RNA-seq (sNuc-Seq) and pseudotime analysis of neurons dissociated from medial ganglionic eminence like IPSC-derived spheroids with doxycycline-inducible activation of AKT. We thus present an inducible, reversible approach for accelerating the maturation of human stem cell derived CIns and studying the influences of this disease-related signaling system in human neurons.
Keywords: maturation, MGE, sNuc-seq

162

POPULATION ANALYSIS USING DESMIN AND CD56 AS MARKERS REVEALED THAT THE TRANSITION FROM PROLIFERATIVE MYOBLASTS TO DIFFERENTIATED MYOBLASTS IS DIRECTLY IRREVERSIBLE

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The development and regeneration process of skeletal muscle has been shown to be regulated by several important transcription factors. However, the MyoD gene is constantly expressed in the process where proliferating myoblasts differentiate to myotube, and there are many unclear points about its differentiation control mechanism. In this study, we performed population analysis by flow cytometry to approach the factors related to the process of transition of myoblasts from proliferative myoblasts to differentiated myoblasts just before myotube formation. We examined whether human skeletal muscle-derived cells could be grouped by immunostaining for several skeletal muscle-related proteins. As a result, we found that Desmin/CD56 co-staining produced three groups: Desmin-negative cells (Group 1), Desmin-positive and CD56 negative (or weakly positive) cells (Group 2), and Desmin-positive and CD56-positive cells (Group 3). It was also found that when differentiation was induced using a serum-free medium, at a low density so that cell fusion did not occur, Group 2 disappeared and Group 1 and Group 3 remained. On the contrary, Group 2 was maintained when FGF-2, EGF, and TGF-BETA1 were added to this differentiation medium. Since these factors have been reported to suppress the differentiation of myoblasts, it was considered that Group 2 corresponds to so-called proliferative myoblasts and Group 3 corresponds to differentiated myoblasts. Furthermore, when the cells in which Group 2 had disappeared due to differentiation induction was cultured under the addition of FGF-2, EGF, and TGF-BETA1, Group 2 did not recover, although CD56 expression was slightly attenuated. As conclusion, we found that the addition of FGF-2, EGF, and TGF-BETA1 suppresses the transition from proliferative myoblasts to differentiated myoblasts, but cannot reverse the differentiated myoblasts to proliferative myoblasts.
Funding Source
Japan Society for the Promotion of Science KAKENHI (grant number JP20K18011)
Keywords: Skeletal muscle, Myoblast, Differentiation

163

AGE-RELATED DYSFUNCTION OF THE DNA DAMAGE RESPONSE IN INTESTINAL STEM CELLS

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Various age-related dysfunctions of adult tissue-resident stem/progenitor cells (TSCs; also known as somatic stem cells) are associated with perturbation of tissue homeostasis. Restoration of stem cell functions has attracted much attention as a promising therapeutic strategy for geriatric diseases. The intestinal epithelium is one of the most rapidly renewing tissues in the body. Lgr5-expressing intestinal stem cells (ISCs) in crypts differentiate into epithelial cells and thereby maintain intestinal homeostasis. Therefore, dysfunction of ISCs may be important for disruption of intestinal homeostasis and subsequent induction of functional disorders. However, the influence of aging on the functions of ISCs and induction of diseases is largely unknown. Recent studies demonstrated that accumulation of senescent cells promotes organismal aging and that senolytics improve age-associated phenotypes and prolong lifespans. Cells become senescent in response to various aging stresses, such as oxidative stress, telomere shortening, inflammation, irradiation,

Poster Abstracts

exposure to chemicals, and the mitotic stress, all of which induce DNA damage. Numerous types of DNA damage occur naturally and are removed by the DNA damage response (DDR). This response induces DNA repair and apoptosis; therefore, its dysregulation leads to accumulation of damaged DNA and consequently cellular dysfunctions, including tumorigenesis. The mutation rate is highest in the small and large intestines. However, the influence of aging on the DDR in ISCs has not been studied. Here, we compared induction of the DDR, inflammation, and mitochondrial biogenesis upon irradiation between young and old mouse ISCs in vivo and reported that their functions were reduced.

Funding Source
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Keywords: stem cell aging, intestinal stem cell, dna damage

164

THE EFFECT OF HUMAN ADIPOSE-DERIVED STEM CELLS (ASCS) ON BIOLOGICAL ACTIVITY OF DERMAL FIBROBLASTS IN AUTOLOGOUS IN VITRO COCULTURE

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Adipose-derived stem cells (ASCs) have a very wide biological activity, hence they can be potentially used in regenerative medicine, plastic surgery and other fields of medicine. In addition, ASCs are relatively easily obtainable from patients and characterized by low immunogenicity. ASCs also play an important role in wound healing mainly due to the secretion of cytokines and growth factors. Due to the increasing number of patients with chronic wounds, skin lesions and other dermatological problems worldwide, methods stimulating skin regeneration are intensively sought for. The aim of the project was to evaluate the influence of human ASCs on the biological activity of dermal fibroblasts in autologous in vitro coculture. ASCs characteristics were confirmed by flow cytometry (expression of CD26, CD73, CD90, CD105, no expression of CD14, CD19, CD45, HLA-DR), in vitro cell differentiation into three lineages and histological analysis. Our preliminary results indicate that ASCs stimulate the biological activity of fibroblasts in vitro. Fibroblasts showed increased proliferation, chemotaxis and collagen production. Moreover, after 48-hour stimulation by ASCs the fibroblasts showed increased expression of the CD26 antigen and stable expression of the CD73, CD90, CD105 antigens. In conclusion, our study indicates that ASCs may influence dermal fibroblasts via paracrine effect. These finding may improve our understanding of the cross-talk between ASCs and skin cells. Our observations also showed that adipose-derived stem cells may be a promising therapeutic tool in regenerative medicine and dermatology.

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Keywords: regeneration, adipose-derived stem cells (ASCs), fibroblasts

Advanced Technologies in Stem Cell Biology

201

A PHENOTYPIC ANALYSIS SYSTEM BASED ON ROBUST NEURAL DIFFERENTIATION USING B-HLH-TYPE TRANSCRIPTION FACTORS

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A large number of reports has been already accumulated on methods to induce differentiation of human ES/iPS cells into various types of neurons. However, due to technical issues, not all of these methods can be easily reproduced in any research or facility. The Dual-SMAD inhibition method, which is often used for neuroectoderm differentiation, is an enormously powerful tool, but the state of intracellular SMAD signaling and brain region patterning in Petri dishes is ever-changing and difficult to control properly. Another issue is that human iPS cells tend to have high clonal variation.To solve these issues, we have focused on and improved a method to drive neural differentiation using transient gene expression by introducing pro-neural factors NEUROG2 or ASCL1 into human ES/iPS cells. We successfully introduced these TetO-driven genes into hES/iPS cells with PiggyBac vector and induced neuronal differentiation by treating doxycycline. In particular, the PiggyBac vector can be used to increase the number of copies of GOI inserted into the genome. The vector in this study also contains drug resistance genes, allowing for drug selection at the iPS cell stage. Therefore, the selected iPS cells can be differentiated into neurons with a high efficiency of almost 100%, and at the same time, they can be grown, maintained, and cryopreserved at a pluripotent stem cell state.Having achieved such a robust induction of neuronal differentiation, our next step is to investigate the production of subtype-specific neurons in a semi-comprehensive manner. In this study, we will introduce our attempt to produce more diverse neurons individually according to the experimental purpose while utilizing scRNA-Seq results.

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Keywords: Transcription factor, Direct differentiation, Neuron

203

REPROGRAMMING OF SOMATIC CELLS BY MRNA TRANSDUCTION

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Somatic cells are reprogrammed by several factors and are converted into induced pluripotent stem (iPS) cells. Various reprogramming methods have been reported so far, such as those using viruses, plasmids, proteins, and chemical compounds. We believe that it is important to produce safer and better quality iPS cells when considering medical applications. And if the recently launched autologous iPS cell service is to become popular in the future, we think that a more user-friendly method of reprogramming is needed.We are currently developing an mRNA-based reprogramming method. It has been already reported that human dermal fibroblasts are reprogrammed by mRNA transduction. We have obtained similar results in our laboratory under the feeder-free culture conditions. The reprogramming efficiency of using mRNA to establish iPS cells from HDFs was a few percent. The next step is to establish iPS cells from peripheral blood mononuclear cells (PBMCs) using mRNA. Since PBMCs are floating cells, it was difficult to introduce mRNA. We examined various culture conditions for PBMCs, and also tried many commercially available RNA transfection reagents. Finally, we succeeded in establishing iPS cells from PBMCs using mRNA. In this meeting, we would like to talk about mRNA-based reprogramming, including the latest findings.

Keywords: Reprogramming, iPS cell, RNA

204

THE EFFECTS OF HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELL CONDITIONED MEDIA PRODUCED WITH FETAL BOVINE SERUM OR HUMAN PLATELET LYSATE ON SKIN REJUVENATION CHARACTERISTICS

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Human mesenchymal stem cell-conditioned medium (MSC-CM) is produced using mesenchymal stem cell culture technology and has various benefits for the skin, including wrinkle removal, skin regeneration, and increased antioxidant activity. Its popularity is thus increasing in the field of functional cosmetics.In this study, we analyzed the effects of fetal bovine serum-supplemented MSC-CM (FBS- MSC-CM) and human platelet lysate-supplemented MSC-CM (hPL-MSC-CM) on skin rejuvenation characteristics. We found that the concentrations of important growth factors (VEGF, TGF-BETA1, and HGF) and secretory proteins for skin regeneration were significantly higher in hPL-MSC-CM than in FBS-MSC-CM. Furthermore, the capacity for inducing proliferation of human dermal fibroblast (HDF) and keratinocytes, the migration ability of HDF, extracellular matrix (ECM) production such as collagen and elastin was higher in hPL-MSC-CM than that in FBS- MSC-CM. These results support the usefulness and high economic value of hPL-MSC-CM as an alternative source of FBS-MSC-CM in the cosmetic industry for skin rejuvenation.

Funding Source
SCM Lifescience research fund
Keywords: Human mesenchymal stem cell, Skin rejuvenation, Human platelet lysate

205

FROM 50 MILLION TO 15 BILLION HUMAN IPS CELLS WITHIN A WEEK : HIGHLY REPRODUCIBLE EXPONENTIAL IPS EXPANSION IN 10L BIOREACTORS WITH MAINTENANCE OF CELL QUALITY

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2D cell culture has been widely used to manufacture the first generation of cell therapies. However, due to the drawbacks of scale-out processes (footprint, workforce use, variability and subsequent QC expenses), the industry is shifting towards the goldstandard for bioproduction scale-up, i.e. bioreactors, with the goal of addressing mass-markets with standardized and affordable products.Here using new C-Stem™ technology based on a high-speed cell encapsulation microfluidics, we report the production of two single batches of 15 billion hiPSCs in 10L bioreactors with an unprecedented 276-fold amplification in less than a week. Data demonstrates high-reproducibility and maintenance of best-in-class cell viability and pluripotency. Also documenting the scale-independent amplification profile obtained with C-Stem™ in 30mL, 500mL, 1.5L and 10L bioreactors, we argue that the C-Stem™ technology is amenable to produce commercial-size batches of stem cells in larger bioreactors.

Funding Source
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Keywords: hiPSC expansion, Scale-up in bioreactor, iPS-derived cell therapy

Poster Abstracts

206

GENERATION OF MATURE COMPACT VENTRICULAR CARDIOMYOCYTES FROM HUMAN PLURIPOTENT STEM CELLS

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Compact cardiomyocytes that make up the ventricular wall of the adult heart represent an important therapeutic target population for modeling and treating cardiovascular diseases. Here we established a differentiation strategy that promotes the specification, proliferation and maturation of compact ventricular cardiomyocytes from hPSCs. The cardiomyocytes generated under these conditions display the ability to use fatty acids as an energy source, a high mitochondrial mass, well-defined sarcomere structures and enhanced contraction force. These ventricular cells undergo metabolic changes indicative of those associated with heart failure when challenged in vitro with pathological stimuli and were found to generate grafts consisting of more mature cells than those derived from immature cardiomyocytes following transplantation into infarcted rat hearts. hPSC-derived atrial cardiomyocytes also responded to the maturation cues identified in this study, indicating that the approach is broadly applicable to different subtypes of the heart. Collectively, these findings highlight the power of recapitulating key aspects of embryonic and postnatal development for generating therapeutically relevant cell types from hPSCs.

Keywords: cardiomyocyte, pluripotent stem cell, maturation

208

EFFECT OF HORMONES AND NUCLEAR RECEPTOR AGONISTS ON MATURATION OF CARDIOMYOCYTES DERIVED FROM PLURIPOTENT STEM CELLS

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Pluripotent stem cell-derived cardiomyocytes (PSC-CMs) are mainly immature in a dish. With this point, the utilities of using PSC-CMs in medical applications are limited. To overcome this issue, imitation of the environments during heart development has been applied into the culturing system such as plating the cells on extracellular matrices, fatty acid supplementation, and hormone treatments. However, no method is available to precisely determine the maturity of the treated PSC-CMs. Here, we first developed a transcriptome-based method to determine the maturation scores of the PSC-CMs. To establish the scoring formula, we performed RNA sequencing of mouse hearts raging from embryo to adult. The maturation scores were calculated by a set of gene expressions multiply by the weight of each gene. The maturation scores gradually and linearly increased from embryo to adult, and the scores of PSC-CMs also increased gradually to the scores of late-embryonic to neonatal hearts as the culture periods were extended, indicating that this method efficiently determines PSC-CMs maturity. Next, our previous study identified that some nuclear receptors (NRs), especially hormone receptors, were activated during pre and postnatal heart development. Thus, we hypothesize that the agonists of these NRs enhance cardiomyocyte maturation. To test the hypothesis, we treated both mouse and human PSC-CMs with hormones and NR agonists. Then, we scored their maturity using the novel method. With two weeks of treatments with the best combination of NR agonists, the maturation scores of mouse PSC-CMs reached the scores of hearts between postnatal days 14 to 28, while ones of human PSC-CMs were around that of postnatal day 10. In this condition, both mouse and human PSC-CMs exhibited rod-like or hypertrophic shape, which improved calcium handling, sarcomere shortening, and mitochondrial function. In conclusion, our study provides robust culture conditions to promote cardiomyocyte maturation in both mouse and human PSC-CMs which could improve the fidelity of using PSC-CMs as a model for cardiac diseases in the future.

Funding Source

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Keywords: Pluripotent stem cell-derived cardiomyocytes (PSC-CMs), Cardiomyocyte maturation, Cardiac disease modeling

209

DEVICE TO MEASURE THE TRANSMITTANCE OF STRATIFIED CELL SHEETS

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The benefits of using mesenchymal stem cells cells sheet (MSCCSs) include an increase in the lifespan of the cells, a decrease in cell biodistribution, and a way to directly target the area requiring cell therapy. Engineering stratified cell sheets (CSs) is a very delicate process that is dependent on different factors and in addition, one challenge has been that successful mechanical harvesting and calculation of the harvesting time of a CS has been unquantifiable and mainly

decided by the manipulator's experience. To solve this problem, we have developed an affordable device that can non-invasively measure the transmittance of a CS. The principle of our device is that the CS is placed on a stage between a light-emitting diode and a light detector which then measures transmittance. Nine hundred measurements were performed over the CSs in order to obtain an accurate transmittance value. CSs were engineered using two different growth culture media with the same adipose stromal cell (ASC) initial seeding (10.2×104 ASCs per cm2). Specific markers of the ASCs were detected after the harvesting of the cell sheets (Positive: CD29, CD73, CD105; negative: CD19, HLA-DR). The transmittance curves for both conditions decreased parallelly from different initial values. The transmittance decrease is related to the growth rate of the cells but is also conversely related to the CS "strength" which can only be noticed by experienced manipulators. As soon as the transmittance was around 75%, the CSs were mechanically harvestable but required higher mechanical skill. From the 10th day in culture, the transmittance of the CSs reached a plateau, indicating that the CSs were at the highest maturity level. In conclusion, this device can measure transmittance which could be used to estimate an optimal time of CSs harvesting and for quality control purposes. This device could also measure the transmittance of other transparent tissues such as the cornea.

Funding Source
Emmaus Medical, Inc.
Keywords: Mesenchymal Stem Cells, Cell Sheet, Transmittance

210

CELL IDENTIFICATION BY INFORMATION THEORETIC APPROACH TO SINGLE CELL RNA-SEQ DATA FOR QUALITY REFERENCE OF STEM CELL THERAPY

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Stem cell therapy requires the quality control of the differentiated cell from stem cell, and it needs the reference of every cell types seen in vivo. To establish the quality reference, it is important to identify precisely the cell types from the gene expression data. Here, we propose the new method to identify the cell type from single cell RNA-seq data by information theoretic approach. The technique enables us to identify cell types without prior knowledge of gene markers.

Keywords: Cell identificarion, Bioinformatics, Single cell RNS-seq

211

SUBTYPE-SPECIFIC IMAGING OF INDUCED GABAERGIC INTERNEURONS FROM CRISPR-MEDIATED REPORTER KNOCK-IN IPS CELLS

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Induced pluripotent stem cells (iPSCs) have facilitated human disease modeling and therapeutic research in vitro. A recent study demonstrated the efficient induction of GABAergic interneurons (INs) from human iPSCs(hiPSCs) by the transient overexpression of ganglionic eminence-fate-determining transcription factors ASCL1 and DLX2 (Yang et al., 2017). While the defects of INs in neuropsychiatry disorders are extensively studied using patient-derived iPSCs (Ishii et al., 2019), the neuronal vulnerability can be different depending on IN subtypes among diseases. INs are heterogenous with different morphological and electrophysiological properties and neuronal circuit formation in the neocortex. Parvalbumin+(PV+) and somatostatin+ (SST+) neurons are both IN subtypes derived from progenitor cells in MGE, but it is poorly understood how the distinct types are emerged, and separated from the same origin. Therefore, it is crucial to discriminate IN subtypes derived from hiPSCs and analyze INs individually for developmental and pathophysiological research. In this study, we established a hiPSC line harboring PV and SST double reporters. The mNeonGreen and tdTomato fluorescent reporter genes were respectively inserted into the direct downstream of PV and SST coding regions with self-cleaving 2A peptide sequences by CRISPR/Cas9-mediated genome editing. For the KI vector construction, we employed a reported robust method based on the Multisite Gateway technology (Yoshimatsu et al. 2019). The functionality of the reporter genes was verified by CRISPR-dCas9 forced gene expression of target genes in the reporter hiPSCs. The above-mentioned method to induce INs was adapted to the reporter hiPSCs to examine reporter gene expression in neuronal cells. For investigating lineage segregation between PV+ and SST+ neurons, organoid formation using the reporter hiPSCs can be effective as organoids can recapitulate various molecular and environmental features of the developing human brain.

Funding Source
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Keywords: GABAergic interneuron, Neuronal differentiation, CRISPR/Cas9

Poster Abstracts

212

SUBCULTURE METHOD OF HUMAN IPS CELLS IN SUSPENSION CULTURE USING A CELL DISPERSION DEVICE

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The stirred suspension culture using a bioreactor system is an efficient method for the large-scale expansion of human iPS cells. In suspension culture, human iPS cells form aggregates that must be dispersed into single cells in order to scale up. In the small-scale cell dispersion step, enzyme solution treatment and pipetting are performed manually. This process was prone to individual procedure differences and prevented expansion into large-scale cultures. We have developed a reactor equipped with a cylinder that rotates at high speed in order to refine manual cell dispersion (cell dispersion tool). There are two types of cell dispersion tools, 20 mL (small scale) and 100 mL (bench top scale), each of which has the ability to process 100 million cells and 1 billion cells. Aggregates of human iPS cells prepared using our suspension culture reactor were dispersed into single cells using a cell dispersion tool. Dispersion of iPS cell aggregates using this tool required no enzymes, only chelating agents. The cell suspension thus prepared was seeded in a larger scale reactor to form aggregates and proliferate similarly. As a result, scale-up suspension culture from 20 million cells to 3 billion cells could be performed without enzyme treatment using suspension culture reactors with 100 mL, 500 mL, and 1500 mL volumes.

Funding Source
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Keywords: iPS cell, Suspension culture, Aggregate

213

GENERATION AND CHARACTERIZATION OF NEURONAL CELLS DIRECTLY DIFFERENTIATED FROM HUMAN T-LYMPHOCYTES

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Induced pluripotent stem cells have already proven to be a promising material for elucidating human neuronal disease models and drug discovery, as well as for cell therapy against injure or deconditioning. However, in order to prepare robust pathological modeling or transplantation resources, it is necessary to first spend several weeks for full reprogramming, several months for quality control, and finally a long time for neuronal differentiation and maturation, which is restrictive not only in terms of the time required but also labor and cost. On the other hand, induced neuronal cells (iN cells) programmed directly from human dermal fibroblasts may be very efficient, taking only a few quick steps. However, obtaining human fibroblasts requires an invasive biopsy, which makes the research difficult. In this study, we focused on the direct conversion of T-lymphocytes prepared from human adult peripheral blood mononuclear cells into properly functioning iN cells. While the gene set (NEUROD1/ASCL1/POU3F2/ZIC1) known to generate fibroblast-derived iN cells alone does not produce large numbers of neurons, we succeeded in effectively producing target cells by simultaneously introducing Yamanaka 4 factors without glial-feeder support. In fact, by mediating the transduction gene set with Sendai virus, an RNA virus with no risk of genomic integration, cells showing electrical activity were obtained within 3 weeks of culture. They showed stereotyped neuronal morphology and expressed several subtypes of neuronal markers. To further understand the details of the emergence process of these iN cells, we performed scRNA-Seq and even studied pseudo-time analysis. In short, this more rapid and less invasive method of iN cell preparation can be used as a resource for neuropathological modeling and cell therapy.

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Keywords: Neuron, Direct conversion, Single-cell RNA-seq

214

A NOVEL ANIMAL ORIGIN-FREE AND STABILIZED HUMAN PLURIPOTENT STEM CELL (HPSC) EXPANSION MEDIUM FOR HIGH QUALITY HPSCS

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To simplify traceability and viral safety concerns associated with hPSC culture conditions, we have developed an animal origin-free (AOF) hPSC maintenance medium, TeSR™-AOF, manufactured under cGMP using animal-free raw materials with traceability to the secondary level of manufacturing. To improve consistency of the culture environment and support reduced feeding schedules with every other day or weekend-free feeding, key media components, including native FGF2 in TeSR™-AOF, are stabilized over 72 hours at 37°C. In addition, buffering capacity was optimized to

stabilize pH under reduced feeding schedules. Transition of hPSC-based therapies to the clinic is reliant on reliable and consistent expansion of high quality hPSCs. To improve expansion across hPSC lines, TeSR™-AOF was optimized to improve plating efficiency compared to low-protein formulations. Typically, plating efficiency was enhanced by 27.1% ± 4.71% (mean ± STDEV; n=3 cell lines); however, in select hPSC lines with historically low plating efficiency in low-protein media formulations, the plating efficiency was improved by 80 to 140% (n=2 cell lines) in TeSR™-AOF. We investigated key cell quality attributes of hPSCs cultured for ≥10 passages in TeSR™-AOF with reduced feeding schedules. hPSC marker expression was assessed by flow cytometry at passages 5 and 10 and hPSCs cultured in TeSR™-AOF maintained an average of 98.3 ± 1.55% OCT4 and 93.4 ± 3.37% TRA-1-60 (n=4). hPSCs maintained in TeSR™-AOF were confirmed karyotypically normal by G-banding and by routinely screening cultures using the hPSC Genetic Analysis Kit (>20 passages, n=4). To assess regenerative potential of hPSCs maintained in TeSR™-AOF, directed differentiation to all three germ layers was performed using the STEMdiff™ Trilineage Kit (n=2). Using the ArciTect™ CRISPR-Cas9 genome editing system, hPSCs cultured in TeSR™-AOF demonstrated 68.6 ± 2.03% gene knock-out efficiency and 9.17 ± 1.77% ssODN knock-in efficiency (n=2). hPSCs in TeSR™-AOF had a cloning efficiency of 33.7 ± 8.04% (n=3) when supplemented with CloneR™2. In summary, TeSR™-AOF was designed with quality and safety in mind and formulated to robustly, improved attachment efficiency, consistency and reproducibility, whilst enabling versatile workflows and preserving high quality hPSCs in long term culture.

Keywords: pluripotent, cell therapy, Animal origin free

215

PHYSIOLOGICAL HYPOXIA MAINTAINED BY NITRIC OXIDE SIGNALING REGULATES LOCAL CRAWLING OF HEMATOPOIETIC STEM CELLS

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Hematopoietic stem cells (HSCs) are retained in the bone marrow (BM) niche in many adult mammals. Oxygen concentration of BM niche is critical for maintaining HSC. Despite recent advances in intravital imaging techniques for visualizing oxygen distribution in BM, subcellular oxygen level and mechanisms underlying tuning oxygen concentration of HSC niche remain unclear. To reveal subcellular oxygen concentration in BM, we used BTPDM1, a cell-permeable, iridium-based phosphorescent probe and phosphorescence lifetime imaging. By intravital oxygen imaging of mouse calvarial BM using BTPDM1, we found that subcellular oxygen concentration was kept at approximately 3% in the entire BM. As a mechanism for maintaining physiological oxygen levels in the BM, we identified the importance of synthesis of nitric oxide (NO), a vasodilator derived from BM arteries. Local administration of L-NAME, a NO synthase inhibitor, reduced oxygen concentration in BM. In vivo HSC tracing analysis using multi-photon laser microscopy showed that NO inhibition also reduces local HSC crawling in BM. Reduction of HSC migration by L-NAME was rescued by administration of NO-donor or 100% oxygen inhalation. To analyze whether HSC migratory activity is affected by oxygen concentration, we compared velocity of HSC in 3% and 1% oxygen concentration by using in vitro HSC migration assay. HSC velocity in 1% oxygen was lower than that in 3% oxygen. By using fluorescent phalloidin as a tracer for actin filaments, we found that actin filaments are more abundant in HSC in 3% oxygen than in 1% oxygen. These results indicate that NO-dependent vasodilation plays a key role in maintaining physiological hypoxia of BM and crawling of HSC by facilitating actin polymerization.

Funding Source

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Keywords: hematopoietic stem cells, intravital imaging, hypoxia

216

SCREENING OF HUMAN IPSCS FOR CHEMICAL TOXICITY PREDICTION BASED ON RNA-SEQ DATA FROM HUMAN ESCS

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In the field of toxicity evaluation of chemicals, there is a strong demand for alternative methods that can replace conventional animal experiments. The highly accurate toxicity prediction system using human ES cells, which we have previously reported, has provided a new perspective on regarding undifferentiated human PSCs as an excellent alternative device. At present, it has been confirmed that KhES-3 cells from Japanese male can predict not only developmental toxicity but also adult toxicity. However, in order to render this prediction system widely acceptable, replacement of human ESCs to iPSCs is required to moderate the ethical problems. Here we successfully screened a healthy Japanese iPS cell line which could replace the KhES-3 cells by the sensitivity scores (IC50) for

Poster Abstracts

24 toxic chemicals. The candidate iPSC line was confirmed to show similar prediction accuracies to those of KhES-3 line in six toxicity categories. In future, we will be able to provide a highly accurate toxicity prediction system with human iPSCs utilizing KhES-3 gene expression database of chemical exposures.

Funding Source
Core Center for iPS Cell Research, Research Center Network for Realizaion of Regenerative Medicine, Japan Agency for Medical Research and Development (AMED), and Joint Research with Shiseido Company, Limited
Keywords: Toxicity prediction, Toxicogenomics database, Animal alternative method

217

LARGE-SCALE EXPANSION OF IPS CELLS IN CLINIMACS PRODIGY® ADHERENT CELL CULTURE SYSTEM

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The CiRA Foundation and its core, Facility for iPS Cell Therapy, is responsible for manufacturing and banking of clinical grade induced pluripotent stem cells (iPSCs). It requires efficient and scalable cell expansion protocols that allow for maintenance of cells morphology, immunophenotype, genetic stability and pluripotency under current good manufacturing practice (cGMP) requirements. We are currently working on the transition from existing manual cell expansion protocols in the iMatrix-511/StemFit® AK03N culture system onto an automated closed culture platform. The CliniMACS Prodigy® Adherent Cell Culture System (Miltenyi Biotec) allows GMP-compliant and scalable iPSC cultivation. Here we report the progress of the process development. The cells from existing stock are initially expanded on the standard 6-well tissue culture plate, harvested and inoculated into the CliniMACS Prodigy® TS 730 tubing set chamber, cultured under the Adherent Cell Culture process, then harvested and inoculated once more into the CellSTACK®-5 Chamber (Corning®), before final harvest and banking. The process allows for the semi-automated expansion of iPSCs from the 1E+05 up to 5E+08 cells scale within about 14 days. Since the CellSTACK® Chambers are available in several sizes our process is highly scalable, and utilization of automated closed system greatly reduces the workload of large-scale expansion of iPSCs.

Funding Source
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Keywords: large scale iPSC expansion, closed culture system, cGMP

Organoids: From Basic Biology to Clinical Translation

301

MOTOR NERVE ORGANOID IS USEFUL TOOL TO ANALYZE AXONAL DEGENERATION OF ALS

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Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder characterized by the death of motor neurons and degeneration of axons. The purpose of this study is to analyze the pathomechanism of motor nerve axon in ALS using human induced pluripotent stem cells (hiPSCs)-derived motor neurons. We identified aberrant increasing of axon branching in FUS-mutant hiPSCs-derived MN axons compared with isogenic controls. We developed a microfluidic device to form motor nerve organoid which can be used to analyze axonal degeneration of stem cell derived motor neurons in vitro. RNA profiling of isolated axons was conducted by applying the microfluidic devices that enable axon bundles to be produced for omics analysis. We identified increased level of Fos-B mRNA, the binding target of FUS, in FUS-mutant MNs. While Fos-B reduction using siRNA or an inhibitor ameliorated the observed aberrant axon branching, Fos-B overexpression resulted in aberrant axon branching in zebrafish model. The commonality of those phenotypes was further confirmed with other ALS causative mutation than FUS. In transcriptome analysis of RNA present in the axon compartment of hiPSC-derived MNs, PHOX2B (paired-like homeobox protein 2B) showed lower expression in TARDBP mutant axons, which was consistent with axon qPCR and in situ hybridization. Analyzing the axonal fraction of hiPSC-derived MNs using microfluidic devices revealed key regulators of ALS-mutant axon. Motor nerve organoid is the useful tool to analyze axonal degeneration of ALS.

Funding Source
Japan Agency for Medical Research and development, AMED; Japanese Ministry of Education, Culture, Sports, Science, and Technology; Health, Labor and Welfare Sciences Research Grants, the Ministry of Health, Labor and Welfare, Japan.
Keywords: motor nerve organoid, amyotrophic lateral sclerosis, iPS cells

302

EFFECTS OF INJECTION OF HUMAN IPS CELL-DERIVED INTESTINAL ORGANOIDS ON THE RECTAL TISSUES IN COLITIS MODEL MICE

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Ulcerative colitis (UC) is one of the inflammatory bowel diseases with repeated remissions and relapses, and its pathogenesis has not yet been clarified. Immunosuppressive drugs have enabled the induction and maintenance of remission in many patients with UC. The degree of healing of the intestinal mucosa is very important to involve in relapse. Transplantation of organoids derived from human intestinal samples has been reported to be effective in healing the colonic mucosa. Therefore, it is expected to be a new treatment for UC. The use of patients-derived organoids is preferable for autotransplantation in preventing immune rejection. However, it may not be effective because of the difficulty of transplantation in someone else and the time and cost required. To overcome this problem, we generated human iPS cell-derived intestinal organoids (hiPSC-HIOs) and evaluated their effects on colitis model mice. First, we differentiated two types of intestinal organoids in the absence (HIO-A) or presence (HIO-B) of small molecule compounds, and investigated whether these hiPSC-HIOs express intestinal cell markers and engraft in vivo. The results showed that the HIO-A had the higher expression of intestinal stem cell markers and the lower expression of intestinal differentiated cell markers than HIO-B. In addition, the HIO-A transplanted under the kidney capsule of mice were more engrafted. Finally, we injected hiPSC-HIOs into the rectum of colitis model mice. The body weight and the disease activity index scores (DAI scores) of the HIO-A-injected mice recovered earlier than those of the sham group. After 9 days of injection, the morphology and the expression of tight junction proteins in the rectal tissues of the organoid-injected mice were restored to similar condition to healthy mice. Moreover, the expression of pro-inflammatory cytokine markers in HIO-A-injected group were lower than the sham group. These results were indicated that hiPSC-HIOs with higher expression of intestinal stem cell markers contribute to improve mucosal damage faster and cleaner than natural healing, which may help prevent UC relapse. We hope that our results will lead to further research on the application of human iPS cell-derived intestinal organoids for UC therapy.

Keywords: human iPS cell, intestinal organoid, colitis therapy

303

THE EPITHELIAL-DERIVED FACTORS AND MECHANICAL ENVIRONMENT INDUCE THE MUSCULARIS MUCOSA OF THE IPSC-DERIVED HUMAN GASTRIC ORGANOID

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The gastric muscularis mucosa (MM) is a thin layer of smooth muscle and is contained in the gastrointestinal wall. It separates the mucosa from the submucosa and enhances contact between the gastrointestinal epithelium and the lumen contents. The human gastric MM has not been clarified how to emerge, although its development has been investigated in mice and chicken. Human gastric organoids (hGOs) have recently been generated from induced pluripotent stem cells (iPSCs) by using activin, noggin and EGF. The epithelium of the organoids has been indicated gastric differentiation. In contrast, subepithelial cells have not shown smooth muscle differentiation, and these hGOs have not been accompanied by MM. These hGOs have not enabled us to investigate how gastric MM differentiate. In this study, we tried to induce hiPSC to differentiate into the hGOs with MM at first. By culturing hGOs for a longer period than reported manuscripts, subepithelial smooth muscle cells have been induced, and they have been considered to be accompanied by MM. The time-course analysis of these hGOs with MM revealed that subepithelial smooth muscle differentiation appeared after about day 21 and followed gastric differentiation of hGOs' epithelium. During differentiation into hGOs with MM, we identified some signaling factors secreted by the organoid epithelium, and the emergence of MM was suppressed by inhibiting them. Furthermore, subepithelial smooth muscle differentiation was promoted when the hGOs protruded through the Matrigel layer and attached to the stiff bottom of the plastic plates. These findings suggested that the epithelial-derived factors and mechanical environment induce the muscularis mucosa of the iPSC-derived human gastric organoids. One of the epithelial-derived factors also expressed in gastric ulcers. This indicated that it could help to regenerate gastric MM.

Keywords: hiPSC, gastric organoid, muscularis mucosa

Poster Abstracts

304

DEVELOPING A 3D HIPSC-DERIVED CULTURE MODEL FOR ENDOGENOUS TAU AGGREGATION

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Tauopathies are neurodegenerative diseases characterized by the intracellular accumulation of tau protein aggregates in the brains. Multiple failures of clinical trials based on the results of mouse models indicated the requirement of more appropriate disease models of human cells. In this study, we aimed to establish 3D human iPSC-derived culture system harboring multiple cell types with close contact and adult tau isoform (3R and 4R) expression which are resembling the in vivo situation because in AD brains 3R and 4R tau are co-aggregated.At first, we introduced MAPT N279K/P301S/IVS10+16 mutations to healthy donor-derived hiPSC by genome-editing technology. Then, cerebral organoids were formed from the hiPSC to induce various neural cell types. Next, the organoids were once dissociated to single cells and plated onto low attachment plates to induce cell clustering. Finally, we obtained multiple cell clusters, i.e. 3D culture model, containing various neural cells with close contact. The scRNA-seq analysis revealed that the 3D culture consisted of excitatory/inhibitory neurons, astrocytes, oligodendrocyte precursor cells, and so on. After the addition of recombinant tau seeds to initiate tau aggregation, we could induce endogenous tau aggregation mainly in the 3D model of mutant hiPSC. In addition, we found that the triple mutation increased the expression of 4R tau, implied the co-expression of 3R and 4R tau with the mutations enhanced tau pathogenesis.Our findings indicated that the close contact of neural cells in the 3D model enabled the propagation of tau aggregates among human neurons and triggered endogenous tau aggregation. This 3D culture model using mutant hiPSC will serve as a platform for both basic research and drug discovery targeting tau aggregation.

Funding Source
JKiC, JSPS, AMED
Keywords: Tau, Alzheimer, Aggregation

306

VALPROIC ACID SUPPRESSES THE TISSUE-RECONSTRUCTING ABILITY OF COLORECTAL CANCER STEM CELLS BY INHIBITION OF GSK3

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Cancer stem cells (CSCs) can reconstruct cancer tissues, are responsible for recurrence and metastasis, and contribute to resistance to treatment. Therefore, developing therapies against CSCs is essential for treating unresectable cancers. Previously, we generated induced colorectal cancer stem cells (iCSCs) by introducing OCT3/4, KLF4, and SOX2 into colorectal cancer cell lines. Unlike their parental lines, they can form organoid and xenograft, which recapitulates human colon cancer tissues. In this study, we aimed to identify critical factors in colorectal CSCs using iCSCs and iCSC-derived organoids, evaluated the effects of drugs targeting these factors and confirmed the drug efficacy in patient-derived organoids.To identify the molecular mechanisms that promote the properties of CSCs, we compared the global expression pattern of iCSCs with that of the parental cells by a microarray. We identified several probes which showed >2-fold higher expression in the iCSCs than the parental cells with an FDR < 0.05. Among them, we focused on RCAN2, a known negative regulator of calcineurin. Calcineurin was reported to translocate NFAT from the cytoplasm to the nucleus, and GSK3 has an opposite function. A calcineurin-inhibitor FK506 enhanced the CSC properties of iCSCs. Conversely, GSK3 inhibition by VPA and CHIR99021 impeded the organoid-forming ability and expansion of iCSCs. GSK3 inhibitor and FK506 showed the opposite effect regarding the NFATc3 localization in the iCSC. These data suggested that NFAT localization regulated by calcineurin and GSK3 plays crucial roles in the tissue-reconstructing ability of colon CSCs, and VPA can be a candidate in stem cell-targeting therapy.Then, to confirm the effect of VPA on clinical colorectal cancer cells, we established ten patient-derived colorectal cancer organoids. When VPA was administered to each organoid for ten days, the number of viable cells decreased significantly in eight cases, and the remaining two cases also showed a downward trend. CHIR99021 also reduced the viable cell number, supporting the effect of VPA via GSK3 inhibition in patient-derived cells. VPA may be helpful as a therapeutic agent for colorectal cancer.

Keywords: organoid, VPA, cancer stem cell

307

SUPRAMOLECULAR HYDROGEL ENCAPSULATION OF KIDNEY ORGANOIDS INCREASES GLOMERULI FORMATION

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The differentiation of hiPSCs towards renal lineages leads to tissue that highly resembles the mammalian kidney. These hiPSC-derived tissues are known as kidney organoids and contain renal progenitor cell populations that develop into nephrons. Kidney organoids hold translational promise as a regenerative therapy for patients suffering from renal failure. However, the nephrons found in kidney organoids are not properly segmented, which may negatively impact clinical function. The segmentation of nephrons is influenced by a proximal-distal gradient of Wnt- and Notch signaling, which determines the final identity of differentiating nephron progenitor cells (NPCs). The canonical Wnt signaling pathway is tuned by a wide array of cues, including mechanotransducive forces from the surrounding environment. Mechanotransduction induces nuclear translocation of YAP, where the protein is capable of forming suppressor complexes for numerous Wnt-related genes. As such, a sufficient amount of mechanotransduction may skewer NPC differentiation towards proximal segments of the nephron. Here, we take the first step towards investigating the role of biomaterials, and their respective mechanical forces, on lineage commitment of NPCs within organoids. To this end, kidney organoids were encapsulated in a fully synthetic hydrogel at different time points. These hydrogels are composed of ureido pyrimidinone (UPy)-based molecules, which are chemical moieties capable of self-assembling into a dynamic, fiber-like network via supramolecular interactions. Furthermore, by coupling integrin-binding peptides to UPy-moieties, it is possible to bio-functionalize the hydrogel network to allow cell-material interactions leading to mechanotransduction. These properties together result in a fully synthetic hydrogel in which kidney organoids remained viable and contained segmented nephrons. However, encapsulation of kidney organoids within UPy-hydrogels led to a sharp increase of glomeruli, while maintaining the same amount of proximal- and distal nephron segments. These results demonstrate that lineage commitment of NPCs is susceptible to changes in the surrounding mechanical environment and may be directed in this fashion.

Funding Source

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Keywords: Organoid, Hydrogel, Glomerulus

308

ON-CHIP GLOMERULAR FILTRATION BARRIER CONSTRUCTED BY CO-CULTURE OF KIDNEY ORGANOID-DERIVED PODOCYTES AND HUVECS FOR FUNCTIONAL EVALUATION AND CYTOTOXICITY ASSESSMENT OF PAN

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The main function of the glomerulus is the selective filtration of plasma components and waste products from the blood, which is carried out by the glomerular filtration barrier consisting of vascular endothelial cells, glomerular basement membrane, and podocytes. However, this filtration barrier is impaired by some diseases and medications, causing irreversible damage. Therefore, nephrotoxicity tests are necessary for the development of new drugs, but conventional screening methods using animal models cannot accurately evaluate the toxicity to human glomeruli. In this study, we constructed an on-chip glomerular filtration barrier using human iPS cell-derived podocytes. To construct a glomerular filtration barrier on-chip, we fabricated a device with three parallel channels made of PDMS by soft lithography. Next, human umbilical vein endothelial cells (HUVECs) were introduced to the first channel. The middle channel was filled with a gel that mimicked a glomerular basement membrane. The third channel was prefilled with podocytes derived from kidney organoids generated from human iPS cells. To evaluate the glomerular filtration barrier for permeability, fluorescently labeled inulin and albumin were introduced from the endothelial side of the flow path after 48 hours of incubation. Also, 24 hours after the construction of the glomerular filtration barrier, PAN (Puromycin Aminonucleoside), a glomerular disrupting substance, was introduced into the vascular side of the flow path, and after another 24 hours of incubation, permeability was evaluated using inulin and albumin as well. The results showed that without PAN treatment, the permeability of albumin was about 1/3 of that of inulin, and selective filtration was performed. On the other hand, when PAN was introduced, there was no difference in the permeability of inulin and albumin, and the overall permeability increased. This suggests that we were able to construct a model of glomerular filtration function

Poster Abstracts

and drug-induced glomerular damage with this device. The on-chip glomerular filtration barrier constructed in this study using iPS-derived podocytes will serve as a basis for a model to evaluate glomerular damage in future nephrotoxicity tests.

Funding Source
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Keywords: Kidney organoid, Microphysiological systems, Nephrotoxicity

309

SELF-ORGANIZING SINUSOIDAL VESSELS IN LIVER ORGANOIDS USING THE INVERTED MULTILAYERED AIR-LIQUID INTERFACE 3D PERFUSION (IMALI) CULTURE

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The liver produces coagulation factors related to hemostatic function. In recent years, the development of organoid technology, which allows the generation of complex and multi-cellular structures derived from stem cells, has been rapidly evolving with expectations for the understanding of disease pathology, drug development, and regenerative medicine. However, reported organoids of the liver are limited in part by the absence of essential supporting structures, for example, the organ-specific and hierarchical vasculature (sinusoidal, portal, and central venous endothelium), thereby, limiting their therapeutic use against diseases associated with hemostatic and coagulation deficit.To circumvent these challenges, we report the Inverted Multilayered Air-Liquid Interface 3D culture (IMALI) platform to self-organize human induced pluripotent stem (iPS) cell-derived liver organoids with hierarchical vascular structures. IMALI culture incorporates both arterial and venous vascular endothelial progenitor cells by leveraging boundary conditions with extracellular matrix in the gas-liquid plane. Furthermore, to reproduce the fluid conditions of the localized high load of blood flow during development and vascular network formation, we developed a culture system that alternately loads organoids with laminar and turbulent flow by intermittently increasing the perfusion flow rate. We found that mature hepatocytes which express zone-specific markers such as glutamine synthetase and E-cadherin are widely distributed in alignment with developing CD32b+ vascular networks and that the production of coagulation and complement factors is remarkably enhanced. Secreted coagulation factors are sufficient for correction of in vitro and in vivo clotting time. Given the scarcity of blood-derived coagulation products, an organoid-derived authentic human protein source can potentially be used for the development of diagnostics and therapeutics for disorders affecting the coagulation cascade.

Funding Source
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Keywords: Liver organoid, Coagulation, Endothelial cell

311

HUMAN IPSC-DERIVED PANCREAS ORGANOIDS: MODELLING MODY3 DIABETES IN VITRO

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Maturity-onset diabetes of the young 3 (MODY3) is a rare type of diabetes caused by heterozygous mutations in the hepatocyte nuclear factor 1ALPHA (HNF1A). The p291fsinsC truncating mutation in HNF1a is the most frequent in MODY3 patients, however how this mutation causes MODY3 is not fully understood. In this study, we used MODY3 patients and CRISPR/Cas9 induced iPSC lines to delineate the molecular mechanism behind the p291fsinsC mutation during differentiation of iPSCs into beta-like cells using a novel 3D iPSC-derived pancreas organoid system. Patient and CRISPR/Cas9-derived iPSCs organoids showed a severe reduction in PDX1+ and Nkx6.1+ pancreas progenitors and endocrine cells (INS+, SST+ or GCG+) differentiation. Interestingly, we found that the truncated form of HNF1A interacts with the key pancreas developmental factor HNF1B and inhibits HNF1B-dependent genes. Our study uncovers a novel function of the HNF1a p291fsinsC mutation during pancreas development using a novel iPSC-derived pancreas 3D organoid model. The p291fsinsC mutation results in a previously undescribed pancreatic development defect ranging in severity, explaining the disease heterogeneity observed in MODY3 patients.

Funding Source
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Keywords: Diabetes, Organoids, iPSCs

312

DEVELOPMENT OF HUMAN GUT ORGANOID MODELS BY INCORPORATION OF PSC-DERIVED MACROPHAGES TO INVESTIGATE INTESTINAL IMMUNE FUNCTION

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In order to maintain the intestinal barrier integrity and the plastic intestinal homeostasis, the immunocompetent cells below the intestinal epithelium interact with the epithelial cells. We have developed novel human intestinal organoids (HIOs) from hPSCs under xenogeneic-free culture conditions in vitro (JCI Insight 2017). Xenogeneic-free HIOs (XF-HIOs) are uniquely structured by apical-out mucosal epithelium and complexed mesenchymal including smooth muscles and intestinal nerve cells. The XF-HIOs exert peptide absorption and catalytic activity on xenobiotics (Stemjournal 2021). However, it is still challenging for in vitro gut organoid models to evaluate human gastrointestinal immunological functions. Here, we have developed the organoid technologies to recapitulate the gut immune function by introducing hPSC-derived macrophage-precursors into the gut organoids (MC-XF-HIOs). We examined gene expression levels of macrophages in the MC-XF-HIOs. The macrophages sorted from the organoids by FACS sorting of dissociated single cells from the organoids. PCR array analysis revealed the expression levels of multiple genes involved in macrophage polarization. Furthermore, to assess the abilities of the organoids to produce and secrete soluble cytokines and chemokines, we investigated cytokines and chemokines in the organoids using a bead-based Multiplex cytokine assay. CCL2, CCL3, CXCL10, EGF, EOTAXIN, IL-4, IL-6, IL-8, IL-10, and IL-12 were secreted from the organoids. Macrophages are usually classified into one of two major subtypes, M1 and M2. The hPSC-derived macrophages residing in the XF-HIOs showed several hallmarks of both M1 and M2. Macrophages residing in the organoids are considered to be a heterogeneous cell population, and it is not fully clear how and what levels they recapitulate naïve intestinal macrophages. In order to verify how much the MC-XF-HIOs system reflects human intestinal physiology and pathology, further analysis will be needed.

Funding Source

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Keywords: gut organoids, pluripotent stem cells, gut immunity

313

NF-KB REPORTER INTESTINAL ORGANOIDS: A 3D MODEL FOR STUDYING INTESTINAL INFLAMMATION IN HEALTH AND DISEASE

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The intestinal epithelium constitutes a dynamic physical and protective barrier against harmful and other external stimuli. While inflammation is essential for epithelial barrier protection, when uncontrolled, it can also damage the tissue.The NF-kB signaling pathway is particularly relevant in the intestine. NF-kB activation protects intestinal epithelial cells against various noxious stimuli, contributing to maintaining intestinal barrier integrity. Conversely, the same pathway mediates chronic intestinal inflammatory diseases by inducing pro-inflammatory gene expression. The availability of appropriate in vitro models of the intestinal epithelium is a crucial step for understanding the contribution of NF-kB in physiological and pathological processes and further advancing the development of new therapies and drugs.In this work, we established, characterized, and validated three-dimensional cultures of intestinal organoids derived from a transgenic reporter mouse (Balb/C-NF-kB-RE-Luc). The NF-kB-RE-Luc intestinal organoids recreated the cellular composition of the original tissue, whereas the reporter specificity and sensitivity were similar to the in vivo model. Jejunum-derived NF-kB-RE-Luc-reporter organoids, when stimulated with TNF-ALPHA, provide a reliable useful ex vivo model to evaluate the anti-inflammatory effects of natural and synthetic compounds.These reporter organoids are a useful tool for exploring the epithelial-specific contribution of the NF-kB pathway in intestinal health and disease while reducing the use of laboratory animals.

Funding Source

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Keywords: Mouse intestinal organoids, Intestinal inflammation, NF-kB Pathway

Poster Abstracts

314

GENERATION OF MACROPHAGE-INCORPORATED LUNG ORGANOID FROM HUMAN PLURIPOTENT STEM CELLS

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Differentiation technology from pluripotent stem cells into specific cells has been developed over the past decade, and attempts to disease modeling on organoids have steadily increased. Organoid production has the advantage of being able to discover new drugs by replacing animal experiments, and at the same time, by mimicking the living body to check the physiological activity of cells in the living body in vitro. Lung epithelial cells maintain cell homeostasis while resident immune cells in alveoli and reduce damage to lung cells caused by foreign substances. However, no attempt has been made to introduce immune cells into the lung organoids. The purpose of this technology was to develop macrophage-incorporated lung organoids and to analyze the function and characteristics of innate macrophage. We established a technique to differentiate lung organoids and macrophages from human pluripotent stem cells. Lung organoids expanded steadily upon differentiation day 20 to 30, and subculture was possible. Various lung specific markers (SFTPB, SFTPC, ABCA3) were highly expressed in CD47hiCD26lo-sorted lung organoid but lowly expressed pluripotent stem cell and endoderm markers (OCT4, SOX17) at differentiation day 28. Also, differentiated macrophages were specifically expressed macrophage marker (CD163, CD86, CD11b) from their subtype. Moreover, we attempt to incorporate macrophage in lung organoids. Incorporate macrophages well aggregate into small spots and settle down in lung organoids until 14 days. Through this study, we can take advantage of the interaction by mimicking the microenvironment between the lungs and immune cells in the human body. It will also serve as a framework of modeling lung disease.

Funding Source
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Keywords: Lung organoid, Macrophage, Stem cell differentiation

315

EFFICIENT GENERATION OF LENTOID BODIES FROM HUMAN IPS CELLS USING SEAM METHOD

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Cataract is the leading cause of global blindness, mostly in the developing world. Cataract is a disease in which the lens becomes cloudy due to various causes. Currently, the only way to treat cataracts is surgery using an intraocular lens. Drug therapy is expected as another option. When developing a therapeutic drug for cataract, it is desired to develop an excellent human lens model. Recently, our research group has succeeded in producing ocular organoids from human iPS cells, which we call SEAM (self-formed ectodermal autonomous multi-zone), where major primordia during ocular development such as the cornea, retina and lens can be obtained. In this study, we report on the efficient induction of lens differentiation by applying SEAM. The expression of a series of crystallin proteins, which were components of lentoid bodies, was greatly enhanced compared to the normal SEAM method, which was confirmed by phase-contrast microscopy, quantitative PCR, and immunostaining. This human iPS cell-derived lens model will be a useful tool to elucidate not only the development of the human lens but also the mechanism of cataract development. In the future, the generation of cataract models using our system is expected to lead to the development of drugs for cataract treatment.

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Keywords: human iPS cells, lens, SEAM

316

MODELING ALZHEIMERS DISEASE IN FOREBRAIN ORGANOIDS

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Alzheimer's disease (AD) is the most common cause of age-related neurodegenerative dementia associated with progressive memory impairment and cognitive damage. While access to patient brain tissues is limited, human induced pluripotent stem cell (iPSC) technology is a promising approach for the generation of in vitro human AD models. To study disease mechanisms, develop drug-screening system and evaluate candidate molecules, we generated 3D forebrain organoids from AD-iPSCs and analyzed Ab and Tau pathology. The obtained AD-organoids recapitulated Ab pathology but were missing tau aggregation. Neurodegenerative diseases characterized by the pathological accumulation of tau are collectively termed tauopathy. P301L is the most common mutation in Tau linked to FTDP-17 and has higher aggregation ability than Tau-WT. In order to generate tauopathy models using brain organoids, we overexpressed full length tau carrying the P301L mutation into forebrain organoids. Immunofluorescence showed that phospho-tau and aggregated tau levels were dramatically increased in Tau-P301L-overexpressing organoids compared to GFP transduced controls. Moreover, tau filaments were detected in cell body and neurites by Immunoelectron microscopy. We have successfully recapitulated amyloid b pathology in AD-patient derived brain organoid, and tau pathology in Tau-P301L-overexpressing brain organoids. These models can be used as a platform for studying the molecular processes of Alzheimer's disease, developing drug screening approaches and evaluating candidate molecules.

Funding Source
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Keywords: Brain Organoids, Alzheimers Disease, Tauopathy

317

DECIPHERING EARLY ANGIOGENIC FACTORS OF HUMAN ON-CHIP IPSC-DERIVED CEREBRAL ORGANOIDS

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An important roadblock for further development and implementation of iPSC-derived cerebral organoids for translational research is the lack of efficient vascularization protocols. Although initial angiogenic processes including sprouting of vascular endothelial cells may be initiated in vitro using on-chip culture system, complete vascularization leading to a functional capillary network may only be achieved via transplantation to a rodent brain. Here, we used a combination of an on-chip culture system and region-specific transcriptome analysis to identify molecular program of early angiogenesis in human iPSCs derived cerebral organoids. Brain organoids were cultured on a preformed three-dimensional vasculature established from HUVEC cells for ten days until initial sprouting from vascular cells was visibly detectable (day 35). Several time points (day 28, 31, and 35) were used to identify transcriptome changes related to organoid angiogenic response. To eliminate cells exhibiting response to hypoxia and potentially necrotic core, only 100 µm thick outer cortical layer of cerebral organoids was microdissected using UnipicK+ microdissection system. Isolated total RNA was subjected to a full transcriptome analysis using Agilent whole-genome microarrays harboring 60,000 probes. Differentially regulated genes and pathways specific for angiogenesis and formation of vascular system were identified and are discussed in this presentation. This study provides the first global analysis of cerebral organoid response to preformed three-dimensional vascular network and points out putative modifiers for efficient on-chip vascularization.

Keywords: Microfluidics, Organoid vascularization, Cerebral organoid

Poster Abstracts

318

IN-VITRO GENERATION OF A FUNCTIONAL AND VASCULARIZED LIVER ORGANOID FROM HUMAN IPS CELLS

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Liver diseases are becoming an increasing burden on global health care-systems and economies, and although liver transplantation is the only life-saving solution for end-stage liver diseases, less than 10% of global transplant needs are met each year. To meet the increasing demand for liver transplants, the generation of liver-like tissues from human induced pluripotent stem cells is a promising approach. In this study, we reported a functional human liver organoid with a tissue-like structure using hiPSCs. To create a tissue-like organoid, we cultured hiPSC-derived liver buds on a cell culture insert and found that the survival of hepatic lineage cells and vascularization of ECs was improved with the air-liquid interface culture method. In this organoid, we also noticed that the hepatic cells were segmented into clusters throughout the liver organoid which lacked integration with other cell types. To further promote organoid function and structure, we replaced hepatic endoderm cells with hepatoblasts (HBs) as our hepatic lineage and detected a much clearer tissue-like vascular network. In addition, there was a significant increase in the expression of hepatic-lineage genes as well as an increase in the production of Albumin. In HB organoids, hepatic cells and the non-parenchymal cell types all integrated together to form a uniform tissue without segmentation. The only remaining issue was an unexpected growth of mesenchymal cells (MCs) forming a layer at the bottom of the liver organoid. To prevent the rapid proliferation of MCs, we treated the organoids with a TGFBETA inhibitor. As a result, the mesenchymal cell layer disappeared, and the expression of hepatic-lineage genes as well as the production of Albumin were improved. Surprisingly, TGFBETA inhibition could also help the vessels in our organoid to develop into a more sinusoidal-like structure. In the future, we aim to apply this tissue-like organoid for clinical therapies as well as in-vitro disease modelling.

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Keywords: iPSCs, tissue-like structure, liver organoid

319

ANALYSIS OF HYPOTHALAMUS-PITUITARY DEVELOPMENT USING SPATIAL TRANSCRIPTOME

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Accumulating evidence has revealed cellular heterogeneity in organs and the importance of cell-cell interactions in regulating organ functions. Spatial transcriptome analysis provides a novel platform for studying cell-cell interactions at the gene expression level. The hypothalamus-pituitary axis plays an essential role in regulating various biological functions, including homeostasis, growth, and reproduction. During the embryonic period, the pituitary develops from oral ectoderm in contact with the adjacent hypothalamus. By receiving several signaling molecules from the hypothalamus, oral ectoderm invaginates towards the hypothalamus and forms a pouch-like structure, named Rathke's pouch, which contains pituitary progenitor cells. However, precise mechanisms underlying the cell fate specification and morphological change of hypothalamus and pituitary are largely unknown. The induction method of hypothalamus-pituitary organoids from human (h)iPSCs can recapitulate the tissue interaction in vitro. In this study, we performed spatial transcriptome profiling of hiPSC-derived hypothalamus-pituitary organoid to investigate the tissue interactions involved in pituitary progenitor cell differentiation. Our results demonstrated that the hypothalamus cell population contacting pituitary progenitor cells showed an enriched expression of extracellular factor genes, including growth factors and extracellular matrix. The knockout in hiPSCs of several of these genes resulted in impaired pituitary cell differentiation, suggesting that these factors released from the hypothalamus might regulate pituitary progenitor cell differentiation in a paracrine manner. These genes are potential causal gene candidates for congenital hypopituitarism. In conclusion, spatial transcriptome profiling provides a novel platform for analyzing the tissue interaction networks and identifying novel factors involved in pituitary development.

Keywords: Pituitary, Organoid, Transcriptomics

320

STAGE-SPECIFIC CHANGES IN MITOCHONDRIAL DYNAMICS REVEALED IN DIFFERENT OXYGEN CONDITION USING HUMAN BRAIN ORGANOID MODEL

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Mitochondria play a pivotal role in a variety of processes including calcium signaling and cell apoptosis. The role of mitochondria dynamics in neural cell fate is also well documented. Our previous experiments on different neuronal differentiation models showed that normoxic oxygen conditions (5% O2) impact neural to glial cell fate by increasing expression of the astrocytic marker, GFAP and lowering expression of the neuronal marker, BETAtubIII. In this work, we used a brain organoid model from human induced pluripotent stem cells (hiPSCs) at three different stages of development - 11-day neurospheres, 44-days (44D-BO) and 4-month brain organoids (4M-BO) grown in two different oxygen conditions: 5 and 21% O2 to decipher mitochondrial morphology and network connectivity at single-cell level. Our mitochondria analysis framework revealed changes in mitochondrial networks parameters throughout brain organoid development, which is accompanied with increased expression of neuronal (BETATubI-II) and glial (GFAP) markers. Moreover, further analyses revealed that normoxic oxygen condition affects key parameters of the mitochondrial morphology in stage-specific manner. Changes of mitochondrial morphology and connectivity are the most noticeable at the stage 44D-BO. Furtermore, analysis of large-scale mitochondria interaction networks (MINs) within confocal three-dimensional mitochondrial immunofluorescence images identified significantly altered motifs and network features (such as average shortest path length, modularity, information content) in 5% O2 compared to 21% O2. These results suggest that oxygen conditions influence neural fate by inducing changes in mitochondrial dynamics. Overall, our framework offers a powerful tool to elucidate mitochondrial fate/morphology at a single-cell level at different stages of neuronal differentiation in the brain organoid model.

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Keywords: human cerebral organoids, mitochondrial biogenesis, oxygen tension

Stem Cell-Based Disease Modelling and Drug Development

401

IN VITRO MODEL OF AUTOIMMUNE DISEASE MADE FROM PATIENT DERIVED IPS CELLS

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Vogt-Koyanagi-Harada disease (VKH) is an autoimmune disease, in which patient's melanocytes are attacked by their own immune system. When melanocytes in the choroid of the eye are attacked, it develops uveitis (an inflammatory disorder in the eye), which in the worst case leads to blindness. Autoimmune diseases are generally intractable and their causes are not identified well. As autoimmune diseases are disorder of the immune system, they have strong correlation with the type of human leucocyte antigen (HLA) encoded in the genome. In the case of VKH, it has a strong correlation with HLA-DR4 that is mostly expressed in Asian population. While all the VKH patients express HLA-DR4, not all the HLA-DR4 possessors necessary develop VKH, which indicates HLA-type is not the only determinant of the disease. This together with the onset of VKH that is most likely in adulthood, suggests some environmental factors in addition to genetic factors are complexly involved in the development of the disorder. It is even possible that the causal antigen (the peptide sequence recognized by the immune system) may differ patient by patient. Thus, in this study we aim to develop a patient-specific in vitro model of VKH uveitis, utilizing patient derived iPS cells. For this purpose, we generated iPS cells from T-lymphocytes isolated from a certain VKH uveitis patient, and then differentiated them into melanocyte-like cells that showed expressions of melanocyte markers TYR and MITF, by modifying previously reported protocols for melanocyte induction. After confirming the expression of HLA-DR4, we are going to co-culture the induced melanocytes with the lymphocytes of the same patient to see whether the melanocytes will be attacked, reproducing the in vivo condition of VKH. With this in vitro model of a VKH patient, our ultimate goal is to identify the causal antigen of that particular patient and develop a personalized drug peptide that blocks the immune reaction against the causal antigen. We believe this approach utilizing patient-derived iPS cells to generate the target cells, and co-culturing them with the lymphocytes drawn from the same patient, should be applicable to other autoimmune diseases for the discovery of drug for efficient and safe cure of each patient.

Funding Source
RIKEN Incentive Research Project FY2021 (YNF), JSPS KAKENHI Grant Number 21H03097 (YNF)
Keywords: autoimmune disease, in vitro model, patient derived iPS cells

Poster Abstracts

403

MODELING BRAIN OVERGROWTH IN AUTISM USING HUMAN PLURIPOTENT STEM CELLS

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Approximately 15-20% of individuals with Autism Spectrum Disorder (ASD) have disproportionate megalencephaly (ASD-DM), with disproportionate enlargement in both gray and white matter volume. Individuals with ASD-DM have more severe behavioral and cognitive problems and are less responsive to standard therapeutic interventions, leading to very poor prognoses relative to individuals with ASD and normal head circumferences. Increases in brain size often precede clinical symptoms, suggesting that understanding the underlying mechanisms regulating brain overgrowth could provide a window of opportunity for intervention or mitigation of symptoms. Here, we generated ~40 human iPSC lines from cohorts of children (2-4 years old) with complete clinical and phenotypic data, including A) ASD-DM; B) ASD subjects with normal sized brains, ASD-N; C) Typically developing (TD) subjects with DM, TD-DM; and and D) TD subjects with normal sized brains, TD-N. We differentiated each of the iPSC lines into neural progenitor cells (NPCs) and oligodendrocyte progenitor cells (OPCs) and investigated changes contributing to brain overgrowth. We observe increased proliferation and suppressed phagocytosis of NPCs/OPCs by macrophages in ASD-DM. RNA-sequencing of the differentiated progenitor cells reveals important signaling mechanisms related to the neuroimmune system in regulating cellular phagocytosis. In prior work, we have demonstrated that CD47 (a 'don't eat me' signal) is overexpressed in both NPCs and OPCs in 16p11.2 deletion carriers with macrocephaly contributing to reduced phagocytosis in vitro and in vivo. Treatment of 16p11.2 deletion NPCs and OPCs with an anti-CD47 antibody to block CD47 restores phagocytosis to control levels in cellular and mouse models. Here, we show that similar neuroimmune mechanisms regulate cellular homeostasis in idiopathic forms of autism and highlight new forms of therapy for selected autistic individuals with brain overgrowth early in the disease.

Funding Source

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Keywords: autism, disease modeling, iPSCs

404

RESTORATION OF THE DEFECT IN RADIAL GLIAL FIBER CELL MIGRATION AND CORTICAL PLATE ORGANIZATION IN BRAIN ORGANOID MODEL OF FUKUYAMA MUSCULAR DYSTROPHY

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Fukuyama congenital muscular dystrophy (FCMD) is a severe, intractable genetic disease that affects the skeletal muscle, eyes, and brain and is attributed to a defect in alpha dystroglycan (ALPHADG) O-mannosyl glycosylation. We previously established disease models of FCMD; however, they did not fully recapitulate the phenotypes observed in human patients. In this study, we generated induced pluripotent stem cells (iPSCs) from a human FCMD patient and differentiated these cells into three-dimensional brain organoids and skeletal muscle. The brain organoids successfully mimicked patient phenotypes not reliably reproduced by existing models, including decreased ALPHADG glycosylation and abnormal radial glial cell (RG) fiber migration. A basic polycyclic compound restored ALPHADG glycosylation in the brain and muscle models tested and partially rescued the abnormal RG migration observed in cortical organoids. Therefore, our study underscores the importance of ALPHADG O-mannosyl glycans for normal RG architecture and proper neuronal migration in corticogenesis.

Funding Source

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Keywords: Fukuyama muscular dystrophy, alpha dystroglycanopathy, brain organoid

405

DYSFUNCTION OF THE PROTEOGLYCAN TSUKUSHI CAUSES HYDROCEPHALUS THROUGH ALTERED NEUROGENESIS IN THE SUBVENTRICULAR ZONE IN MICE

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We have been studying the molecular function of Tsukushi (TSK), which is a soluble molecule belonging to the Small Leucine-Rich Proteoglycan family (Ahmad et al., 2018). TSK is expressed in pericytes on the blood vessels and ependymal cells in the subventricular zone (SVZ) of the lateral ventricle (LV) of the mouse brain. We analyzed brain morphology and expression of marker genes in TSK knockout mice (TSK-/-) brain. TSK-/- mice developed hydrocephalus after birth and this phenotype became even more severe at later stages. We found aberrant cell proliferation and cell death at SVZ were induced by dysregulated Wnt signaling in TSK-/-. Both overexpression of TSK protein using transgenic mice in TSK-/- background and direct injection of TSK protein into the LV of TSK-/- brain rescued the LV expansion in TSK-/-. We performed sequencing of peripheral blood DNA from 13 hydrocephalus patients with unknown reason and found 3 heterozygous nucleotides changes within TSK coding region, which introduce missense mutations that are predicted to cause amino acid changes in TSK protein sequence. Our results suggest that TSK is involved in the pathogenesis of hydrocephalus in human patients. We would like to discuss the developmental mechanism of hydrocephalus in the absence of TSK, therapeutic potential of TSK to rescue hydrocephalus and determine the percentage of patients carrying mutated TSK gene to establish TSK as a marker for hydrocephalus diagnosis.

Funding Source
KAKENHI (22122009), Kumamoto University Advanced Research Project Stem Cell-Based Tissue Regeneration Research and Education Unit, Program for Leading Graduate Schools HIGO Program in Kumamoto University and Japan Agency for Medical Research and Development.
Keywords: Tsukushi, Hydrocephalus, neural stem cell

406

ROPINIROLE HYDROCHLORIDE IDENTIFIED BY IPSC DRUG DISCOVERY FOR AMYOTROPHIC LATERAL SCLEROSIS: A RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE 1/2A TRIAL

Morimoto, Satoru[1,3], Takahashi, Shinichi[1,2,3], Ito, Daisuke[3], Daté, Yugaku[3], Okada, Kensuke[3], Chai, Muh Chyi[1,4], Nishiyama, Ayumi[5], Suzuki, Naoki[5], Hirai, Miwa[6], Kabe, Yasuaki[6], Suematsu, Makoto[6], Jinzaki, Masahiro[7], Aoki, Masashi[5], Sato, Yasunori[8], Nakahara, Jin[3], Suzuki, Norihiro[3], Okano, Hideyuki[1]

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Ropinirole Hydrochloride Remedy for Amyotrophic Lateral Sclerosis (ROPALS) trial is an investigator-initiated, phase 1/2a, randomized, double-blind, placebo-controlled trial that evaluated the safety and efficacy of ropinirole hydrochloride in patients with ALS (UMIN000034954, JMA-IIA00397). ROPALS trial is also aimed at assessing the feasibility of iPSC-based drug repositioning. Participants are randomly assigned to ropinirole hydrochloride or placebo, administered orally for 24 weeks in the double-blind period followed by a 24-week open-label active extension period. The primary endopints were safety and tolerability. The secondary endpoints include the ALS Functional Rating Scale-Revised (ALSFRS-R) score, combined assessment of function and survival (CAFS), and a composite endpoint for the muscle strength, respiratory, and bulbar functions. Twenty participants received ropinirole hydrochloride (n=13) and placebo (n=7). The incidence of adverse events was similar in both groups, and most of them had been known previously. The DELTAALSFRS-R and the CAFS score favored the ropinirole hydrochloride group, further supported by increased physical activity and muscle strength. Moreover, ropinirole hydrochloride treatment was associated with extended time-to-first disease progression events (median progression time: 28 weeks; $p = 0.008$) and increased time to %FVC ≤ 50% ($p = 0.010$). This trial showed a consistent trend across various efficacy endpoints that collectively favors ropinirole hydrochloride. Thus, ropinirole hydrochloride is possibly a safe and effective therapy for ALS. Furthermore, as an exploratory study, we have established iPSCs from the blood cells of all participants and are trying to identify stratification factors to distinguish responders / suboptimal responders for ropinirole hydrochloride. The iPSC-based drug discovery is an innovative tool for drug discovery of neurodegenerative diseases.

Funding Source
This study was funded by The Japan Agency for Medical Research and Development and K Pharma Inc.
Keywords: ALS, Ropinirole, iPSC-based drug discovery

Poster Abstracts

407

GENERATION AND ANALYSIS OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CORTICAL NEURONS HARBORING PRESENILIN CONDITIONAL KNOCKOUT ALLELES

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Alzheimer's disease (AD) is characterized neuropathologically by the presence of amyloid plaques and neurofibrillary tangles as well as the loss of synapses and neurons. Mutations in the amyloid precursor protein (APP) and presenilin (PS) have been linked to familial forms of AD (FAD), accounting for approximately ~2% of AD patients. In particular, more than 200 distinct mutations in the PS genes have the majority of all identified causative mutations in FAD, highlighting an importance of PS mutation in the AD pathogenesis probably by affecting GAMMA-secretase proteolytic activity, which then leads to an increase of more toxic BETA-amyloid (ABETA) peptides, especially ABETA42 or longer forms. Despite extensive investigations of pathogenic mechanism of PS mutations thus far, normal physiological functions of PS in mature neurons, especially human neurons, remain to be determined. However, detailed examination of PS physiological roles in mature neurons is often hampered by experimental hurdles, since simple PS knockout impairs Notch signaling pathway lethally during neural development. In this study, to investigate whether PS plays essential physiological roles in human cortical mature neurons, we generated PS1 conditional knockout (cKO) induced pluripotent stem cells (iPSCs) with or without additional PS2 knockout by CRISPR/Cas9 system, in which PS1 can be ablated selectively under an introduction of Cre recombinase. We then differentiated the PS cKO iPSCs into human cortical neurons in vitro, and ablated PS1 proteins by infection of lentivirus expressing Cre. Whereas no gross morphological alteration was observed between PS-null neurons and PS-intact control neurons, ABETA generation was robustly reduced in PS-null neurons, along with a concomitant accumulation of APP-CTFs. Using this novel stem cell-based tool, we would discuss important physiological PS functions in human mature neurons, dysfunction of which could possibly underlie AD pathogenesis.

Funding Source
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The Japan Agency for Medical Research and Development
Keywords: Induced pluripotent stem cell, Alzheimer's disease, Presenilin

408

IPSC-BASED DISEASE MODELING FOR LATE-ONSET NEURODEGENERATIVE DISEASES USING A CHEMICAL COMPOUND ACCELERATING SENESCENCE

Shiga, Takahiro[1], Miyoshi, Sakura[2], Tamune, Hidetaka[3], Kumersarkar, Avijite[4], Nakai, Kento[4], Kuzumaki, Naoko[2,5], Ishikawa, Ke-Ichi[1,6], Baba, Kazuyoshi[4], Okabe, Shigeo[3], Hattori, Nobutaka[6], Okano, Hideyuki[2], Akamatsu, Wado[1]

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Patient-specific iPS cells are a valuable tool for models of neurological diseases, but the problems with late-onset neurodegenerative such as Parkinson's disease and Alzheimer's disease are that they require a prolonged culture period to detect the disease phenotype. In this study, we screened compounds that promote the maturation and differentiation of iPSC-derived neurons. We used a lentiviral synapsin-promoter GFP reporter as an indicator of the maturation of iPSC-derived neurons. We screened inhibitors that promote the maturation of iPS cell-derived neurons and found that an ATM kinase inhibitor (JA1), promotes not only the maturation but also the senescence of iPS cell-derived neurons. Comprehensive gene expression analysis showed that this ATM kinase inhibitor was effective in changing young fibroblasts into old fibroblast-like cells. This suggests that it is capable of inducing senescence in a variety of cells. When JA1 was added to the culture medium, it significantly shortened the time to phenotypic expression in neurological disease-specific iPS cell models of Alzheimer's disease and Parkinson's disease. This compound can easily accelerate neuronal differentiation and maturation without any gene transfection and is useful for reproducing pathological conditions specific to neurodegenerative diseases in a shorter period than conventional cultivation. The effect of JA1 has a great benefit in improving the research efficiency of disease model iPS cells and this compound is an extremely useful tool in aging research.
Keywords: Senescence, Parkinson's disease, Alzheimer's disease

409

INVOLVEMENT OF RNA VIRUS INFECTION PATHWAY IN DIFFUSE- AND INTESTINAL-TYPE GASTRIC CANCER

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Epithelial-mesenchymal transition (EMT) and cancer stem cells (CSCs) have common cellular phenotype features. Gene expression signature and molecular network pathways in diffuse- and intestinal-gastric cancer (GC), and mesenchymal stem cells (MSCs) have been investigated to understand the EMT and CSCs. Data in Gene Expression Omnibus (GEO) was analyzed in Ingenuity Pathway Analysis (IPA). Since the involvement of RNA virus infection pathway has been found in diffuse- and intestinal-type GC, we focused on the relationship between diffuse- and intestinal-type GC and RNA virus infection. In the "Coronavirus Pathogenesis Pathway", NPM1 was involved in Hepatitis C virus-related hepatocellular carcinoma. Several molecules, including SMAD3, NFKAPPAB and JNK were related to canonical pathways "Regulation of the EMT Pathway" and "Regulation of the EMT by Growth Factors Pathway", and activated in diffuse-type GC compared to intestinal-type GC. Direct RNA-RNA interactions of microRNA targeting identified in coronavirus pathogenesis pathway included let-7, mir-10, mir-15, mir-155, mir-17, mir-181, mir-19, mir-25, mir-30, and mir-34. Molecular pathways activated in RNA virus infection, as well as diffuse- and intestinal-type GC may be an interesting cue for understanding the disease progress and identifying drug targets.

Funding Source
Japan Agency for Medical Research and Development (AMED), JSPS KAKENHI, Ministry of Health, Labour, and Welfare (MHLW)
Keywords: EMT, gastric cancer, molecular network

410

IDENTIFICATION OF THERAPEUTIC AGENTS FOR MITOCHONDRIAL CLEARANCE DISORDER IN PARKINSON'S DISESE

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Parkinson's disease (PD) is a neurodegenerative disease caused by selective loss of midbrain dopaminergic neurons. Although more than 90% of PD cases are sporadic without any identified causative genes, iPSC-based models of familial PDs with specific cellular defects are useful for disease modeling and drug screening. We have established and reported disease-specific iPSCs from two types of the familial PDs, PARK2, caused by PRKN mutation, and PARK6, caused by PINK1 mutation, with impaired mitochondria clearance. Then, several PD-related phenotypes including impaired mitochondrial clearance and increased cell vulnerability in PARK2 and PARK6 dopaminergic neurons were quantified automatically with an imaging cytometer. We performed library screening (320 compounds) for their ability to ameliorate multiple phenotypes and identified four candidate drugs. We then confirmed that these candidate drugs could recover PD-phenotypes of PARK6 fly models and apoptotic phenotypes in iPSC- derived neurons derived from sporadic PD patients. The high-throughput phenotype detection system is an effective tool for drug screening to explore disease-modifying drugs in PD.

Keywords: Parkinson's disease, patient-specific iPSCs, mitochondrial clearance

Poster Abstracts

411

ANTIOXIDANT EFFECTS OF TAURINE IN THE MELAS PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS AND THE SUPPRESSION OF EMT SIGNALS IN RETINAL PIGMENT EPITHELIUM

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Mitochondria play an important role in cellular energy production through oxidative phosphorylation. Several studies suggested the relationship between mitochondrial dysfunction and aging-related diseases, including age-related macular degeneration (AMD). Mitochondrial dysfunction could induce apoptosis or the dysfunction of retinal pigment epithelium (RPE) which supports retinal photoreceptors and eventually causes loss of central vision. And mitochondria disease (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes, MELAS) patients often present with central retinal dystrophy. To elucidate the retinal disease mechanisms in MELAS patients, we utilized MELAS patient-derived induced pluripotent stem cells (MELAS-iPSCs) with mitochondria DNA (mtDNA)-mutation, A3243G. The drug screening system by the live/dead cell imaging indicated MELAS iPSCs were vulnerable to glucose deficiency, which was partially rescued by taurine treatment. Metabolomic analyses showed that the reduced glutathione (GSH) ratio to oxidized (GSSG) decreased in MELAS iPSCs, which was also rescued by taurine treatment. MELAS-iPSCs were differentiated into retinal organoids and retinal pigment epithelium (MELAS-RPE). The mutation ratios in mtDNA in MELAS-RPE were confirmed by qPCR, those were maintained through several passages. The OCR was reduced in MELAS-RPE as compared with those in control RPE. It is known that Epithelial-Mesenchymal Transition (EMT) of the RPE is involved in retinal diseases such as AMD. And the EMT signals were activated in MELAS-RPE which were suppressed by taurine treatment. In the current study, we have investigated the pathogenic mechanisms of MELAS at the molecular and biochemical levels in MELAS-iPSCs and -RPE to advance our understanding of the pathogenesis of mitochondrial disorders and the therapeutic effects of taurine.

Funding Source
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Keywords: Mitochondria, reactive oxygen species, retinal pigment epithelium

412

TRANSCRIPTOME-WIDE MAPPING OF FUS-INTERACTING RNAS IN HUMAN IPS CELL MODELS OF FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

Yano, Masato[1,2], Nogami, Masahiro[3,4,5], Matsuo, Tsuyoshi[3,4], Kamei, Takayuki[5], Nishida, Mayumi[5], Nakanishi, Atsushi[4,5], Ogi, Kazuhiro[3,4,5], Hayakawa-Yano, Yoshika[1,2], Okano, Hideyuki[2]

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Fused in sarcoma/translated in liposarcoma (FUS) is a causative gene of familial amyotrophic lateral sclerosis (fALS). Mutated FUS causes accumulation of DNA damage stress and stress granule (SG) formation, etc., thereby results in motor neuron (MN) death. We have used iPS cell-derived motor neurons as a cellular model to explore the causes of motor neuron death and elucidate the hub genes, related to DNA damage response pathway in the molecular aetiology influenced by mutant FUS using the *iBRN method. To further understand the direct link to the molecular aetiology in human iPSCs derived MNs with FUS mutation, we performed HITS-CLIP, a technology of protein-RNA interaction at the transcriptome-wide. Transcriptome-wide mapping of wild type and mutated FUS revealed that genomic distribution of FUS interacting RNAs were quite different between wild type and mutated FUS proteins. Interestingly, nuclear RNAs including snRNA and nuclear long noncoding RNA (lncRNA), such as MALAT and NEAT1 were dramatically down-regulated in mutated FUS CLIP in comparison with wild type FUS CLIP, while cytosolic RNA molecules were up-regulated in mutated FUS CLIP including lncRNAs with RNA buffering action. These dataset strongly suggest that the difference of RNA ligands between wild type and mutated FUS might be associated with the cause of molecular pathology and DNA damage response.*iBRN ; "Non- biased" Bayesian gene regulatory network analysis based on induced pluripotent stem cell (iPSC)-derived cell model1.

Funding Source
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Keywords: FUS, CLIP, ALS

413

USEFULNESS OF MELANOCYTES DIFFERENTIATED FROM INDUCED PLURIPOTENT STEM CELLS FOR RESERCH ON PATHOLOGY OF XERODERMA PIGMENTOSUM

Takemori, Chihiro[1], Koyanagi-Aoi, Michiyo[2,3], Fukumoto, Takeshi[1], Kunisada, Makoto[1], Hosaka, Chieko[1], Aoi, Takashi[2,3], Nishigori, Chikako[1,3]

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Xeroderma Pigmentosum (XP) is a rare, autosomal recessive, hereditary DNA repair disorder characterized by extreme hypersensitivity to ultraviolet radiation (UVR). XP complementation group A (XP-A) is the most frequent type in Japan, and patients with XP-A present most severe cutaneous and neurological symptoms due to nucleotide excision repair deficiency. Here, we succeeded in establishment of induced pluripotent stem cells (iPSCs) from XP-A patients and generated melanocytes via melanocyte precursor cells (MPCs), which are self-renewing cell lineage and can differentiate into melanocyte in a week after GSK3BETA inhibition. The use of MPCs makes it relatively easy to obtain melanocytes. XP-A-iPSC-derived melanocytes (XP-A-iMCs) appeared morphologically similar to normal human epidermal melanocytes and expressed melanocyte markers. To elucidate the molecular mechanism of the disease, we performed microarray and analyzed difference of gene expressions between XP-A-iMCs and healthy-control-iPSC-derived melanocytes (HC-iMCs) at 12 hours after high dose (150 J/m2) UV-B irradiation. The results showed that the major GO term categories for genes specifically upregulated in XP-A-iMCs were cell proliferation and death, and that for genes specifically downregulated in XP-A-iMCs was cell cycle regulation. This gene expression profile might explain part of the pigmentary characteristic disorders observed in patients with XP-A.

Keywords: Xeroderma Pigmentosum, melanocyte precursor cell, microarray analysis

414

MODELING OF NEURODEGENERATIVE DISEASES USING DIRECTLY CONVERTED NEURONS FROM URINE-DERIVED CELLS

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The technology of induced pluripotent stem cells (iPSCs) enabled us to model neurodegenerative diseases using human cells. However, the aging signatures are almost cancelled in iPSCs, which is a limitation to study aging-dependent diseases using iPSCs. Thus, we aimed to develop aged neurons using human cells not via iPSCs. To achieve the aim, we examined the direct neuronal conversion of urine-derived cells (UDCs) that can be extracted from human urine and collected multiple times in non-invasive ways that enables us to follow individual person's aging.After several modification of the direct conversion method for fibroblasts, we succeeded to induce neuron-like morphological changes in UDCs (UDC-iNs). Neuronal markers including microtubule-associated protein tau were expressed in the UDC-iNs, and the frequency of Ca oscillation of the cells was much faster than the original UDCs. In addition, transcriptome analysis revealed neuronal profiles of the UDC-iNs. Using the newly established conversion method, we succeeded to establish UDC-iNs from the donors at various ages, 2 to 89 years old. DNA methylation analysis of the UDCs from those donors illustrated the donor's age-dependent methylation in UDCs and the preservation of methylation status even after the conversion to UDC-iNs. The survival rate of UDC-iNs was declined over time, but the survival rate was increased by tau reduction, indicating that tau expression was detrimental to UDC-iNs. Preliminary study showed that the survival rate of UDC-iNs from Niemann-Pick type C, mild cognitive impairment, and Alzheimer's disease were less than controls. However, more donors will be required to conclude.These data implied the potential of UDC-iNs as a prediction platform for individual's neuronal aging.

Funding Source
AMED, JSPS, KGRI

Keywords: Urine derived cell, Neurodegenerative disease, Direct conversion

Poster Abstracts

415

PHENOTYPIC DRUG SCREENING FOR ADPKD USING KIDNEY ORGANOIDS DERIVED FROM DISEASE-SPECIFIC HUMAN IPSCS

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease. It is characterized by multiple renal cysts and bilateral kidney enlargement, leading to end-stage kidney disease. Although many disease models have been proposed for ADPKD, the precise mechanisms of the renal cystogenesis remain unknown, and no definitive therapies are currently available. To identify the key factors of renal cystogenesis that may have therapeutic implications, genetically relevant human models are needed. Herein, we established kidney organoid models generated from two kinds of disease-specific human induced pluripotent stem cells (hiPSCs), PKD1 gene-edited and ADPKD patient-derived hiPSCs. A stepwise differentiation method that we have recently reported was applied to generate kidney organoids. We found that forskolin, a stimulator of cAMP signaling, facilitates the reproduction of cystic phenotypes within the kidney organoids depending on the PKD1 genotype. Importantly, ADPKD patient-derived as well as heterozygous PKD1 gene-edited kidney organoids recapitulated cystogenesis in vitro. We further utilized ADPKD patient-derived kidney organoids to examine the effects of known inhibitors of cystogenesis. The results suggested the possibility of organoid-based phenotypic drug screening for ADPKD. To enhance the efficacy of this approach, we developed a high-throughput screening (HTS) platform by combining suspension cultures of kidney organoids and a robotic liquid handling system. Finally, using ADPKD patient-derived organoids, we performed a phenotypic screening of 1,223 pharmaceutical compounds to identify candidate drugs that inhibit forskolin-induced cystogenesis. In conclusion, we established novel models for ADPKD using kidney organoids differentiated from disease-specific hiPSCs and developed a phenotypic drug screening platform. This strategy will contribute to identifying novel therapeutic targets for ADPKD treatment.

Funding Source

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Keywords: ADPKD, Disease modeling, Drug screening

416

ELUCIDATING EARLY PATHOPHYSIOLOGY OF SPINAL-BULBAR MUSCULAR ATROPHY USING DISEASE-SPECIFIC IPSCS

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Spinal-bulbar muscular atrophy (SBMA) is an adult-onset neuromuscular degenerative disease caused by CAG repeat expansion in androgen receptor (AR) gene. So far findings from mice models have indicated that testosterone-dependent mutant AR aggregations play important roles in neuronal dysfunction and degeneration. However, the phenotypes of mice models are different from those of patients in several aspects. Here, we generated induced pluripotent stem cells (iPSCs) from SBMA patients and established more accurate disease models to investigate the pathogenesis of SBMA. iPSCs were established from four SBMA patients and three age-matched controls, and were differentiated into motor neurons (MNs) for the pathophysiological analysis. Mutant AR aggregations were not detected in 4-week SBMA-MN culture by immunocytochemistry or Western blot analysis, while alteration of the expressions of disease-associated genes including CALCA (Calcitonin related polypeptide alpha: CGRP-1) and TBETAR2 was detected. Those two genes are known to be associated with the early pathology of SBMA, suggesting this model recapitulates early pathophysiology of SBMA. We also identified ER stress as a disease accelerating factor that significantly enhanced the phenotypes of SBMA-MNs. Transcriptome analysis revealed several disease-associated molecules that induced neurodegenerative phenotypes in control-MNs. Furthermore, the phenotypes in SBMA-MNs were rescued by the inhibitors of identified molecules. Using this disease model, uncovering early pathophysiology of SBMA, and identifying novel biomarkers and therapeutic targets are expected.

Funding Source

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Keywords: iPSC-derived motor neurons, spinal-bulbar muscular atrophy, motor neuron disease

417

EFFICIENT, REPRODUCIBLE, AND FUNCTIONAL DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS (IPSCS) INTO SKELETAL MUSCLES FOR MUSCULAR DISEASE MODELING

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Pathophysiological analysis and drug discovery targeting human diseases require disease models that better recapitulate patients' pathology. Disease-specific iPSCs could potentially recapitulate disease pathology more accurately than existing models when iPSCs were differentiated into relevant affected cell types. Thus, successful modeling of muscular diseases requires an efficient differentiation of iPSCs into skeletal muscles (SkMs). For this purpose, Dox-inducible MYOD1 expression system has been widely used, however, it requires time- and labor-consuming clone selection procedures for efficient differentiation, and needs to overcome clonal variations. Here, we established bulk SkMs differentiation system without clone selection by modifying the previously reported Dox-inducible MYOD1 expression system. First, bulk MYOD1-iPSCs were established by the selection with Puromycin or G418 and were compared with clonally established MYOD1-iPSCs. As a result, bulk MYOD1-iPSCs established with puromycin selection showed similar transgene (Tg) expression and differentiation efficiency to clonally established MYOD1-iPSCs and generated more than 80% of Myogenin and MHC positive cells within seven days from the days of differentiation. On the other hand, bulk MYOD1-iPSCs with G418 selection showed less Tg expression and differentiation efficiency compared with clonally established MYOD1-iPSCs. Moreover, we confirmed similar and efficient differentiation of several iPSC lines into SkMs using bulk MYOD1-iPSCs with puromycin selection, suggesting that this system is reproducible and may overcome clonal variations. Finally, three-dimensional muscle tissues were fabricated from bulk MYOD1-iPSCs with puromycin selection, which exhibited contractile force by electrical stimulation, indicating their functionality. Together, these results suggest that our bulk differentiation system is capable of efficiently generating functional SkMs, and requires less time-labor, facilitating the generation of disease models for the pathophysiological analysis of muscular disorders.

Funding Source
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Keywords: Bulk Culture, Skeletal Muscle, Disease Modeling

418

NAD REGULATES KEY METABOLIC FUNCTIONS IN IPSC DERIVED NEURONS

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Aging increases the incidence of neurodegenerative diseases, such as amyotrophic lateral sclerosis, Alzheimer's, Parkinson's, or Huntington's disease. Recent studies have revealed that the decline of cellular nicotinamide adenine dinucleotide (NAD) levels is involved in aging-related disorders. Accordingly, therapeutic approaches boosting cellular NAD have been reported to prevent these disorders. Here, we hypothesized that NAD and its related metabolites regulate the vulnerability of neurons to stress factors related to neurodegenerative diseases. First, we successfully differentiated human-induced pluripotent stem cells (hiPSC) into neurons by modifying a previously reported protocol using Tet-On-driven expression of Neurogenin2 gene (Ngn2) and neuronal microRNAs (miR-9/9*-124) (Ishikawa M et al., Cells 2020). Next, we measured intracellular NAD levels in iPSC and iPSC-derived neurons by high-performance liquid chromatography. We also evaluated the oxygen consumption rate (OCR) in iPSC-derived neurons using a metabolic extracellular flux analyzer. A specific inhibitor of NAD biogenesis was used to understand the decrease of intracellular NAD concentration. Flux analyzer analysis revealed that the inhibition of NAD biogenesis by chemical compounds reduced the maximal OCR after FCCP treatment. Following experiments revealed that the Mitochondrial Membrane potential (MMP) was also decreased. Finally, capillary electrophoresis-mass spectrometry (CE-MS)-based metabolome analysis was used to evaluate the importance of intracellular NAD levels in hiPSC-derived neurons. This analysis revealed that NAD biogenesis inhibition induced a partial decrease of glycolysis and reduced the amount of energy generated through the electron transport chain (ETC) that performs oxidative phosphorylation (OXPHOS). These findings illustrate that hiPSC-derived neurons are regulated by NAD concentration changes at multiple levels, indicating that the neuronal resilience regulated by NAD could be a potential target for neuronal aging.

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Keywords: iPSC, neuron, metabolism

Poster Abstracts

419

USING ANCESTRY-INFORMED HUMAN INDUCED PLURIPOTENT STEM CELL MODELING TO ELUCIDATE MOLECULAR MECHANISMS UNDERPINNING EWS/FLI TRANSFORMATION IN EWING SARCOMA

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Ewing sarcoma (ES) is a rare but deadly bone and soft tissue tumor primarily diagnosed during the second and third decades of life. ES is characterized by translocation-induced fusions between EWSR1 and ETS family members-typically FLI1-which act as aberrant transcription factors that dysregulate global gene expression via binding to GGAA repeat elements throughout the genome. Unusual in global representation, ES has far higher incidence in populations of European ancestry; in-fact, ES is diagnosed 10X more frequently in children of European descent compared to children of African ancestry. To understand the molecular underpinnings for this disparity, we obtained induced pluripotent stem cells (iPSC) from individuals of known ancestral descent based on principle component analysis (PCA) comparing genotype information from the database of genotypes and phenotypes (dbGaP) on our purchased iPSC lines with the HapMap dataset to quantify ancestry. We obtained two lines each of ~100% European and African ancestry, and four lines with intermediate European/African admixture (45%-90% African). As the EWS/FLI fusion protein is normally toxic to most cell types, we first sought to determine whether tolerance to the EWS/FLI protein differed based on ancestry. To do this end, we differentiated iPSC from each ancestry into neural crest cells (iNCC), a proposed cell-of-origin for ES, and transduced them with a lentivirus expressing either a GFP reporter or a GFP-2A-EWS/FLI cassette. We monitored each line for tolerance to the EWS/FLI fusion protein via flow cytometry analysis for the GFP reporter. While the GFP control vector was maintained at a high frequency of ~95% over 21 days regardless of ancestry, we found that iNCC derived from European lines maintained a significantly higher frequency of cells expressing GFP-EWS/FLI than the pure African lines. Intriguingly, admixed lines maintained an intermediate frequency of GFP-EWS/FLI positive cells. To elucidate the molecular underpinnings of this phenomenon, we are conducting a battery of genomic assays including ATAC-seq, RNA-seq, and CUT&TAG-based profiling of global EWS/FLI occupation for each ancestral iNCC line over a time course following EWS/FLI transduction. The assays are underway and updated results will be presented. Our results show the feasibility of using ancestry-informed iPSC to study genotype to phenotype differences in ES and will be used to identify new targetable pathways for the treatment of ES.

Funding Source
American Institute for Radiologic Pathology
Keywords: Bottom-up Cancer Modeling, Ewing sarcoma, Ancestry Informed iPSC

420

IPSC TECHNOLOGY REVEALS MITOCHONDRIAL DEFECTS CAUSE OF ASTROGLIOPATHY IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS AND PARKINSONISM-DEMENTIA COMPLEX IN THE KII PENINSULA

Leventoux, Nicolas[1], Morimoto, Satoru[1], Ishikawa, Mitsuru[1], Endo, Fumito[2], Shibata, Shinsuke[1], Yamanaka, Koji[2], Kuzuhara, Shigeki[3], Kokubo, Yasumasa[4], Okano, Hideyuki[1]

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In the southern-coast area of the Kii peninsula in Japan, patients are suffering from amyotrophic lateral sclerosis (ALS) and Parkinsonism-dementia complex (PDC) - Kii ALS/PDC - an incurable pathology at the origin of neurodegeneration in multiple systems. Although of unknown aetiology, astrocytic abnormalities have been noted in this disease, such as reactive astrocytes and aging-related tau astrogliopathy. To investigate this disease, we first developped a protocol to differentiate induced pluripotent stem cells (iPSCs) into astrocytes (iPasts).After publishing our protocol, we established iPSCs cell lines derived from five patients with Kii ALS/PDC and generated iPasts from them (Kii iPasts). We succeeded to recapitulate, in vitro, in these cell lines, several pathological phenotypes such as reactivation or reduced glutamate uptake capacity, compare to control iPasts.iPSCs technology enabled to compare transcriptomes of Kii and healthy control iPasts by RNA sequencing. We particularly focus on one gene drastically downregulated, involved in mitochondrial metabolism,

and sometimes previously reported as ALS- and PD-linked. The lentiviral overexpression of this target gene completed our experiments using a molecule to activate mitochondria, and confirmed that astrocytic functions such as glutamate uptake and mitochondria respiration could be rescued in some Kii-iPasts cell lines.In conclusion, we consider our results as innovative to explore mitochondria as a therapeutic target in Kii ALS/PDC disease.

Funding Source
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Keywords: Amyotrophic Lateral Sclerosis & Parkinson disease, Mitochondria, iPSC

422

INDUCED MARGINAL CELLS OF INNER EAR FROM HUMAN IPS CELLS AS THE DISEASE MODELS FOR HEARING LOSS

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Hearing loss is one of the most common sensory impairments in humans. Sensorineural hearing loss, which is mainly caused by the damage of cochlear cells is hardly treated because most of damaged inner ear cells cannot be regenerated in adult human. In addition, cochlea is not readily accessible thus it is not easy to obtain disease cells of deafened patients by biopsy to analyze them. In such organs, human stem cell-based approaches contribute to understand the pathophysiology and development of therapeutic treatments.Marginal cells in mammalian cochlea are the epithelial cells located in stria vascularis, connected each other by tight junction and express many ion-transport-related proteins at either apical or basolateral membrane. By transporting ions unidirectionally, marginal cells generate endocochlear potential in cochlea, which is essential for the hair cells to generate action potentials during the mechanotransduction in hearing. Mutations in genes expressed in marginal cells are known to cause hereditary hearing loss. Impaired marginal cells are also implicated in sudden hearing loss, endolymphatic hydrops, presbycusis and drug-induced hearing loss. Therefore, in vitro human marginal cell-models would be useful to study marginal cell-related hearing loss and develop novel therapies.Here we report a method for differentiating human pluripotent stem cells into marginal cell-like cells (iMCs). iMCs differentiated using our methods express multiple marginal cell markers, including specific ion transporters, and tight junction proteins, suggesting that iMCs are pharmacokinetically functional. To the best of our knowledge, this is the first in vitro model of human marginal cells. We believe the method is of great importance in auditory science and can be utilized not only for the analyses of pathophysiology but the future drug discovery/development in multiple etiology of hearing loss.

Funding Source
This work was supported by JSPS KAKENHI (19K24038).
Keywords: induced pluripotent stem cells, hearing loss, epithelial cells

423

REDUCED PHOX2B STABILITY CAUSES AXONAL GROWTH IMPAIRMENT IN MOTOR NEURONS WITH ALS-LINKED TARDBP MUTATIONS

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Poster Abstracts

Amyotrophic lateral sclerosis (ALS) is a progressive, and incurable adult-onset neurodegenerative disease, leading to motor neurons (MNs) degeneration, muscle atrophy, and finally death due to respiratory dysfunction. The precise mechanisms for the selective MNs vulnerability in ALS are unclear. One of the causative genes for ALS is transactive response DNA binding protein (TARDBP), which encodes transactive response DNA binding protein 43 kDa (TDP-43). TDP-43 is an RNA-binding protein and is thought to be involved in ALS, as TARDBP mutations have been reported to cause abnormalities in RNA metabolism, such as changes in mRNA stability. Moreover, axonal structure abnormalities precede neuronal deaths, suggesting that axonal pathology may be the earliest sign of ALS, which is so called "dying-back hypothesis". On the basis of these findings, the purpose of this study is to find novel modulatory genes in human MN axon-related pathology affected by TARDBP mutations. Human induced pluripotent stem cells (iPSCs) were established from peripheral blood mononuclear cells of familial ALS patients with a TARDBP mutation and induced to differentiate into MNs. The neurite length of TARDBP-mutant MNs was significantly shorter than that of control MNs without TARDBP mutations by measuring fluorescence after lentivirus vector transfections. Next, RNA sequencing (RNA-Seq) of MN axon fractions was performed using microfluidic devices to identify disease-related genes. RNA-Seq revealed reduced paired-like homeobox protein 2B (PHOX2B) expression in TARDBP-mutant axons. Quantitative PCR and in situ hybridization with iPSC-derived MNs were used to confirm a decrease in PHOX2B expression. Also, PHOX2B mRNA stability was reduced in TARDBP-mutant MNs. TARDBP knockdown with siRNA reduced PHOX2B mRNA expression. Moreover, PHOX2B knockdown with siRNA reduced neurite length in human MNs. Finally, phox2b knockdown in zebrafish with morpholino oligonucleotide induced short spinal axons and impaired escape responses. PHOX2B is highly expressed in other types of neurons maintained even after ALS progression. TARDBP mutation-induced MN axonal vulnerability may be mediated by PHOX2B downregulation.

Funding Source
Grants-in-Aid for research on rare and intractable diseases; Research Committee on Establishment of Novel Treatments for Amyotrophic Lateral Sclerosis; the Research Center Network for Realization Research Centers/Projects of Regenerative Medicine and Practical Research Project for Rare/Intractable Diseases from Japan Agency for Medical Research and development, AMED; Japanese Ministry of Education, Culture, Sports, Science, and Technology, Clinical Research, Innovation and Education Center, Tohoku University Hospital (CRIETO).
Keywords: ALS (amyotrophic lateral sclerosis), TARDBP (transactive response DNA binding protein), PHOX2B (paired-like homeobox protein 2B)

424

PATHOPHYSIOLOGICAL MODEL USING IPSC-DERIVED CORTICAL NEURONS FROM FTD PATIENTS WITH C9ORF72 REPEAT EXPANSION

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The C9ORF72 hexanucleotide repeat expansion is the most common genetic cause of familial frontotemporal dementia (FTD), which mainly affects the frontal and temporal lobes of the cerebral cortex. Some studies have recently suggested that one of the major pathomechanisms underlying FTD is the generation of toxic dipeptide repeat (DPR) proteins produced by C9ORF72 expansion. However, DPR-driven pathogenesis of FTD has not been fully verified in human neural cell models. While the development of human induced pluripotent stem cell (iPSC) technology has enabled the generation of patient-derived neural cells in a dish, there are no reports on iPSC-based modeling of C9ORF72-mediated FTD. In this study, we aimed to generate pathophysiological disease models using iPSCs-derived cortical neurons from FTD patients with C9ORF72 repeat expansion. First, we succeeded in generating frontal lobe-specific neurons from patient-derived iPSCs by modulating Wnt and FGF8 signaling pathway. Gene expression patterns of generated neurons were closely similar to those of human embryonic frontal lobes. Next, we found that p62 protein, which associated with autophagy, were accumulated in frontal lobe-specific neurons derived from FTD patient iPSCs, whereas such phenotypes were not detected in neurons with other brain region identities than the frontal lobe, suggesting that the frontal lobe-specific phenotypes of FTD could be recapitulated in our culture system. p62 accumulation was also observed when DPR proteins were overexpressed in neurons from healthy control iPSCs, which indicates that DPR protein toxicity would primarily underlie the FTD pathomechanisms. Further studies into the DPR protein toxicity by C9ORF72 repeat expansions should be explored in this iPSC-based FTD models.

Keywords: C9ORF72, iPSC, Frontotemporal dementia (FTD)

425

DYNAMIC CHANGES IN THE EXPRESSION OF NEUROPEPTIDES AND THEIR RECEPTORS IN GABAERGIC AND DOPAMINERGIC NEURONS DERIVED FROM IPS CELLS OF PARKINSON'S DISEASE PATIENTS

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Parkinson's disease (PD) is known to be associated with motor and non-motor symptoms, including depression, sleep disorder, dementia, digestive disturbance and sensory neuropathy. In PD, degeneration of dopaminergic neuron in the substantia nigra is known to be associated with general motor impairment, while non-motility symptoms in PD may be related to non-dopaminergic neurons, such as GABAergic neurons. The molecular composition of dopaminergic and GABAergic neurons includes the expression of neuropeptides and their receptors, which cooperatively modulate neuronal responses. In the present study, we investigated possible changes in the expression of neuropeptides and their receptors in dopaminergic and GABAergic neurons derived from induced pluripotent stem cells (iPSCs) of PD. A dramatic decrease in mRNA and protein levels of ghrelin receptor, which is associated with various brain functions, such as food intake, metabolism and reward, was found in PARK2-specific iPSC-derived dopaminergic neurons. Furthermore, the expression level of somatostatin, which is highly concentrated in a large proportion of GABAergic neurons and is responsible for the emotional processes that exert anxiolytic and anti-depressant effects, was reduced in differentiated GABAergic interneurons from PARK2-specific iPSCs derived from PD patients. These finding suggest that the deficiency of neuropeptides and their receptors in dopaminergic and GABAergic neurons may lead to the motor and non-motor symptoms of PD via regulation of the excitatory-inhibitory imbalance of the neural network.

Keywords: iPSCs, Parkinson's disease, Neuropeptides

426

GENERATION OF AN IN VITRO DISEASE MODEL OF ANIRIDIA BY INTRODUCING HETEROZYGOUS LOSS-OF-FUNCTION MUTATION OF PAX6 GENE IN HUMAN IPS CELLS

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Aniridia is a rare congenital disorder with bilateral iris hypoplasia or aplasia caused by the heterozygous loss-of-function mutation of PAX6 gene, which functions as a transcription factor and plays a pivotal role during embryogenesis. The mutations lead to the reduction of PAX6 gene expression down to 50% of normal level and may have a non-negligible impact on the expression of its downstream genes, possibly leading to the emergence of the disease phenotypes of aniridia. However, it is still unclear how and why the 50% reduction of the PAX6 gene expression result in the disease phenotypes of aniridia. Loss-of-function mutation of PAX6 gene was introduced to human induced pluripotent stem cells by CRISPR-CAS9 gene editing technique. The generated cell lines were differentiated into ocular cells through on-dish culture method to induce the formation of self-formed ectodermal autonomous multizones (SEAMs) and analyzed.Human iPS cell lines lacking functional PAX6 gene at one (PAX6+/- hiPS) or two alleles (PAX6-/- hiPS cells) have successfully been generated. The PAX6-/- cells were not able to form SEAMs, likely reflecting the disease situation of homozygous small eye (Sey/Sey) mice. The PAX6+/- hiPS cells demonstrated less efficient differentiation into corneal epithelial cells, likely reflecting the disease situation of clinically observed corneal epithelial stem cell deficiency in aniridia patients. The generated PAX6+/- hiPS cells seem to be useful as an in vitro model of aniridia and may contribute to further understanding of the disease mechanism of aniridia as well as the function of PAX6 gene.

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Keywords: aniridia, PAX6 gene, iPS cells

Poster Abstracts

427

INVESTIGATION OF THE FUNCTIONAL CHANGE IN ASTROCYTES DIFFERENTIATED FROM PARKINSON'S DISEASE-SPECIFIC IPS CELLS: EVALUATION OF PARKIN-DEPENDENT AND -INDEPENDENT MECHANISMS

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The loss of nigrostriatal dopamine neurons in Parkinson's disease depends on the involvement of non-autonomous cellular responses. In this study, astrocyte induction from Parkin mutant-related Parkinson's disease (PARK2)-specific iPS cells (PARK2-iPSCs) and analysis of neuroprotective factors localized to astrocytes were performed to investigate the changes in the neuronal extracellular microenvironment under Parkinsonism. We first attempted to induce differentiated astrocytes from healthy individuals and PARK2-iPSCs through neural stem cells that showed specificity in the midbrain region that is considered to be the primary site of Parkinson's disease. The present results showed mature differentiation into GFAP-positive astrocytes from healthy iPS cell-derived differentiated cells, whereas PARK2-iPSCs-derived differentiated cells showed low expression of GFAP and S100BETA in mature astrocytes, suggesting that PARK2-iPSCs-derived astrocytes may be immature. Under these conditions, we found alterations in the expression of NRF2 signal-related genes, which are critical for neuroprotective effects in immature astrocytes derived from PARK2-iPSCs, and decreased expression of neuroprotective factors such as NRF2 and Metallothionein 1F (MT1F). Furthermore, immature astrocytes were induced in the process of astrocytic differentiation from iPS cell lines lacking Parkin (isogenic control), whereas no decrease in Nrf2 signal-related gene expression was observed in these differentiated cells. These results suggest that Parkin-dependent astrocyte immaturity and Parkin-independent disruption of neuroprotective activity may be associated with Parkinson's disease.

Funding Source

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Keywords: iPSCs, Parkinson's disease, Astrocytes

428

DEVELOPMENT OF ALGORITHMS FOR AI DRUG DISCOVERY AND IMPLEMENTATION ON ALS IPSC PANEL

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During the past several years, many compounds have been developed for intractable diseases, yet many diseases remain without a treatment. Compound screening, especially phenotypic screening with human induced pluripotent stem cells (iPSCs), is a useful tool for discovering new candidate drugs and disease pathways even if the disease mechanism has not been completely clarified. Nevertheless, it is still a major challenge, as it is extremely time-consuming and very costly to evaluate millions of compounds. Machine learning is expected to improve low throughput and high assay cost in cell-based phenotypic screening. However, it is still a challenge to apply machine learning to achieving sufficiently complex phenotypic screening due to imbalanced datasets, non-linear prediction, and unpredictability of new chemotypes. We developed a prediction model based on a heat diffusion equation (PM-HDE) to address this issue. The algorithm was verified as feasible for virtual compound screening using biotest data of 946 assay systems registered with PubChem. Then, PM-HDE was applied to actual screening. Based on supervised learning of the data of about fifty thousand compounds from biological phenotypic screening with iPSCs, virtual screening of >1.6 million compounds was implemented. Finally, we confirmed that PM-HDE enriched the hit compounds and identified new chemotypes. Furthermore, the compounds identified by PM-HDE demonstrated broad and potent effectiveness against motor neuron death in clones derived from various sporadic ALS patients. This prediction model could overcome the inflexibility in machine learning, and our approach combined with iPSC-based phenotypic screen and machine learning could be a powerful tool for identifying promising leads and a novel platform for drug discovery.

Keywords: ALS, machine learning, drug development

429

ESTABLISHMENT OF IPSC-DERIVED NEUROMUSCULAR CO-CULTURE MODEL FOR THE ANALYSIS OF NON-CELL AUTONOMOUS NEURO-DEGENERATION IN MOTOR NEURON DISEASES

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Recently, non-cell autonomous neurodegeneration by skeletal muscles was shown to play crucial roles in various motor neuron diseases, such as amyotrophic lateral sclerosis (ALS), spinal bulbar muscular atrophy (SBMA), and spinal muscular atrophy (SMA), and, neuro-muscular interaction has been expected as a novel therapeutic target. However, detailed molecular mechanisms remains to be fully elucidated due to the lack of appropriate disease models. Here, we established an iPSC-based neuro-muscular co-culture model, in which functional neuromuscular junctions (NMJs) were formed and dynamics of NMJs could be visualized by fluorescent reporter. We first introduced AChR (Acetylcholine receptor) reporter (AεG) into human myoblast cell line Hu5/E18 to visualize NMJs (Hu5/E18-AεG). When Hu5/E18-AεG-derived myotubes were co-cultured with iPSC-derived motor neurons, clustering of AεG was detected in myotubes at the axonal projection site. We next co-cultured iPSC-derived skeletal muscles with motor neurons. Skeletal muscles were differentiated from human iPSCs by DOX-inducible MyoD1 expression by the modification of previously reported protocol. Then, iPSC-derived skeletal were infected by lentivirus expressing AεG, and co-cultured with iPSC-derived motor neurons labelled by HB9e438::mRFP. As a result, significant clustering of AεG signals was detected in mRFP-labeled motor neuronal projection site. By time-lapse imaging, dynamics of NMJs were successfully visualized, which was applicable to the analysis of neuro-muscular pathology. Moreover, functionality of neuro-muscular synapses was confirmed by the analysis using optogenetics and micro-electrode arrays (MEA). Taking advantage of this neuro-muscular model, we are currently differentiating disease specific iPSCs of motor neuron diseases into both motor neurons and skeletal muscles, and co-culturing them to elucidate neuromuscular pathology and to identify novel therapeutic targets.

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Keywords: Neuromuscular junction, Micro-electrode array, Imaging

430

MULTICELLULAR MODELING OF CILIOPATHY BY COMBINING IPS CELLS AND MICROFLUIDIC AIRWAY-ON-A-CHIP TECHNOLOGY

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Multiciliated airway cells play a crucial lung function that facilitates the eliination of inhaled pathogens and foreign matter from the airway tract. Coordinated ciliary beating among cells contributes to unidirectional mocociliary flow. We previously reported a method of stepwise differentiated iPSCs to airway cells, although it has been difficult to recapitulate it in vitro using human induced pluripotent stem cells (iPSCs). . In the present study, we challenged to apply airway-on-a-chip technology to control the intercellular coordination of ciliary beating. After an airway epithelial cell sheet cultured on an airway chip with fluid shear stress (FSS) for two weeks, the cell sheets were taken out from the device and fluorescent microbeads were placed on the cell sheet, demonstrating that the microbeads were flowed to the same direction of the medium flow during airway chip culture. In addition, the subcellular localization of VANGL1 among cells were aligned at the opposite side of microbead flows, indicating the global axis of multicellular planar cell polarity (PCP) were formed. Surprisingly, FSS could promote ciliogenesis more strongly than air-liquid interface or airway chip culture without FSS. Next, we applied those findings to disease modeling of primary ciliary dyskinesia (PCD), a genetic disease characterized by impaired ciliary movement. We started to work on PCD disease modeling by using iPSCs derived from patient's blood cells as well as genetic knockout iPSCs of PCD causative genes. We found that FSS promoted formation of the global PCP axis even in PCD patient-derived multiciliated airway cells. Conclusively,

Poster Abstracts

airway-on-a-chip technology seems to be beneficial for regulating intercellular ciliary beating, suggesting that mechanical stimulation might enhance the quality of airway disease modeling of iPSCs and be beneficial for elucidating the pathological mechanisms of various refractory lung diseases and the future regenerative medicine.

Funding Source
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Keywords: airway-on-a-chip, coordinated ciliary beating, planar cell polarity

431

GENERATION OF THE HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED GLIAL CELL MODELS FOR ANALYZING THE APOLIPOPROTEIN E FUNCTIONS IN ALZHEIMER DISEASE

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The number of patients suffered from Alzheimer's Disease (AD) is increasing. The detailed mechanism of AD onset is not yet elucidated, and it is necessary to clarify the pathogenic mechanism for developing the effective therapy. Among the three alleles (E2, E3, E4) of the Apolipoprotein E (APOE) gene expressing in astrocytes and microglia in the brains, APOE4 is the most well-known risk factor for sporadic AD that occupies almost all of AD patients. However, it remains unclear whether and how ApoE4 isoform negatively impacts the physiological functions in human astrocyte and/or microglia. To analyze the functions of ApoE isoforms during the course of sporadic AD pathogenesis, in this study, we first plan to develop the in vitro human astrocyte and microglia models derived from human induced pluripotent stem cells (hiPSCs). We converted APOE ε3/ε3 hiPSCs derived from the healthy subject to APOE ε4/ε4 by CRISPR/CAS9-mediated genome editing. Astrocytes and microglia were induced from APOE ε3/ε3 or APOE ε4/ε4 hiPSCs, using highly efficient differentiation protocols we established. We analyzed gene expression of APOE ε3/ε3 or APOE ε4/ε4 astrocytes and microglia and identified some differentially expressed genes. We next evaluated the function of lipid transport of APOE ε3/ε3 or APOE ε4/ε4 astrocytes. While the molecular weights of lipoproteins with ApoE3 or ApoE4 were quite similar, intracellular lipid accumulation of APOE ε4/ε4 glial cells was different from APOE ε3/ε3 cells. In conclusion, the hiPSC-derived glial models in this study are the promising tool for elucidating the functions of ApoE isoforms in sporadic AD pathogenesis.

Funding Source
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Keywords: Apolipoprotein E, Alzheimer's disease, iPS cell derived glial cells

432

GENERATION OF HYPOTHALAMIC MODEL FOR PRADER-WILLI SYNDROME USING PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS

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Hypothalamic neurons from human pluripotent stem cells were technically difficult to generate for its complex and various types of cells. Recently, methods for inducing hypothalamic neurons from induced pluripotent stem cells (iPSCs) have been reported. Using these methods, we can make models of diseases presenting with hypothalamic dysfunction and elucidate the mechanisms. Prader-Willi Syndrome (PWS) is a genomic imprinting disorder caused by loss of function of paternally expressed genes (PEGs) in chromosome 15q11-13 region. Hyperphagia, hypogonadism, and short stature due to growth hormone deficiency are major clinical manifestations of PWS, and these phenotypes have been thought to be closely related to hypothalamic dysfunction. However, little is known about the cellular and molecular pathophysiology in PWS. Here, we established models of PWS using hypothalamic neurons from patient-derived iPSCs. We generated iPS cell lines from two PWS patients. Those karyotypes are 46,XX.del(15)(q11.2q13) and 46,XX,upd(15)mat. Optimizing the medium condition, we have succeeded in generating RAX(+), NKX2.1(+), OTP(+) hypothalamic progenitor cells from those PWS-iPSCs as well as several hypothalamic neurons including POMC, AGRP, NPY positive cells which are crucial for the regulation of feeding. Moreover, we found NDN, MAGEL2, and MKRN3, PEGs in chromosome 15q11-13, were highly expressed in control hypothalamic neurons, but not in PWS hypothalamic neurons. Notably, these PEGs are known to be highly expressed in hypothalamus and related to PWS clinical symptoms. This indicates that these PWS cell lines genetically recapitulate disease-specific features. Abnormal cell differentiation and exacerbated inflammatory responses have been reportedly observed in PWS hypothalamus. Using these in vitro models, we are going to reveal in vitrophenotypes and pathophysiology of PWS.

Keywords: hypothalamus, Prader-Willi syndrome, imprinting disease

433

QUANTIFICATION OF NEURONAL, SYNAPTIC, AND NETWORK FUNCTION IN STEM CELL MODELS OF NEURAL DEVELOPMENT AND DISEASE

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Induced pluripotent stem cell (iPSC) technology has allowed complex human biology to be reproduced in vitro at high throughput scales. Furthermore, advances in genetics and proteomics have accelerated development of neural disease-in-a-dish models, while 3D cell preparations (e.g. organoids) have enabled mature models that better recapitulate human development.For the optimization, validation, and utilization of complex in vitro neuronal models, it is critical to characterize the function of neurons, synapses, and networks over time. Here, we present data supporting the use of label-free, multi-well microelectrode array (MEA) technology as an efficient approach for reliable and repeated quantification of cellular and network function across development and in disease.First, the Maestro system was used to characterize human iPSC-derived cortical organoid function across 10 months. Organoids exhibited increasingly complex spiking activity over time, with network events becoming more frequent. Local field potentials were also compared to electroencephalograms from premature neonates, revealing that the development of complex network oscillations in cortical organoids mimic the network dynamics of early human brain development. Multi-well MEA was also used to validate two iPSC disease-in-a-dish models. In a model of Dravet syndrome, patient-specific hiPSC-derived neurons were tracked for 36 days in vitro. Dravet syndrome cultures exhibited significantly higher mean firing rate, network burst duration, and network burst density compared to wild type controls, reflecting an epileptic phenotype. Similarly, in a model of Fragile X syndrome, hiPSC-derived neurons exhibited reduced FMR1 mRNA and FMRP expression compared to isogenic controls. Reduced expression corresponded to increased neural activity after DIV21. The disease phenotype was reversed with addition of FMR1 mRNA or co-culture with control neurons, both in a dose-dependent manner. By bringing human biology to a dish, hiPSC-derived neurons deliver biologically-relevant models for studying neural development and disease. The Maestro multi-well MEA platform enables label-free chronic tracking of neural network function ideal for validating neuronal models and developing novel therapeutics.

Keywords: Organoids, neurodevelopment, disease modeling

434

EVALUATION OF ALZHEIMER'S DISEASE MODEL PHENOTYPES AND THE EFFECTS OF 17B-ESTRADIOL ON NEURONS DERIVED FROM HUMAN IPS CELLS

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Alzheimer's disease (AD) is a neurodegenerative disease that is associated with cognitive decline and has a high prevalence of more than 30 million patients worldwide. While several clinical trials on AD treatments have failed to achieve the pleasant outcomes, it is believed that the heterogenous nature of disease mechanism of AD determines the different drug responses and disease mechanisms among the AD patients. Therefore, the generation of the robust disease model of AD as a platform for understanding AD heterogeneity has been strongly suggested. Here, we generated the iPSCs-derived cortical neurons including the excitatory and inhibitory neurons through the newly established feeder-free induction method. The donors are comprised of familial and sporadic subtypes. These iPSC-derived neurons demonstrated the disease phenotypes of AD such as increased amyloid-beta (ABETA) peptides evaluated by ELISA of secreted ABETA42 and ABETA40, neuronal hyperactivation evaluated by calcium imaging using Fluo-8 indicator, and morphological changes of the neurites. Besides, we evaluated the non-cell autonomous effect of sex dimorphism coming from sex hormone by treating the with 17b-estradiol, we showed that 17b-estradiol treatment for 15 minutes increased neuronal activity. These findings indicated that (i) the disease modeling of AD from hiPSC can be achieved by this newly established feeder-free induction method, (ii) hiPSC-derived AD neurons respond to sex steroid hormones, (iii) the current AD models can be used to investigate both autonomous and non-autonomous effects coming from sex dimorphism.

Funding Source
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Keywords: iPSCs, AD, 17b-estradiol

Poster Abstracts

Tissue Engineering

501

IN VITRO CONSTRUCTION OF LIVER TISSUE WITH BILIARY DRAINAGE CHANNEL USING CHEMICALLY INDUCED LIVER PROGENITOR CELLS (CLIPS)

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We have been working on liver tissue regeneration with vascular induction as an extension. Among them, in order to create a bile excretion pathway from the hepatocyte, attempts were made using primary bile duct epithelial cell isolation and fetal hepatoblast isolation without success. Finally, bile duct cells were converted from rat mature hepatocytes through chemically induced liver progenitor cells called CLiPs. By co-culturing with cells in 3D, it was confirmed in vitro that the bile excreted from rat hepatocytes is excreted into the CLiPs-derived bile duct. The integrated tubule-hepatocyte tissue was able to transport the bile, as quantified by the cholyl-lysyl-fluorescein assay, which was not observed in the un-cocultured structure or in the biliary cell monolayer. Furthermore, this in vitro integrated tubule-hepatocyte tissue exhibited an upregulation of hepatic marker genes. Together, these findings demonstrated the efficiency of the CLiPs-derived tubular biliary-duct-like structures regarding the accumulation and transport of bile.Finally, aged hepatocytes could be converted into CLiPs (Aged-CLiPs) expressing progenitor cell markers, but with a relatively low proliferative rate compared with young hepatocytes. Aged-CLiPs possessed both hepatocyte and cholangiocyte maturation capacity. HGF facilitated CLiPs conversion in aged hepatocytes, which was partly related to the activation of Erk1 and Akt1 signaling. Aged rat hepatocytes have retained reprogramming plasticity as evidenced by CLiPs conversion in culture. HGF promoted proliferation and CLiPs conversion in aged hepatocytes. Hepatocytes from aged donors may be used as an alternative cell source to mitigate donor shortage. In conclusion, construction of liver tissue with a bile excretion channel is indispensable for drug metabolism research and future regenerative liver construction.

Keywords: CLiPs, Liver tissue, Bile duct

502

METABOLIC CONTROL OF PLURIPOTENT STEM CELLS FOR THE GENERATION OF PANCREATIC BETA-CELL

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Stepwise differentiation protocols are reported for generating human pluripotent stem cell (hPSC)-derived insulin-expressing cells capable of producing and secreting insulin in response to high glucose and are useful as a cell source for regenerative medicine. However, marked variations have been reported in the differentiation capacities among hPSC lines. Elimination of such variations would therefore be useful. Here, we aim to establish a protocol that is widely applicable in various cell lines. We previously reported that pluripotent stem cells rely on methionine metabolism to sustain their pluripotency. We reported that short-term methionine deprival renders pluripotent stem cells in a state poised for differentiation so that the cells showed potentiated differentiation upon signal induction. Here, we found that applying a methionine deprival protocol resulted in potentiated differentiation of hPSCs to pancreatic endocrine BETA cells. We investigated the underlying molecular mechanism using microarray analysis. We identified upregulation of Zn exporter SLC30A1 and a reduced intracellular protein-bound Zn content in hPSCs as a downstream target of methionine deprival. We found that culturing hPSCs at Zn deprival conditions potentiated endoderm differentiation. The Zn deprival procedure is a powerful tool to eliminate undifferentiated hPSCs and potentiate differentiation, combined with methionine deprival. Through metabolite and expression profile analysis, we revealed an interplay between methionine metabolism and Zn signaling in cell fate regulation of hPSCs.

Funding Source
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Keywords: pluripotent stem cells, methionine metabolism, pancreatic endocrine

503

TISSUE ENGINEERING FOR CONSTRUCTING CARDIAC TISSUE FROM PLURIPOTENT STEM CELLS - NOVEL TISSUE MODELS REPRODUCING LETHAL ARRHYTHMIAS -

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Pluripotent stem cells (PSCs) are a powerful tool to develop cellular disease models and cell transplantation therapies as they can give rise to variety of cell types. Nevertheless, to generate higher structures as a tissue, cellular components alone are insufficient. One additional essential piece for the purpose is tissue engineering. We have been investigating cardiovascular cell differentiation and regeneration using PSCs. Combining our advantageous methods for differentiation of cardiomyocytes, endothelial cells, mesenchymal cells, with various tissue engineering technologies including cell sheet formation and gelatin hydrogels, we succeeded in generating human induced pluripotent stem cell (hiPSC)-derived cardiac tissue (HiCT) that consists of layered cardiac tissue sheets including three cardiac cell types. Now a clinical study of HiCT transplantation to patients with severe heart failure (Department of Cardiovascular Surgery, Kyoto University) has been approved from IRB of Kyoto University. As for disease models, we previously reported an in vitro model reproducing a lethal arrhythmia, Torsade de Pointes (TdP). We further added modulations to the model and recently succeeded in reproducing another lethal arrhythmia, ventricular fibrillation (VF) showing critical features as VF, such as VF-like extracellular field potential and breakup of spiral wave reentry. To our knowledge, this is the first in vitro VF model. 3D cardiac tissue structure formation with multiple cell types contributes to explore new advanced in vitro models in which various physiological and pathophysiological phenomena, that can be never induced in models at the cellular level, can be emerged.

Keywords: Pluripotent stem cells, lethal arrhythmia, 3D cardiac tissue

504

CARDIOVASCULAR REGENERATIVE MEDICINE SUPPORTED BY TISSUE ENGINEERING

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There are two approaches in cardiac regenerative medicine; restoring endogenous potential for damage repair, and functional regeneration of damaged tissue by exogenous replenishment of reconstructed tissues. For the former part, we have been working on clinical translation of a drug delivery system based on gelatin hydrogel (GH), a biodegradable biomaterial as a carrier for sustained release of cytokines. After pre-clinical studies, we conducted a clinical trial of the injection of basic fibroblast growth factor (bFGF)-incorporated GH microspheres for patients with critical limb ischemia (CLI), and a following clinical study using bFGF-incorporated GH sheets concomitant with coronary artery bypass surgery for ischemic heart disease (IHD) patients. Clinical trials for CLI exhibited an excellent outcome including improved perfusion and alleviation of clinical symptoms. Clinical study for IHD showed functional recovery and improved blood perfusion. Both studies were free from serious procedure-related adverse events. For the latter part, we have been investigating the replenishment of de novo human heart tissues in diseased hearts and functional recovery using human induced pluripotent stem cell (iPSC)-derived cardiovascular populations (cardiomyocytes and vascular cells), and a formulation of tissue-engineered 3-dimensional structures from the cardiovascular cells. We prepared 5-layered cardiac tissue sheets including various cardiovascular cells from human iPSCs and conducted pre-clinical transplantation studies for animal disease models. The incorporation of GH contributed to increased viability of 5-layered cardiac tissue sheets by mitigating the stacking limitation caused by hypoxia and necrosis. The transplantation of the tissues onto a rat myocardial infarction (MI) model exhibited myocardial restoration accompanied with vascularization among the graft. The transplantation of clinical-scale artificial tissues onto a porcine MI model resulted in a functional recovery and attenuated fibrosis and apoptosis. We are currently preparing a clinical study of the transplantation of the human iPSC-derived cardiac tissues. Thus, tissue engineering-based technologies contribute to cardiovascular regenerative medicine.

Funding Source
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Keywords: Regenerative medicine, Tissue engineering, Stem cells

Poster Abstracts

505

ALIGNMENT CONTROL OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES PROMOTES THE SYNCHRONOUS CARDIOMYOCYTE CONTRACTION IN THE CARDIAC TISSUE

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Alignment, as seen in the native myocardium, is crucial for maintaining the efficient cardiac function. Cardiac function is divided into systolic and diastolic function. However, it remains unclear how the cardiomyocyte alignment influences the cardiac function and the underlying mechanisms. The aims of our study were to fabricate aligned human cardiac tissue and elucidated the effect of alignment control on the contractile properties. When human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) were cultivated on a micro-processed fibrin gel with inverted V-shaped ridges (MFG), the orientation index, which was calculated based on the Fourier analysis using Phalloidin staining images, was significantly higher in hiPSC-CMs on MFG than that on the control fibrin gel (1.5 ± 0.1 vs. 1.2 ± 0.1, $p < 0.001$, $n = 4$). The value was close to the index of an adult rat heart tissue (1.6 ± 0.1), which suggested that hiPSC-CMs on MFG were aligned more uniformly than that on the control fibrin gel, and we succeeded in fabricating the aligned cardiac tissue. Assessing the contractile properties with the direct contractile measurement system, the contractile force, maximum contractile velocity, and relaxation velocity were significantly increased in aligned cardiac tissue compared with non-aligned cardiac tissue at 75 pacing per minutes upon the electrical stimulation (contractile force: 0.9 ± 0.5 mN vs. 0.5 ± 0.3 mN, $p = 0.02$, $n = 11$). The some gene expression profiles were not different between the two groups. However, the motion capture analysis revealed that the cardiomyocytes in the aligned cardiac tissues showed more unidirectional and synchronous contraction than the non-aligned cardiac tissues. It may indicate the cardiac tissue maturation as the electrical integration of cardiomyocytes. Further, the aligned cardiac tissues had more than twice impulse per glucose consumption compared with non-aligned cardiac tissue. In conclusion, cardiomyocyte alignment control promoted the synchronous cardiomyocyte contraction, which might contribute to maintain the efficient cardiac function. Understanding the molecular mechanism of alignment control-mediated synchronous contraction of cardiomyocytes might provide us the new insights on the cardiac function in heart disease with myocardial disarray.

Funding Source

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Keywords: human induced pluripotent stem cell-derived cardiomyocytes, Alignment, Synchronous contraction

506

POTENTIAL OF GELATIN HYDROGEL NONWOVEN FABRICS GENOCEL® AS A 3D CELL CULTURE SCAFFOLD

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One of the realistic strategies to enhance and maintain the biological activities of cells is to achieve a 3-dimensional (3D) cell-cell interaction in the in vivo condition. Several 3D cell culture scaffolds have been prepared from different materials in various designs both for the in vitro and in vivo applications. The porous structure of scaffolds can provide cells with spaces for their proliferation, migration, and differentiation. However, one of the big problems for 3D constructs is the death and functional loss of cells due to the poor supply of nutrients and oxygen to cells. When the thickness of cell constructs becomes larger than 150-200 μm, nutrients and oxygen do not reach to the construct inside, resulting in the cell necrosis inside the construct. Gelatin hydrogel has an ability to allow nutrients and oxygen to permeate. However, in the sponge form, the mechanical strength is poor. As one trial to resolve this issue, gelatin hydrogel non-woven fabrics (GHNF) has been developed. In this study, the potential of GHNF as a 3D cell culture scaffold was evaluated. The GHNF were prepared by a solution blow method of gelatin, following by the dehydrothermal crosslinking. The GHNF showed a mechanical strength strong enough not to allow the shape to deform even in a wet state. The wet GHNF also showed resistance against repeated compression. After human mesenchymal stromal cells (hMSC) were seeded and cultured in the GHNF, the inner cell distribution of GHNF, the apoptosis, hypoxia inducible factor (HIF)-1ALPHA, Ki67 of cells were evaluated. hMSC proliferated inside the GHNF with times while the cells were alive and bio-active even in the GHNF of 1 mm thickness, having a homogeneous distribution of cells in number. The number of apoptosis and HIF-1ALPHA positive cells were significantly low compared with those of polypropylene nonwoven fabrics with the similar fiber diameters and intra-structure. Gelatin fibers were degraded with culture time, and a network-like extracellular matrix (ECM) structure was produced. It is concluded that the GHNF is a promising culture scaffold for 3D cell constructs.

Keywords: 3D cell culture, Gelatin, Extracellular matrix

507

DEVELOPMENT OF TOOTH REGENERATIVE MEDICINE STRATEGIES BY CONTROLLING THE NUMBER OF TEETH USING TARGETED MOLECULAR THERAPY

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Anhidrotic ectodermal dysplasia (EDA), which is a syndromic congenital anodontia, is a rare disease with an incidence of 15.8 per 100,000 live births. Causative genes for congenital anodontia, such as MSX1, WNT10A or RUNX2 have also been identified. However, anodontia appears from early childhood, which is the developmental stage of the jaw, it adversely affects nutritional security and growth. Many attempts have been made to regenerate teeth by tissue engineering techniques, but due to problems such as cost and safety, they have not yet reached clinical application. We found that the USAG-1 protein (BMP / WNT antagonist) gene-deficient mouse formed excess teeth (teeth existing above the normal number of teeth), and this finding shows that one protein affects increase of the number of teeth. We also found that the formation of missing teeth was restored by mating various congenital anodontia model mice (Eda, Runx2 gene-deficient mice, etc.) with USAG-1 gene-deficient mice. Here, we tested whether inhibiting thetopical expression of Usag-1 can reverse arrested tooth formation after Runx2 abrogation. The results showed that local application of Usag-1 Stealth small interfering RNA (siRNA) with cationized gelatin promoted tooth development following Runx2 siRNA-induced agenesis. Furthermore, we prepared anti-USAG-1 neutralizing antibodies, considering the difference in activation mode for BMP and WNT signaling. Anti-USAG-1 neutralizing antibodies were found to ameliorate anodontia with a single intraperitoneal administration in Eda-deficient mice, a model of congenital anodontia. The development of a human anti-USAG-1 antibody / siRNA could be a curative treatment for regenerating one's own teeth by molecular targeted therapy which is different from a current treatment using general artificial teeth.

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Keywords: Tooth regeneration, USAG-1, Targeted molecular therapy

508

GENE DELIVERY FOR CREATION OF HEPATOCYTE SHEETS SECRETING ANGIOGENIC FACTORS

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Creation of liver tissues using hepatocytes is one of promising methods for the treatment of liver diseases such as enzyme deficiencies and hemophilia. Our laboratory developed temperature-responsive cell culture dish for the creation of transplantable hepatocyte sheets. Transplantation of hepatocyte sheets were able to be engrafted in a pre-vascularized subcutaneous site, resulting in long-term secretion of hepatocyte-specific enzyme. For effective engraftment of transplanted tissues, we focused on the fabrication of hepatocyte sheet tissues secreting angiogenic factors using non-viral transfection of pDNA or mRNA encoding VEGF. Transfected hepatocytes with VEGF mRNA rapidly secreted VEGF within 24 hours, resulting in the cumulative VEGF secretion of ca. 140 ng. By contrast, the transfection of VEFG pDNA exhibited much lower secretion of VEGF than that of mRNA. However, the hepatocytes transfected with VEGF pDNA continuously secreted during 7 days of culture and the cumulative secretion of VEGF was reached to ca. 3 mg. Thus, rapid expression of angiogenic factors from non-proliferative hepatocytes can be achieved by using mRNA transfection. A combination of hepatocyte sheet formation using temperature-responsive cell culture surface and mRNA delivery technology is considered to have a potential to provide the engraftment of transplanted liver tissues with maintaining hepatic functions.

Funding Source
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Keywords: Hepatocyte, Gene delivery, Angiogenic factor

Poster Abstracts

509

TISSUE-ENGINEERING OF AN ESOPHAGUS: SCALABLE PROTOCOL FOR REPOPULATING DECELLULARIZED ESOPHAGUS FROM LARGE ANIMALS WITH MUSCLE PROGENITOR CELLS

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Esophagus is a vital organ for the human digestive system, loss of which results in inability to swallow foods. Besides esophageal cancer, which is the commonest cause of losing the oesophagus in adults, long-gap oesophageal atresia represents a clinical need of an innovative strategy to restore the loss of the continuity due to maldevelopment of oesophagus. Aiming to generate a biocompatible graft that can substitute the gap in the oesophagus, we sought to establish an optimum protocol for repopulating decellularised porcine esophagi with muscle progenitor cells. The decellularisation protocol we have established for rodent oesophagi was adapted and modified for scaling up the model. Optimization of the protocol and the bioreactor enabled derivation of extracellular matrix (ECM) scaffolds from various size of pigs (3kg, 10kg, and 25kg), suggesting its applicability to patients of a broad range of ages. Efficient removal of native cells and preservation of ECM microstructure were confirmed by qualitative and quantitative assessment of the scaffolds. Mesoangioblasts (MABs) derived from skeletal muscle biopsies of either humans or pigs are used for giving rise to smooth muscle layer in the scaffolds. Expanded cells were micro-injected into the scaffolds, which was subsequently maintained in the custom designed bioreactor. Based on the findings in the rodent experiments, fibroblasts (FBs) are co-seeded with MABs, with expectation of promoting cell migration. Following the three-day period in proliferation media, smooth muscle differentiation was induced by the differentiation media including TGF-BETA. After incubation for 12 days in total, histology showed migration of cells from injection points along the ECM. Positive expressions of Ki67 and smooth muscle marker proteins in the MABs were indicative of proliferation and early differentiation to smooth muscle cells. Our protocol demonstrated migration and differentiation of muscle progenitor cells in three-dimensional ECM scaffolds, suggesting the potential of tissue-engineering approach to generate functional esophageal grafts comprised of smooth muscle layer, which can be advanced to preclinical in vivo experiments in large animals and eventually translatable to human clinical practice.

Funding Source
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Keywords: Esophageal tissue-engineering, Clinical translation, Decellularization

510

PARACRINE SIGNALING OF HEPATOCYTE GROWTH FACTOR FROM CELL SHEET REDUCES RENAL FIBROSIS IN RAT ISCHEMIA-REPERFUSION INJURY

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Ischemia-reperfusion injury (IRI) is a disease model for both acute kidney injury and chronic kidney disease, which are clinically essential problems to be resolved. We reported that transplantation of hepatocyte growth factor (HGF) -producing cell sheets under the renal capsule improved renal IRI in rats from the acute phase to the chronic phase. However, the mechanism was unknown. Therefore, we evaluated differences in tubular epithelial cell apoptosis after IRI with or without transplantation of HGF-producing cell sheets. On the 7th day after right nephrectomy in a nude rat, two HGF transgenic human mesothelial cells (HGF-tg MC) sheets were transplanted under the left renal capsule. After ischemia with clamped for 60 minutes of renal arteries and veins, reperfusion was performed. The left kidney was collected on days 2, 14, and 28 after IRI. Apoptosis of tubular epithelial cells was evaluated by performing Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining on renal paraffin-embedded sections. HGF-tg MC sheets group was compared to other groups; sham surgery (sham); IRI with no treatment (NT); IRI and intravenous administration of recombinant human HGF protein (IV HGF); or IRI with non-transgenic MC sheet transplanted under the renal capsule (MC sheet). TUNEL staining-positive cells were significantly suppressed in the HGF-tg MC and MC sheet group compared to the NT and IV HGF groups. Transplantation of subcapsular HGF-producing cell sheets may reduce tubular epithelial cell apoptosis. Considering that HGF-producing mesenchymal stem cell sheets ameliorate the diminish of reno-microvasculature after IRI, reducing apoptosis in epithelial cells may also associate with reducing renal fibrosis after IRI.

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Keywords: Cell sheet, Hepatocyte growth factor, Ischemia-reperfusion injury

511

TOPICAL APPLICATION OF GELATIN HYDROGEL MICROSPHERES INCORPORATING CISPLATIN FOR BONE METASTASIS ENHANCED ANTI-TUMOR EFFECTS WITH LESS SIDE EFFECTS

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Management of bone metastasis is essential to maintain the patients´ quality of life. Thus, it is highly desirable to develop a more effective and safer local treatment for bone metastasis. The purpose of this study was to investigate the anti-tumor effects and safety of gelatin hydrogel microspheres incorporating cisplatin (GM-CDDP), which we developed as a sustained release system. First, we assessed GM-CDDP for its in vitro degradability and potential for sustained release. Initial bursts were observed within 2 h and CDDP was released gradually with gelatin hydrogel degradation. Second, in vivo anti-tumor and side effects were evaluated using a mirine bone metastasis model of MDA-MB-231 human breast cancer cells incorporating GFP. All mice were assigned to the following four groups: local administration of GM; intraperitoneal injection of free CDDP solution; local administration of free CDDP solution; local administration of GM-CDDP (all, 2mg/kg calculated for CDDP). Mice in each group were euthanzied at 2 weeks (n=5) or 4 weeks (n=5) after treatment. The tumor volume was assessed using an in vivo imaging system. The residual bone volume was calculated using a μCT. Local administration of GM-CDDP significantly suppressed tumor growth and bone osteolysis compared with the other groups (all, $p<0.05$). Histological analysis revealed significantly increased apoptosis with GM-CDDP than the others, suggesting greater delivery and longer exposure of CDDP induced tumor cells apoptosis. Also, local administration of GM-CDDP significantly reduced loss of body weight and elevation of blood urea nitrogen compared with the intraperitoneal injection group ($p<0.05$). Furthermore, topical safety for nervous system was confirmed by von-Frey test using a rat laminectomy model. In conclusion, this study suggested that local administration of GM-CDDP can achieve higher anti-tumor effects and lesser side effects compared to the control and local or systemic administration of CDDP.

Funding Source

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Keywords: Bone metastasis, Drug delivery system, Gelatin hydrogel

512

EXPLOITING THE ROLE OF METABOLIC CUES DURING THE CONVERSION OF MOUSE FIBROBLASTS INTO INDUCED CARDIOMYOCYTES

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Cardiovascular diseases (CVD) are the leading cause of mortality in developed countries. CVD pathologies are typically characterized by the loss of cardiomyocytes which leads to heart failure. Whereas following injury the capacity for regeneration of adult mammalian heart is limited, the neonatal heart is capable of substantial regeneration, but this capacity is lost at postnatal stages. Interestingly, this is accompanied by a shift in the metabolic pathways and energetic fuels preferentially used by cardiomyocytes from embryonic glucose-driven anaerobic glycolysis to adult oxidation of substrates in the mitochondria.Cardiac fibroblasts (CFs) contribute to scar formation after cardiac injury in the human heart. The use of direct reprogramming of resident CFs by cardiogenic transcription factors (TFs) into induced cardiac-like myocytes (iCLMs) has emerged as an attractive strategy. In this approach, a specific combination of TFs, Mef2c, Gata4, and Tbx5 (MGT), can create functional beating cardiomyocytes directly from mouse postnatal fibroblasts in vitro and in vivo. In direct cardiac conversion, several epigenetic barriers need to be overcame to allow the switch from fibroblast to cardiac transcriptional program with, for instance, reduction of H3K27me3 marks in MGT-induced cardiomyocytes.Metabolites are key players in genetic and epigenetic expression programs, as the regulation of histone marks by serving as substrates or cofactors for chromatin-modifying enzymes with, for instance, acetylation and demethylation of histones relying in the availability of acetyl-CoA and the Krebs cycle-derived a-ketoglutarate, respectively. Importantly, lipids and glucose are important sources of acetyl-CoA and have been implicated in histone acetylation landscape transitions driving skeletal muscle stem cell fate and regeneration. Here we intend to explore the impact of age and nutritional availability in the transitions driving transdifferentiation of fibroblasts into iCLMs in vitro. Our preliminary data suggests metabolic cues in the retroviral-induced MGT transduction of MEFs, raising the possibility of metabolite-driven conversion of MEFs into iCLMs.

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Keywords: Cardiac transdifferentiation, Mef2c, Gata4, and Tbx5 (MGT), Metabolism

Poster Abstracts

513

MICROFLUIDIC-GENERATED COLLAGEN MICROSPHERE FOR CONTROLLED FORMATION OF EMBRYOID BODY AND ENHANCED HEPATIC DIFFERENTIATION OF STEM CELLS

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End-stage liver failure is associated with high rates of morbidity and mortality and liver transplantation is the only effective treatment currently. To expand the source of available hepatocytes, stem cell-derived hepatocyte-like cells (HLC) has been generated by researchers. Nevertheless, traditional 2D differentiation methods produce HLC similar to fetal hepatocytes rather than mature hepatocytes. Therefore, promoting functional maturation of HLC is necessary. Some studies have reported that 3D differentiation such as using hydrogel or embryoid body could promote maturation of differentiated HLC. However, there are some challenges associated with 3D culture, such as insufficient oxygen and nutrient supply due to the large size of hydrogel and embryonic bodies (EB), non-uniform size and distributions of EB etc. Microfluidics technique offers high-throughput generation of size-controllable microspheres. Here, we utilized microfluidics technique to encapsulate embryonic stem cells (ESC) into collagen microspheres for 3D differentiation. Our results suggested uniform-sized EB were formed after ESC were encapsulated in collagen microspheres that were later differentiated to HLC with high efficiency. Compared with 3D encapsulation in a bulk collagen hydrogel and 2D differentiation, our approach resulted in HLC with higher expression of mature hepatocyte markers such as ALB and lower expression of hepatoblast marker such as AFP. The HLC were also functional by secreting albumin and urea, as well as storing glycogen. Implantation of the HLC into mice liver demonstrated integration with native tissue after 4 weeks. Our studies on microfluidic-assisted stem cell differentiation can provide relatively mature HLC in a scalable manner and facilitate the development of liver tissue engineering and regenerative medicine.

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Keywords: Liver tissue engineering, Microfluidics, 3D culture

514

TRANSPLANTATION OF COMBINED MUSCLE CELL-DERIVED EXTRACELLULAR VESICLES AND GELATIN HYDROGELIMPROVES BONE REPAIR DELAYED IN DIABETIC MICE

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Extracellular vesicles (EVs) play a vital role in physiological and pathophysiological processes. Recent studies suggested that muscle and bone interact mechanically and functionally, and muscle and bone are remotely linked to each other via humoral factors. We recently reported that EVs from muscle cells (Myo-EVs) suppress osteoclast formation. We therefore examined the effects of transplantation of Myo-EVs soaked gelatin hydrogel sheets on the bone repair in diabetic mice. Gelatin hydrogel sheets were used for controlled release of Myo-EV at bone defect site. Myo-EVs were isolated from the conditioned medium of C2C12 cells by ultracentrifugation. Diabetes was induced in female C57BL/6J mice (8 weeks-old) by intraperitoneal injections of streptozotosin (STZ).
Mice with blood glucose levels greater than 300 mg/dl were considered diabetic. At 2 weeks after induction of diabetes, a bone defect surgery was performed in the right femurs of the mice. Myo-EVs or saline was dropped onto freeze-dried gelatin hydrogel sheets, and the sheets was placed on the bone defect. The femurs were scanned using an X-ray CT system. Immunostaining of section of the femurs were performed against Osterix and F4/80 for detecting osteoblasts and macrophages. Total RNA was extracted from the bone tissue around the defect and real-time PCR was performed. Bone repair was delayed by STZ-treatment, and Myo-EV enhanced bone repair in diabetic mice on day 9 after surgery. The number of Osterix and F4/80-positive cells at the damaged site on day 4 was decreased by STZ-treatment, and was enhanced by Myo-EV transplantation. The mRNA level of osteocalcin, bone formation marker, of the bone tissue on day 4 was decreased by STZ-treatment, and was enhanced by Myo-EV transplantation. Taken together, Myo-EVs might improve bone repair delayed by diabetic state by inducing osteoblasts and macrophages at the bone defect site.

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Keywords: Extracellular vesicles, Gelatin hydrogel, Muscle-bone interaction

515

FABRICATION OF FUNCTIONAL VASCULATURE IN HUMAN RENAL ORGANOID IN VITRO

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Kidney is an important organ for maintaining homeostasis, especially blood condition in the body. No directly treatment was established for kidney disease leaded by diabetes, hyper pressure and congenital genetic disease. Rodent models were useful for estimation of novel renal disease treatment. To confirm findings of xenogenic model results, in vitro human renal disease model from hES and hiPS cells also help us for well understand of molecular mechanism of treatment. For examination renal function with the organoid in vitro, perfusion systems have been reported for renal organoid cultivation. Since, there is a problem that appropriate perfusion volume was differ in renal tubules from in blood vasculature, a strategy to overcome this issue will be perfusion via vasculature in renal organoid in vitro. Recently, three-dimensional tissue perfusion system via vasculature in vitro was reported with biomaterials and decellularization technique, often perfused through HUVEC network structure. However, it was not clear the connectability between renal vasculature plexus of renal organoid and artificial HUVEC networks in vitro. In this study, with auto-vascularized renal organoid was induced from human iPS cells, it was co-cultured with network forming HUVEC for building up in vitro functional renal organoid model with vasculature perfusion. HUVEC networks with cell sheets were co-culture with renal organoid in static culture and perfusion culture. The results showed HUVEC network decreased after co-cultivation. The network structure was retained by perfusion cultivation. It was indicated that perfusion stress suppressed the diminish of vasculature networks in renal organoid and HUVEC co-culture structure in vitro. In future, urine producible renal organoid will establish in vitro with vasculature perfusable cultivation.

Funding Source

Grants-in-Aid for Scientific Research

Keywords: organoid, Functional vascularization, in vitro

516

SUPRAMOLECULAR HYDROGELS FOR IPSC CULTURE AND DIFFERENTIATION TOWARDS KIDNEY ORGANOIDS

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In most studies induced pluripotent stem cells (iPSCs) are cultured on polystyrene surfaces coated with Matrigel or other ECM proteins for the differentiation towards kidney organoids. Interestingly, recently it has been shown that surface stiffness influences the differentiation and eventually kidney organoid formation. Supramolecular biomaterials can be used to exactly control the bioactivity and stiffness of a material, offering an interesting platform for the culture and differentiation of iPSCs towards kidney organoids. In this study, the culture of iPSCs on supramolecular ureido-pyrimidinone (UPy) modified poly(ethylene glycol (PEG) based hydrogels was investigated, using the full potential of non-covalent interactions to introduce bioactivity and to change the mechanical properties. First, the bioactivity was introduced via adding supramolecular bioactive cues, such as the Arg-Gly-Asp (RGD) peptide sequence, to the pristine hydrogel, and via mixing of the pristine hydrogel with Matrigel. As controls, coatings and bulk hydrogels of Matrigel were used, resulting in monolayers and hollow spheroids, respectively, after 3 days. On the pristine supramolecular hydrogel a high concentration of Matrigel was required for iPSC adhesion, survival, and SOX2/OCT4 maintenance. The next step is the introduction of synthetic additive analogues of Matrigel. Next, the mechanical properties of the supramolecular hydrogels were tuned using different wt% of the hydrogels. While on Matrigel gels the cells do not spread but instead form hollow spheroids, the cells attach and spread on the lower wt% UPy-gels with Matrigel as bioactive cue. After 3 days the cells formed large clusters, both attaching to the gel and forming spheroids. On higher wt% UPy-gels, iPSCs either die or form cell clusters. It is hypothesized that the preference for lower wt% gels could either originate from a lower stiffness or from a lower PEG-concentration in the gels. In conclusion, iPSCs cultured on top of our UPy-hydrogels require a material with a high concentration of bioactive cues. Next, the search for different bioactive cues will be continued, focusing on natural proteins and combinations of synthetic peptides. In addition, also the culture of differentiated cells (e.g. nephron progenitor cells) on these materials will be examined.

Funding Source

NWO

Keywords: iPSC, biomaterials, kidney organoids

Poster Abstracts

517

THE COMBINED THERAPY OF PRP AND BASIC FGF USING GELATIN HYDROGEL SHEET FOR ROTATOR CUFF HEALING IN RAT MODEL

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Retears have been a common complication after surgical repair of large and massive rotator cuff tears. It was reported that basic fibroblast growth factor (b-FGF) and platelet rich plasma (PRP) was enhanced rotator cuff tear healing previously. The purpose of this study was to evaluate the efficacy of PRP and b-FGF using gelatin hydrogel sheet (GHS) which can preserve and release sustainably until degradation at the early healing period of rotator cuff tear using a rat model. To create a rotator cuff defect, the infraspinatus tendon of rat was resected from the greater tuberosity. Infraspinatus tendons were repaired and covered with GHS impregnated with PBS, b-FGF, PRP, and b-FGF+PRP. Histological examinations and mechanical examinations were performed. At 2 weeks, the tendon maturing scores using hematoxylin eosin staining of b-FGF+PRP group was statistically higher than other groups. In the safranin O staining, strong proteoglycan staining was observed at enthesis in b-FGF+PRP group compared with other groups. Vascular staining with isolectin B4 in b-FGF+PRP group was higher than in other groups at 2 weeks. The number of Col2-positive cells was significantly greater in b-FGF+PRP group than in other groups. The ultimate failure load of b-FGF+PRP group was significantly higher than control group. In b-FGF and PRP group, tendon maturing and fibrocartilage regeneration at the enthesis was promoted and mechanical strength was higher than in other groups. It was suggested that b-FGF and PRP enhance both tendon and bone-tendon junction healing, and b-FGF and PRP were synergistic.

Keywords: platelet rich plasma, basic fibroblast growth factor, gelatin hydrogel sheet

518

TISSUE ENGINEERING APPLICATIONS OF GELATIN HYDROGEL NONWOVEN FABRICS GENOCEL®

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As a 3D cell culture scaffold, gelatin hydrogel nonwoven fabrics (GHNF) have been designed. Here, some applications of GHNF for tissue engineering are introduced. Currently, hydrogel sponges are often used to allow cells to enhance their ability for wound healing. However, the conventional hydrogel sponges do not always have mechanical strength enough to maintain inner porous structures necessary for cell migration and proliferation. GHNF, prepared by a solution blow of gelatin and the subsequent dehydrothermal crosslinking, have an excellent mechanical property to resolve this issue. When GHNF were transplanted subcutaneously into mice, cells were migrated into the spaces among the gelatin fibers of GHNF. The gelatin fibers were degraded with time while the cell migrated proliferated to form a tissue-like structure. GHNF allowed basic fibroblast growth factor (bFGF) to release in a similar manner to the gelatin hydrogels which had been used in clinical trials. These results indicate that GHNF have a clinical promising and practical potential to accelerate the cell-based tissue regeneration and repairing. Another potential of GHNF is to combine with cell sheets which are used as transplant therapies and tissue models for regenerative therapies and drug discovery research. In the cell sheet technology of tissue engineering, the shrinkage of cell sheets in detachment from the culture dish, remains a problem to be resolved. It has been demonstrated that this shrinkage not only reduces the area of cell sheets available, but also often affects cell functions. Following GHNF fragmented fibers were mixed with cells to form cell sheets, cell sheets incorporating GHNF fibers suppressed the shrinkage of cell sheets detached in a remarked contrast with fibers-free cell sheets. In addition, the cell sheets incorporating gelatin fibers gave cells an aerobic condition which is a better condition to enhance and maintain their functions. The simple incorporation of GHNF fragmented fibers allowed cell sheets to improve the biological conditions of cells. From the experimental results obtained, it is concluded that the GHNF are a promising and useful biomaterial for tissue engineering and regenerative medicine.

Keywords: Gelatin, Wound healing, Cell sheet

519

FABRICATION AND CHARACTERIZATION OF LAMINATED 3D GEL BEAD SCAFFOLD FOR BONE TISSUE ENGINEERING

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In this study, 3D gel beads were prepared via the gelation of sodium alginate (0.5, 1.0, 2.0, 3.0 vol%) and CaCl2 solutions (1.0 mass%). The 3D gel beads were laminated and added to the sodium alginate solution. The shape stability and spherical morphology of the 3D gel beads were optimized by evaluating the effect of varying sodium alginate concentrations. The diameters of the 3D gel beads were measured using Image J, and their sphericities were calculated based on the ratio of their major and minor axes. The results of the bead sizes showed that the diameter of the beads increased with increasing concentration of sodium alginate. The sphericity was also approximately 1.0 when the concentration of sodium alginate increased. These results indicated that the shape stability depended on the concentration of sodium alginate. The 3D gel beads with 2.0 and 3.0 vol% were used to fabricate a laminated 3D gel bead scaffold. The compressive mechanical property and biocompatibility of the laminated 3D gel bead scaffold were optimized by evaluating the effect of varying concentrations on the 3D gel beads and their surrounding alginate solution. The compressive mechanical tests were performed using a conventional mechanical machine, and the load-displacement measurements were recorded on a computer. The compressive elastic moduli were calculated based on the slopes of the initial linear portions of the stress-strain curves. The pre-osteoblastic cell line, MC3T3-E1, was used in this study. The cell proliferation and alkaline phosphatase (ALP) activity were evaluated using a spectrophotometric plate reader. The compressive modulus of the 3.0 vol% 3D gel beads was higher than that of the 2.0 vol% 3D gel beads. The cell proliferation and ALP activity increased with increasing concentrations of the sodium alginate and surrounding alginate solution. In conclusion, the increase in the concentrations of the sodium alginate and surrounding alginate solution improved the shape stability, mechanical properties, cell proliferation, and differentiation of the 3D gel beads.

Funding Source
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Keywords: scaffold, gel beads, tissue engineering

520

ANALYSIS OF RAPID GROWING CHONDROCYTE FOR THE CAPACITY TO REGENERATE CARTILAGE

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Cartilage is a non-vascular tissue that is difficult to repair by itself. In the field of cartilage therapy, Brittberg et al. reported in 1994 the use of autologous chondrocyte implantation (ACI) to treat localized defects in articular cartilage. Thereafter, many studies have since been reported to introduce cartilage regeneration therapies into clinical practice. However, it is still difficult to regenerate homogeneous cartilage equivalent to physiological cartilage because cells with various cartilage matrix-producing abilities exist in the isolated chondrocytes. Therefore, the selection of appropriate cell population is essential for the regeneration of high-quality cartilage. We have previously reported a method to enrich chondrogenic cells according to their proliferation rates. In this study, we optimized the preparative conditions for the rapid cell group which is the rapidly dividing population in the cultured chondrocytes and analyzed the properties of the rapid cell group. Human auricular chondrocytes were fluorescently labeled with CFSE and analyzed by flow cytometry, focusing on cell morphology. The morphological differences between the rapid and slow cell groups were most prominent at 2 days after CFSE labeling, while cells at 4 days were analyzed in the previous study. To optimize the timing of cell collection, the rapid and slow cell groups were harvested according to the fluorescence intensity at 2 days and 4 days. The rapid cell group at 2 days showed the highest proliferation rate. Pellet culture revealed that the rapid cell group produced more GAGs per cell than the slow cell group. To compare the ability for cartilage regeneration in vivo, the cells were seeded on PLLA scaffolds, and transplanted into nude mice. GAG production was greatest in the rapid cell group at 2 days, indicating a high chondrogenic potential. The difference in gene expression between the rapid cell group and the slow cell group at 2 days was analyzed by Affymetrix GeneChip array. The results showed that the rapid cell group had better proliferation and a more undifferentiated cell population. We also found a gene that could be a new marker to enrich chondrocytes with high cartilage matrix production capacity. These results obtained by this study indicated the usefulness of the rapid cell group for cartilage regeneration.

Funding Source
JSPS KAKENHI Grants-in-Aid for Scientific Research (B)

Keywords: chondrocyte, CFSE, flow cytometry

Poster Abstracts

521

PRECLINICAL EXAMINATION OF THE SAFETY AND EFFICACY OF AUTOLOGOUS IRIS PIGMENT EPITHELIAL CELL SHEET IMPLANTATION FOR CHORIORETINAL ATROPHY

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Retinal pigment epithelium (RPE) is essential for maintaining homeostasis of photoreceptor cells. RPE dysfunction causes various chorioretinal diseases including retinitis pigmentosa, age-related macular degeneration, and pathologic myopia. For regenerative medicine to replenish RPE in eyes with chorioretinal atrophy, autologous or allogeneic, somatic or stem cell-derived RPE cells in the form of cell suspension or sheet have been previously tested. The use of iris pigment epithelium (IPE) cells instead of RPE cells can take advantages of autologous, somatic cells. Also we possess our own method to prepare a cell sheet. Here, we report preclinical studies regarding safety and efficacy of autologous IPE cell sheet. To assess tumorigenicity in vitro, a chromosomal aberration test and a colony formation test with a soft agar medium were performed using over-cultured IPE cells. No chromosomal aberration or colony formation was observed. Next, to evaluate the safety and efficacy in vivo, a cell sheet prepared from commercially available human IPE cells was implanted into the subretinal space in nude rats. No death or deteriorated conditions were observed during the observation period. Body weight, general conditions, ophthalmological examination, optical coherence
tomography, and necropsy revealed no remarkable changes associated with the cell sheet. Next, an IPE cell sheet was implanted into the subretinal space in Royal College of Surgeons (RCS) rats with experimental retinal degeneration. A sham surgery was performed in the control group. Eyes were enucleated in weeks 4 and 13 to histologically evaluate the short-term and long-term efficacy of the IPE cell sheet implantation, respectively. The short-term and long-term protection of the outer retinal atrophy was observed. Other additional essential studies to initiate the clinical study were performed, warranting safety and efficacy (data not shown). Finally, a phase I/IIa clinical study of the IPE cell sheet (PBR-001) was initiated in patients with myopic chorioretinal atrophy. As the primary endpoint, the rate (%) of change in the area of chorioretinal atrophy before and after implantation will be calculated and compared to that in the fellow eyes.

Keywords: chorioretinal atrophy, iris pigment epithelial cell sheet transplantation, retinal pigment epithelium

522

IDENTIFICATION OF NEW POLYMER MIXTURE TO ENHANCE THE ENGRAFTMENT EFFICIENCY OF IPS CELL-DERIVED MUSCLE STEM CELL IN THE DIAPHRAGM OF DUCHENNE MUSCULAR DYSTROPHY MODEL MICE

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Duchenne muscular dystrophy (DMD) is an X-linked genetic disease characterized by muscle weakness and atrophy due to mutation of DMD gene which encodes DYSTROPHN. DMD patients need respiratory supports and suffer from cardiomyopathy in early teens. The main causes of death are heart failure, respiratory failure and respiratory infection. There is no efficient treatment of DMD except palliative therapy. Cell transplantation therapy by muscle stem cell (MuSC) is considered as one of the future curative therapy for DMD. We have been investigating to develop the cell transplantation therapy by iPS cell-derived muscle stem cells (iMuSCs). Since it was reported that MuSC cannot pass through a vessel wall, direct injection into diseased muscle is necessary to engraft instead of intra-arterial transplantation. Therefore, direct injection into the diaphragm, which is main respiratory muscle, is necessary to develop an effective therapeutic approach for respiratory disfunction of DMD. This study aims to develop the strategy of iMuSC transplantation into the diaphragm. The stereomicroscopy and tiny needle enable us to inject cells into the diaphragm of NOG-mdx mice, which is an immunodeficient DMD model mice. Using mouse primary satellite cells, direct injection into the diaphragm was feasible and the engrafted satellite cells regenerated skeletal muscle of NOG-mdx mice. Next, we tried to apply the transplantation protocol to iMuSC, however, iMuSC transplantation into the diaphragm was failure. We hypothesized that the transplanted cells were washed out due to the rapid movement of the diaphragm. To retain the transplanted cell in the injection site, some polymer mixtures were tested as cell suspension reagent. Hu5/KD3, an immortalized human myoblast, was mixed with some polymer mixtures and transplanted into the hindlimb muscle to validate the engraftment efficiency. Some polymer mixtures increased the engraftment efficiency of Hu5/KD3 significantly. Moreover, one of the polymer mixtures increased the efficiency of iMuSCs transplantation into the diaphragm significantly. Our finding will promote to establish cell transplantation therapy into the diaphragm for ameliorating respiratory function of DMD.

Keywords: Duchenne muscular dystrophy, Cell transplantation therapy, Polymer mixture

523

APPLICATION OF SILK-ELASTIN AS INJECTABLE HYDROGEL FOR CELL TRANSPLANTATION

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Silk-elastin is a genetically designed de novo protein that consists of tandem repeats of silk fibroin polypeptide (GAGAGS) and elastin-like polypeptide (GVGVP) block. This de novo protein exhibits a characteristic of irreversible sol-to-gel transition which is accelerated to complete within several hours at body temperature. It also shows a high biocompatibility and the elasticity of elastin-derived properties, and the mechanical and tensile strength of silk fibroin-derived properties. It is demonstrated that the silk-elastin solution and the sponge-shaped silk-elastin material can be applied to promote wound healing. We have focused on the high biocompatibility and irreversible sol-to-gel transition property of silk-elastin for another application, which is the silk-elastin-based injectable hydrogel. Considering the nature of silk-elastin solution, it is expected to construct the in situ gelation material at the injected site, which can be applied to the cell transplantation of regenerative therapy. The gelation ability of silk-elastin solution was examined by the incubation of solution at 37 °C. At a higher concentration than 12.5 wt%, the silk-elastin solution was naturally solidified to form the hydrogel within 3 h. The cell viability of MC3T3-E1 cells incorporated in the silk-elastin hydrogel was observed by changing the concentration of the silk-elastin solution used. At higher concentrations, the cell viability after 1 day decreased to be around 50%, whereas the viability of cells incorporated in 12.5 wt% of silk-elastin hydrogel showed about 70%. From these results, it is concluded that the silk-elastin hydrogel formed at a suitable concentration is promising as an injectable biomaterial for the cell encapsulation of regenerative therapy.

Keywords: Silk-elastin, Injectable hydrogel, Cell transplantation

Gene Therapy and Genome Editing

602

DIRECT IN VIVO REPROGRAMMING OF FIBROBLASTS INTO CARDIOMYOCYTES AS A THERAPEUTIC STRATEGY FOR HEART FAILURE

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Cardiovascular diseases remain a leading cause of death worldwide, with the number of heart failure patients increasing rapidly in aging societies. Myocardial cells damaged by myocardial infarction (MI) are replaced by mature scar tissue by activated fibroblasts, and the heart with reduced contractility becomes heart failure in the chronic phase. Direct cardiac reprogramming holds great promise for regenerative medicine. Resident cardiac fibroblasts (CFs) can be directly reprogrammed into cardiomyocyte-like cells (iCMs) by overexpression of cardiac transcription factors in infarcted hearts; however, all in vivo studies were performed in acute MI models with gene delivery, and it remains undetermined whether in vivo reprogramming is effective in heart failure. Here, we generated transgenic mice to address this issue. We found that in vivo reprogramming resulted in the conversion of CFs to cardiomyocytes in heart failure, improved cardiac function and reduced mature scar area. Single-cell analysis revealed that extracellular matrix (ECM)-related genes and TGFBETA signaling-related genes were repressed in reprogrammed CFs, while the expression of ECM-degradation-related genes was induced. Thus, in vivo cardiac reprogramming improves heart failure by converting resident CFs into iCMs and by anti-fibrotic effects.

Funding Source

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Keywords: Direct reprogramming, Fibroblast, Heart failure

Poster Abstracts

603

DEVELOPMENT OF GENOME EDITING THERAPY FOR DUCHENNE MUSCULAR DYSTROPHY UTILIZING IPS CELL MODEL

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Duchenne Muscular Dystrophy (DMD) is one of the most common and severe inherited neuromuscular disease caused by the loss-of-function mutations in the Dystrophin gene on X chromosome. Currently, no effective cure available for DMD, including gene augmentation therapy, since large size of the Dystrophin gene hamper the delivery by viral vectors. Exon skipping to modulate mRNA splicing patterns using antisense oligonucleotide drug is a promising approach, however, the effect of antisense oligos is transient.Recent progress on targeted gene editing by engineered nucleases, such as CRISPR-Cas9, have evolutionally broaden our ability to precisely modify the genomic sequence at desired locus, including genetic correction of Dystrophin gene. However, in vivo delivery of CRISPR-Cas9 is a major challenge for successful genome editing therapy, as most widely used AAV vector has some drawbacks, such as risk of genomic integration and immunological response. To test the effectiveness of novel non-viral CRISPR-Cas9 delivery system, we took advantages of patient-derived induced pluripotent stem cells (iPSCs) as a platform for testing various gene correction approaches. We derived xeno-free, feeder-free, and integration-free iPS cell lines from DMD patients with a mutation in the Dystrophin gene. After differentiation into skeletal muscle by MYOD1 over-expression, we investigated the efficiencies of genome editing, exon skipping, and Dystrophin protein recovery induced by virus-like particle or lipid nanoparticle delivery of Cas9/gRNA. In addition, the delivery efficiency was further assessed by a humanized model mouse of DMD. We demonstrate that, despite of its transient nature of Cas9/gRNA expression, single injection was sufficient to induce long-term (several months or more) restoration of the Dystrophin protein in our mouse model. I would like to share our latest progress on CRISPR-Cas9 delivery technology that hold a great promise for future genome editing therapy of DMD.

Funding Source
T-CiRA, AMED
Keywords: CRISPR-Cas9, delivery, Muscular dystrophy

605

PUBLIC INPUT ON HHGE: THE ELSI DIVIDE

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The International Commission on the Clinical Use of Heritable Human Genome Editing (HHGE) was convened in response to rogue genomic editing of human embryos. The final report referenced a Call for Public Input survey on HHGE's technological and ethical implications, but findings were not disclosed. A Freedom of Information request yielded 61 survey respondents who were categorized as Affiliated with the HHGE Industry (AFF; n=23), Not Affiliated (NAFF; n=31), or Unknown (n=7). Our analysis focuses on questions dealing with ethical, legal, and societal implications (ELSI) of HHGE. Collectively, respondents replied to ELSI questions more frequently than technical ones (72.8% v 31.4%), with NAFF respondents answering more often than AFF respondents (81.7% v 58.0%). In a catch-all 'other comment' question, ELSI concerns were cited nine times more often than technical ones, with 'need for broad societal input' cited most frequently (27.4%). Regarding 'evidence needed to proceed to first in human use,' respondents overall cited Safety (43.1%), Efficacy (25.7%), and ELSI Concerns (24.8%) as critical, but NAFF respondents cited ELSI Concerns three times more frequently than AFF respondents. Concerns with informed consent (IC) included it being Highly Problematic/Impossible (37.8%), being linked to Other ELSI Issues (25.7%), and having Long-Term Monitoring problems (23.0%). NAFF respondents cited more IC concerns than AFF respondents (71.4% v 17.9%). HHGE Governance was cited as being Extremely Important (48.8%), requiring Broad Societal Input (26.8%) and Sanction Ability (22.2%); affiliation was not a factor. This previously unpublished survey data, although small in dataset size, indicates societal understanding of the importance of ELSI issues in HHGE. An 'ELSI Divide,' however, was evident between stakeholders and non-stakeholders, and a case is made for meaningful inclusion of non-stakeholders in HHGE oversight, ensuring that ELSI concerns are robustly represented.

Keywords: Ethics, Social Considerations, Governance

606

MIR-92A-3P INSPIRED SHRNA EXBITS PRO-CHONDROGENIC EFFECTS IN ASCS AND CHONDROCYTE PROTECTIVE EFFECTS IN OA CHONDROCYTES

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Osteoarthritis is a disabling disease that severely compromises patient's labor force and normal life. To alleviate OA progression, stem cell therapy as well as RNAi therapy, an emerging and promising technique, has been widely researched in recent years, and among numerous choices, miR-92a-3p was reported to be pro-chondrogenic and anti-inflammative in chondrogenic BMSCs. Here in our study, to figure out whether miR-92a-3p exhibits pro-chondrogenic and chondrocyte protective effects in adipose-derived stem cells (ASCs) and OA chondrocytes respectively, and to evaluate the possibility for future use in human patients, miR-92a-3p artificial mimic were transfected into ASCs, however we found miR-92a-3p mimic expression level as well as Col-2 expression level firstly rose and then dropped rapidly over time. Due to the cluster structure of miR-25-3p/32-5p/92-3p/363-3p/367-3p and to avoid severe off-target effects, a design using sh-RNA structure was used in order to observe long-term effects of mature miR-92a-3p sequence. In our study we found sh-92a-3p based on miR-92a-3p increased Col-2 expression level in ASCs in normal 2D culture, and in OA chondrocytes, sh-92a-3p decreased the dropping speed of Col-2, Sox-9, COMP and ACAN expression. In vivo experiments, we found sh-92a-3p treated ASCs intra-articular injections alleviated cartilage damage more than ASCs intra-articular injections alone in surgically induced OA mice. Also this effects caused by sh-92a-3p can be transferred from ASCs to OA chondrocytes in a trans-well system, indicating exosomes, as a stable carriers of siRNA, might mediate the transportation of small RNAs from ASCs to OA chondrocytes. Our study provided an atypical thought in shRNA/siRNA designs, and in our study sh-92a-3p showed a longer cartilage and chondrocyte protective effects than miR-92a-3p mimic.

Funding Source

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Keywords: Osteoarthritis, miR-92a-3p, Adipose-derived stem cells

607

EFFICIENT AND SCALABLE HUMAN IPS CELL ENGINEERING BY ELECTROPORATION OF LARGE DNA PLASMIDS, MESSENGER RNA OR CRISPR-CAS9 RIBONUCLEOPROTEIN COMPLEXES

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Human induced pluripotent stem cells (iPSCs) have tremendous promise for basic research and clinical application. To maximize their potential, iPSCs can be engineered using a variety of molecular tools such as large DNA plasmids, mRNA, and protein for cellular differentiation, disease modeling, drug screening, and generation of universal donor cells. However, delivering these molecular tools with high efficiency into iPSCs is challenging, which results in laborious and time-consuming screening of clones of interest. Here, using MaxCyte's scalable ExPERT STx electroporation platform, we show large DNA plasmids (> 9 kb), a 10 kb Cas9-GFP plasmid and mRNA were delivered with over 90% efficiency into human iPSCs. Furthermore, using a GFP iPSC reporter line, electroporation of the CRISPR-Cas9 ribonucleoprotein complex and ssODN resulted in homology directed repair to convert GFP to BFP with greater than 40% efficiency. Altogether, these results demonstrate that electroporation is a versatile approach for achieving highly efficient delivery of large DNA plasmids, mRNA, and ribonucleoprotein complexes into iPSCs.

Funding Source

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Keywords: genome editing, electroporation, iPSC

Poster Abstracts

Stem Cell-Based Therapies

702

EXPANDABLE SENDAI-VIRUS-REPROGRAMMED HUMAN IPSC- NEURONAL PRECURSORS: IN VIVO POST-GRAFTING SAFETY CHARACTERIZATION IN RATS AND ADULT PIG

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One of the challenges in clinical translation of cell-replacement therapies is the definition of optimal cell generation and storage/recovery protocols which would permit a rapid preparation of cell-treatment products for patient administration. Besides, the availability of injection devices that are simple to use is critical for potential future dissemination of any spinally-targeted cell-replacement therapy into general medical practice. Here, we report on the use of manual selection protocol for generating expandable, and stable human NPCs from induced pluripotent stem cells. Established NPCs showed normal karyotype, expression of typical NPCs markers at the proliferative stage, and ability to generate functional, calcium oscillating GABAergic or glutamatergic neurons after in vitro differentiation. Grafted NPCs into the striatum or spinal cord of immunodeficient rats showed progressive maturation and expression of early and late human-specific neuronal and glial markers at 2 or 6 months post-grafting. No tumor formation was seen in NPCs-grafted brain or spinal cord samples. Moreover, we compared the engraftment properties of established human induced pluripotent stem cells (hiPSCs)-derived neural precursors (NPCs) cell line once cells were harvested fresh from the cell culture or previously frozen and then grafted into striata or spinal cord of the immunodeficient rat. A newly developed human spinal injection device equipped with a spinal cord pulsation-cancelation magnetic needle was also tested for its safety in an adult immunosuppressed pig. Previously frozen NPCs showed similar post-grafting survival and differentiation profile as was seen for freshly-harvested cells. Testing of human injection device showed acceptable safety with no detectable surgical procedure or spinal NPCs injection-related side effects.

Keywords: human induced pluripotent stem cells, Neural Stem Cells, Spinal Cord Injury

703

COMPETENCY OF IPSC-DERIVED RETINAS IN MHC-MISMATCHED TRANSPLANTATION

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The use of ESC/iPSC-derived retinal organoids (ESC/iPSC-retinas) in transplantation, for its multiple advantages including the low immunogenicity in vitro we recently reported, is a promising approach to restore visual function in diseases associated with photoreceptor degeneration. As it is essential to reduce the risk of immune rejection for a successful cell therapy in regenerative medicine, we further investigated the tolerance of the use of Major Histocompatibility Complex (MHC)-mismatched ESC/iPSC-retinas in allogeneic transplantation. We first comparatively observed the survival and maturation of macaque monkey iPSC-retinas transplanted subretinally into laser-induced retinal degeneration models of MHC-matched and MHC-mismatched macaque monkeys without systemic immunosuppression. No evident clinical signs of rejection were observed by OCT and fundus examination, however, mild rejection response in three of four MHC-mismatched monkeys were identified in co-culture test of recipient peripheral blood mononuclear cells (PBMC) and iPSC-retinas. The grafts survived and matured with retinal cell marker expressions in both MHC-matched and mismatched transplantation despite moderate infiltration of CD3 positive lymphocytes in the choroid near the graft in three of four MHC-mismatched monkeys. We then examined the effect of MHC matching on the functional integration of mouse ESC-retinas into the host retinal network using multi-electrode array (MEA) recording for light response. Mouse ESC-retinas (originating from C57BL/6) were transplanted into the end-stage retinal degeneration models (rd1) of C57BL/6 and C3H/Hej strains as MHC-matched and mismatched transplantation, respectively, without systemic immune-suppression. Both groups showed improved light responses in host retinal ganglion cells. These results suggested that the use of MHC-mismatched ESC/iPSC-retinas for transplantation is immunologically tolerable and thus may be applied for clinical studies under strict supervision with careful monitoring and appropriate assessment for the event of rejection.

Funding Source
AMED, DSP
Keywords: ESC/iPSC-retina, rejection, MHC

704

PRE-CLINICAL STUDY OF IPSC-DERIVED CORNEAL ENDOTHELIAL SUBSTITUTE TRANSPLANTATION

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We are currently preparing a clinical study on the use of iPSC-derived corneal endothelial cell substitutes (CLS001) to treat corneal endothelial dysfunction (bullous keratopathy). CLS001 cells are derived from clinical grade (QHJI01s04) iPSCs. Confluent CLS001 cells form a hexagonal monolayer with Na, K-ATPase alpha 1 subunit expression (ATP1A1), tight junctions, N-cadherin adherence junction formation, and nuclear PITX2 expression, all of which are characteristics of corneal endothelial cells. CLS001 cells can be cryopreserved until transplantation. Residual undifferentiated iPSCs was below 0.01%, and both in vitro and in vivo tumorigenesis studies were negative for teratoma formation or de novo tumors due to transformation. Proof-of-concept studies were performed in a monkey bullous keratopathy model, where CLS001-transplanted eyes significantly reduced corneal edema compared to control. We are planning to conduct clinical trials following regulatory approval.

Funding Source
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Keywords: iPS cells, Cell therapy, Cornea

705

SMALL EXTRACELLULAR VESICLES DERIVED FROM INTERFERON-GAMMA PRE-CONDITIONED MESENCHYMAL STROMAL CELLS EFFECTIVELY TREAT LIVER FIBROSIS

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Currently, there are no approved drugs for treating liver fibrosis and promoting regeneration. Therapeutic mechanism of mesenchymal stromal cells (MSCs) for cirrhosis is not fully established and recently, small extracellular vesicles (sEVs) from MSCs are focused attention. Here, we show the therapeutic effect of sEVs derived from interferon-GAMMA (IFN-GAMMA) pre-conditioned MSCs (GAMMA-sEVs) for cirrhosis. The effect of GAMMA-sEVs for macrophage polarization, motility and phagocytosis ability, the proteome analysis of GAMMA-sEVs, the effect of GAMMA-sEVs agianst hepatic stellate cells (HSCs) activation, and the therapeutic effects of MSCs and sEVs with or without IFN-GAMMA stimulation in cirrhosis mouse model using intravital imaging technique and single-cell transcriptome analysis were evaluated. GAMMA-sEVs can effectively induce anti-inflammatory macrophages in vitro and can enhance the motility and phagocytic ability. Proteome analysis of MSC derived sEVs could revealed the attractive proteins including annexin-A1 etc. after IFN-GAMMA stimulation. However, GAMMA-sEVs do not prevent the activation of HSCs cells in vitro suggesting that GAMMA-sEVs do not have the direct inhibition of HSC activation. Furthermore, GAMMA-sEVs could improve the inflammation and fibrosis in cirrhosis mouse at dose dependent manner with collecting the extrahepatic macrophages into the damaged area. Single cell transcriptome analysis revealed the wide range of effects such as induction of anti-inflammatory macrophages and regulatory T cell were observed after GAMMA-sEVs administration. Our results that induction of GAMMA-sEVs by pre-conditioning could change the quality of sEVs and resulted in efficient cirrhosis tissue repair showed new therapeutic insight.

Funding Source
This study was supported by AMED, AMED BINDS, KAKEN and InterStem Co.
Keywords: extracellular vesicle, mesenchymal stem cell, macrophage

706

GENERATION OF HUMAN IPS CELL-DERIVED LEYDIG CELLS

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We have successfully induced testosterone-producing Leydig cells from human iPS cells (hiPSC) by forcibly expressing NR5A1 and embryoid body (EB) formation. However, the amount of the testosterone secreted by the resulting cell population was low, and the differentiation efficiency, changes in morphology, and gene expression pattern of the cells in the differentiation process have been unclear. To improve the Leydig cell induction method, and to clarify the efficiency of differentiation and the differentiation process. We optimized the cell density and duration of embryoid body formation, and the culture conditions in the later stage of differentiation, by assessing the concentration of testosterone in the culture supernatant and the expression of known marker genes. Changes in the morphology of cells during differentiation were visualized using a microscope with time-lapse function. Comprehensive gene expression patterns at several time points during the differentiation process were evaluated by RNA-seq. We have established a new differentiation method resulting in

Poster Abstracts

more than 10-fold higher testosterone secretion than the conventional method. The differentiation efficiency was more than 99%. The morphological changes during the differentiation process into Leydig cells were captured over time, and we found the morphological signatures of the cells at each differentiation stage. We clarified the changes in the gene expression patterns of cells during the differentiation process, and we identified several specific marker genes for each differentiation stage. We successfully improved the Leydig cell differentiation method. The efficiency of differentiation and changes in morphology and gene expression patterns during the differentiation process were clarified. Now we are planning to evaluate in vivo function and safeness of the hiPSC-derived Leydig cells in transplantation experiments using animals.

Keywords: Leydig cell, iPS cells, testosterone

707

CLINICAL TRIAL OF BONE REGENERATION THERAPY TO FRACTURE NON-UNION PATIENTS BY TRANSPLANTATION OF AUTOLOGOUS PERIPHERAL BLOOD CD34-POSITIVE CELLS

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Fracture nonunion is a challenging problem, and development of a new treatment strategy is expected. We previously demonstrated that transplantation of autologous peripheral blood CD34-positive cells led healing of intractable fractures via regeneration of both bone and blood vessels in animal studies. Based on these pre-clinical findings, we have conducted a phase I/II clinical trial: Autologous CD34-positive cell transplantation for bone and vascular healing in patients with nonunion fracture, under the approval of Review Committee for Clinical Research Using Human Stem Cells and supported by a grant of the Coordination, Support and Training Program for Translational Research Program from the MEXT. We have completed the assessment of safety and efficacy of this cell therapy for seven patients. Then we advanced to the next phase clinical trial to lead this cell therapy to an established medicine. This research plan was accepted by the Research Project for Practical Application of Regenerative Medicine of AMED. We conducted the phase III clinical trial as a multi-center investigator initiated clinical trial. Autologous CD34-positive cell transplantation to fifteen patients of tibia nonunion and ten patients of femur nonunion, and one-year follow-up after the cell transplantation was completed. All fractures healed. The main outcome was set as the shortening of the time to heal in tibia nonunion patients. Radiographic assessment by the third party proved that the time to heal was statistically shorter in the CD34-positive cell transplanted patients compared to the historical control. This cell therapy for treating intractable fracture nonunions has no competing therapies. The cell can be harvested less invasively, and this cell therapy accompanies no ethical issues. This project will provide a significant social impact because this clinical trial is highly expected to be approved and develop a novel regeneration therapy first in Japan around the world.

Funding Source
This study was supported by the grant from the Japan Agency for Medical Research and Development (AMED).
Keywords: Fracture nonunion, Bone regeneration, CD34-positive cell

708

DEVELOPMENT OF NON-INVASIVE AND EFFECTIVE PERIPHERAL BLOOD CELL THERAPY FOR VASCULAR AND TISSUE REGENERATIVE THERAPY

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After the discovery of endothelial progenitor cells (EPCs), autologous mononuclear cells (MNCs) have been used for clinical vascular regenerative therapy. However, the efficacy of autologous EPC therapy is limited for diabetic patients because of the deficiency in the number and function of EPCs. To resolve this problem, we have developed serum free ex vivo expansion system called Quantity and Quality Control Culture System (QQc) that could potentiate the vasculogenic property of diabetic EPCs and peripheral blood monocytes and lymphocytes for enhanced vasculogenesis and wound healing. In order to start a clinical trial of cell therapy using QQc cultured peripheral blood MNCs (MNC-QQc) for non-healing diabetic wounds, we underwent the pre-clinical study in vitro and in vivo to evaluate the efficacy of MNC-QQc cells and various safety tests to overcome the Japanese regulations of new regenerative therapy law. With approval from the government, we have started the phase I physician based clinical trial from January 2015 to 2017. In the future, we plan to get pharmaceutical approval as

regenerative therapy product to deliver an outpatient based simple, safe and effective vascular and regenerative therapy for patients with non-healing extremity wounds. To achieve this purpose, we refined the QQc methods and developed RE01 cells. RE01 can be generated more stably overcoming patient variability and have higher vasculogenic potential than MNC-QQ cells. Here, we will introduce challenges and the future of world`s first non-invasive and effective peripheral blood cell therapy for vascular and tissue regenerative therapy with RE01 cells.

Keywords: Peripheral blood MNCs, vasculogenesis, ischemic ulcer

709

CELL SURFACE MARKERS EXPRESSED BY AUTOLOGOUS AND ALLOGENEIC CHONDROCYTE SHEETS

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Chondrocyte sheets fabricated on temperature-responsive culture inserts have the potential to treat cartilage defects associated with osteoarthritis of the knee. To date, clinical trials have been conducted to evaluate the safety and efficacy of both autologous chondrocyte sheets fabricated from patient-derived cartilage and allogeneic chondrocyte sheets fabricated from polydactyly-derived cartilage. More recently, iPS cell-derived hyaline cartilaginous tissue developed at CiRA has attracted attention as a novel cell source. Through xenogeneic transplantation models, we have evaluated the efficacy of autologous and allogeneic chondrocyte sheets and found that efficacy varies with donors and cell sources. To identify efficacy markers for chondrocyte sheets, in this study we performed a comprehensive analysis of cell surface markers expressed by autologous chondrocyte sheets fabricated from patient-derived cartilage and allogeneic chondrocyte sheets fabricated from either polydactyly-derived cartilage or iPS cell-derived cartilage. All three types of sheets met the ISCT's MSC minimum criteria (CD73+/CD90+/CD105+/CD11b-/CD14-/CD19-/CD34-/CD45-/HLA-DR-) and also expressed cell surface markers such as CD13/CD29/CD44/CD46/CD47/CD49c/CD49e/CD55/CD81/CD140b/CD147/CD164 at over 95% positivity. Compared to allogeneic chondrocyte sheets, autologous chondrocyte sheets exhibited significantly higher median fluorescent intensities for cell surface markers such as CD73/CD90/CD105. The expressions of cell surface markers such as CD10/CD26/CD49a/CD49b/CD80/CD106/CD107a/CD107b/CD106/CD201/CD227/GD2/SSEA-4 were variable among the three types of sheets. We identified cell surface markers that vary in expression by cell sources. In the future, we will determine whether these cell surface makers can be used to predict the efficacy of chondrocyte sheets.

Funding Source
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Keywords: osteoarthritis, chondrocyte sheet, cell surface marker

710

THREE-DIMENSIONAL EXPANSION AND IN VIVO TRANSPLANTATION OF LUNG PROGENITORS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS VIA CORE-SHELL HYDROGEL MICROFIBERS

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The lung is an organ that lacks regenerative capacity and there are many intractable diseases such as pulmonary fibrosis and COPD. Therefore, it is important to take a regenerative medicine approach, for which human induced pluripotent stem cell (hiPSC) -derived lung cells are a promising cell source. We have reported that lung progenitors and type II alveolar epithelial cells (AEC2s), which are tissue stem cells of the lung, could be induced from hiPSCs. In the present study, we generated hiPSC-derived lung progenitors (hLPs) as previously reported and injected those hLPs intratracheally into naphthalene- and irradiation-injured immunodeficient mice. After two months, we evaluated the murine lungs by immunofluorescence staining and RNA-seq. Immunofluorescence staining showed a lot of human cytokeratin positive cell clusters in the murine lungs, confirming the engraftment of hiPSC-derived cells, and double-positive cells with human cytokeratin and SFTPC, known as an AEC2 marker, were also observed. RNA-seq analysis of FACS-isolated hiPSC-derived cells displayed the expression

Poster Abstracts

of different respiratory epithelial cell markers, including AEC2 and club cell ones. These results suggested the differentiation of hiPSC-derived cells in vivo into various respiratory epithelial cells, such as alveolar epithelial cells and club cells. Next, we established a three-dimensional expansion culture system of hLPs via core-shell hydrogel microfibers for scalable culture in anticipation of cell therapy. Those expanded hLPs expressed the progenitor markers NKX2.1 and SOX9, and maintained the bipotency for differentiation into alveolar and airway epithelial cells. Finally, we injected hLPs expanded by microfibers into the murine lungs and confirmed engraftment of those human cells after two months. This study supports that our methods have the potential for contributing to regenerative medicine to cure refractory lung diseases.

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Keywords: Pluripotent stem cells, Xenotransplantation, Core-shell hydrogel microfibers

712

HUMAN ADIPOSE-DERIVED STEM CELL TRANSPLANTATION ACCELERATES HEALING OF MURINE ACHILLES TENDON MODEL

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Flexsor tendon injury sometimes results in limited hand functions. For the purpose of evaluating effects of stem cell transplantation including recent transcriptome analyses, murine models have an advantage of genetic engineering among various species. However, because of their smallness, previous reports for studying tendon healing in mice involved sometimes no tendon sutures, and joint fixation has not been performed. On the other hand, mechanical stress is mentioned to have a very important role in tendon healing. So we developed and reported a murine Achilles tendon suture model with ankle fixation (mAT model) which mimicked one of clinical procedures. In this study, human adipose derived stem cells (hASCs) were transplanted to mAT model and the effects were evaluated in vivo. hASCs were transplanted to mAT model in hASC-transplanted group and compared with control. The ankle was fixed in plantarflexed position in our procedure postoperatively. Range of motion (ROM) of plantarflexion of the ankles and usage of the Achilles tendons by their behaviour were recorded and histological and gene expression analyses were performed. The hASC-transplanted group showed significantly larger ROM of plantarflexion of the ankle than control ($p<0.05$). Usage of the Achilles tendon was accelerated in the hASC-transplanted group than in control($p<0.05$). Histological analyses revealed that the hASC-transplanted group showed significantly higher density of collagen fibres ($p<0.05$) and significantly lower area of ectopic chondrogenesis ($p<0.05$). The hASC-transplanted group showed significantly higher expressions of Tgfb1 and Tnc at postoperative day 7 and COL1A1, COL1A2 and COL3A1 at postoperative week 6 compared to the control (each $p<0.05$). Our results suggested that ASCs can ameliorate inflammation in acute phase and cure the tendon tissue better in our mAT model.

Funding Source
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Keywords: tendon regeneration, adipose-derived stem cells, murine Achilles tendon suture model

713

CANINE BONE MARROW MESENCHYMAL STROMAL/STEM CELL DERIVED EXTRACELLULAR VESICLES ENCAPSULATED IN CATIONIC GELATIN HYDROGELS SUPPRESS INFLAMMATION ON MICROGLIAL CELLS

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Bone marrow mesenchymal stem/stromal cells (BM-MSCs) have been used as cell sources for treating dogs with naturally-occurring diseases. Extracellular vesicles (EVs) derived from BM-MSCs are now recognized as pivotal to modulating the immune response and supporting tissue repair. However, maintaining the retention of EVs over time after transplantation is a major challenge in the clinical application of BM-MSC derived EVs. The objective of this study was to investigate whether canine BM-MSC derived EVs encapsulated cationic gelatin hydrogels could increase retention and suppress inflammation on microglial cells. Our results showed that the hydrogel could encapsulate the EVs

effectively and sustain the release of EVs. Released EVs reduced the levels of IL-1BETA by microglial cells in response to LPS stimulation. The strategy used in this study may promote the development for assessing and enhancing the therapeutic effects of BM-MSC derived EVs.

Funding Source
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Keywords: extracellular vesicle, gelatin hydrogel, mesenchymal stem/stromal cell

714

PROFILING AND COMPOUND-BASED REDUCTION OF ACCOMPANIED NON-ENDOCRINE CELLS IN ISLET-LIKE ENDOCRINE CELLS DIFFERENTIATED FROM HUMAN IPSC

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Differentiation method of pancreatic endocrine cells from human pluripotent stem cells has been well investigated for cell therapy against type 1 diabetes. Although non-endocrine cells are inevitable contaminant by-products of the differentiation process, the comprehensive profile of the cell type remains to be further elucidated. Here, we cataloged non-endocrine cells using single cell transcriptomic analysis in induced pluripotent stem cells (iPSC)-derived pancreatic islet cells (iPIC) and the derivatives. We found that non-endocrine cells consisted of heterogeneous proliferating cells and intermediate cells expressing genes in peri-pancreatic tissues including FGB and AGR2 despite the high association with pancreas and pancreatic islet. Likewise, non-endocrine cells expressed FGFR isoforms, PLK1 and LDHB which were not dominant in endocrine cells, so that inhibition of pathways involving these genes by chemical inhibitors reduced non-endocrine cells in the late process of endocrine cell differentiation. These findings provide useful insights into cell purification approach and contribute to the improvement of mass production of endocrine cells for stem cell-derived cell therapy.

Funding Source
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Keywords: Stem cell-derived cell therapy, Pancreatic islet cells, Compound-based purification

715

DEVELOPMENT OF IPSC-BASED CELL THERAPY FOR CKD

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Chronic kidney disease (CKD) causes enormous medical and economical problems worldwide. With few curative treatments except for renal transplantation, which is hampered by serious donor organ shortage, regenerative medicine is being pursued. In order to improve the quality of life (QOL) of CKD patients and reduce the medical costs associated with dialysis therapy, our group is aiming to develop a novel cell therapy that prevents the progression of CKD using embryonic nephron progenitor cells (NPCs) differentiated from human induced pluripotent stem cells (hiPSCs). To prepare hiPSC-derived NPCs, we have established a highly efficient differentiation method using an original 6-step treatment in 2D culture, a purification method using a specific cell surface antigen and an in vitro expansion culture method. We found that the renal subcapsular transplantation of these hiPSC-derived NPCs ameliorates kidney injury both biochemically and histologically in several mouse kidney disease models including an acute kidney injury (AKI) model induced by ischemia reperfusion injury. The transplanted hiPSC-derived NPCs do not migrate into the renal parenchyma of host mice, suggesting paracrine effects by renotrophic factors secreted from the hiPSC-derived NPCs as the main mechanism of the therapeutic effects. We are currently examining the therapeutic potential of hiPSC-derived NPCs in mouse CKD models that enable the long-term evaluation of kidney function. By identifying and analyzing the main responsible factors, we aim to elucidate the detailed mechanisms of the therapeutic effects. We are also developing a dedicated cell infusion device and mass production system for hiPSC-derived NPCs in view of clinical application. In this presentation, we will show our recent results and ongoing works towards the first-in-human clinical trial of iPSC-based cell therapy against CKD.

Poster Abstracts

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Keywords: CKD, iPS cell, Cell therapy

716

TRANSPLANTATION OF THE IPSC-DERIVED CARDIOMYOCYTE PATCH ALTERS ECM COMPOSITION DIRECTLY AFFECTING CARDIAC FIBROBLASTS AFTER MYOCARDIAL INFARCTION

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Acute myocardial infarction (AMI) followed by chronic heart failure is the main cause of mortality of heart diseases. Last decade, reparative cell transplantation therapies, including stem cell and stem cell-derived cardiomyocyte transplantation, are developed to improve heart function after AMI. While the therapeutic effect of transplantation of the stem cells, such as bone marrow cells and cardiac mesenchymal cells, are shown to be presumably due to consequence of an acute sterile immune response, the mode of action of stem cell-derived cardiomyocyte transplantation therapy remains elusive. Human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM) patch is transplanted into the immunodeficient rat after AMI. Then functional recovery of the CM patch transplanted heart compared to control animal was examined. Under the condition, transcriptome analysis of human derived cells and rat derived cells were examined. Moreover, co-culture experiments with hiPSC-CMs and cardiac fibroblasts (CFs) were carried out. Transplantation of the hiPSC-CM patch onto the damaged heart after AMI increased the ratio of collagen type I. Additionally, transcriptome analysis of the hiPSC-CMs and the host rat tissues highlighted that TGF-BETA signaling in the hiPSC-CM patch transplanted heart was increased. Moreover, we confirmed that TGF-BETA1 was secreted from the hiPSC-CM patch. By using the in vitro co-culture system with transwell, we revealed that hiPSC-CMs induced collagen type I production by CFs, resulting in improved alignment of the collagen fibers at the infarcted zone. Furthermore, elasticity of the heart was enhanced in the hiPSC-CMs patch transplanted heart compared to control. Our results suggest the direct effect of iPSC-CM patch on CFs to improve elasticity via modulating collagen type I production in the CFs in the damaged heart after AMI.

Funding Source
Cuorips Inc.
Keywords: hiPSC-derived cardiomyocyte, cardiac fibroblast, TGF-b

717

DRUG DISCOVERY AND DEVELOPMENT FOR THE HEARING LOSS USING PATIENT-DERIVED IPSCS: A PHASE I/IIA CLINICAL TRIAL FOR PENDRED SYNDROME/DFNB4

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Hearing loss is one of the most common sensory impairments. Pendred syndrome/DFNB 4 (PDS) is a disorder with fluctuating and progressive hearing loss, vertigo, and thyroid goiter and the mutations in SLC26A4 gene are typically identified. We found pathophysiology of a neurodegenerative disorder in PDS patient derived cochlear cells that were induced via induced pluripotent stem cells (iPSCs) and found sirolimus, an mTOR inhibitor, as an inhibitor of cell death with the minimum effective concentration less than 1/10 of the approved dose for other diseases. Given that there is no rational standard therapy for PDS, we planned a study to examine effects of low dose oral administration of sirolimus for the fluctuating and progressive hearing loss, and the balance disorder of PDS by daily monitor of their audio-vestibular symptoms. This double-blind, placebo-controlled trial was carried out with 16 of outpatients with fluctuating hearing diagnosed as PDS in SLC26A4 genetic testing aged in between 7 and 50 years old at the time of consent and given either placebo or sirolimus tablet (NPC-12T). In NPC-12T placebo arm, placebo will be given for 36 weeks; In active substance arm, placebo will be given for 12 weeks and the NPC-12T for 24 weeks.

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Keywords: induced pluripotent stem cells, hearing loss, Pendred syndrome

718

PRECLINICAL STUDY OF THE COMBINATION THERAPY OF IPS-NKT CELLS AND ALPHA-GALCER-LOADED ANTIGEN-PRESENTING CELLS

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Activated NKT cells show NK-like cytotoxicity toward cancer cells. Moreover, NKT cells produce abundant cytokines in response to T-cell receptor (TCR) stimulation by recognizing glycolipids such as ALPHA-galactosylceramide (ALPHA-GalCer) presented on CD1d. Because activated NKT cells activate other cytotoxic immune cells, NKT cells play an important role in cancer immunity. However, the number of NKT cells is small in peripheral blood. Then, we developed iPS cell-derived NKT cells (iPS-NKT cells) to overcome the small number of NKT cells for cell therapy. Previous early phase clinical trials showed that the combination therapy with ex vivo expanded NKT cells and ALPHA-GalCer-pulsed antigen-presenting cells had an anti-tumor immune response. Therefore, we performed a preclinical study of the combination therapy with ALPHA-GalCer-loaded dendritic cells (DC/Gal) and iPS-NKT cells instead of autologous NKT cells. To assess the anti-tumor efficacy of the combination therapy, we injected human iPS-NKT cells and mouse DC/Gal intratumorally in patient-derived lung cancer xenograft NSG mice. The combination therapy suppressed tumor size compared with the iPS-NKT monotherapy. Next, we performed a safety assay. No clinical graft-versus-host disease occurred in both the iPS-NKT cell monotherapy and the combination therapy groups. To assess the possibility of cytokine release syndrome, we checked inflammatory cytokine levels of IL-1b and IL-6 in Ja18-KO (NKT deficient) C57BL/6 mice after injected human iPS-NKT cells and mouse DC/Gal intravenously. No statistical difference was observed in the cytokine levels between the iPS-NKT cell monotherapy and the combination therapy groups. In summary, the DC/Gal combination improved the anti-tumor effect of iPS-NKT cells without severe adverse effects in a xenograft model.

Funding Source
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Keywords: iPS cells, NKT cells, cancer immunotherapy

719

POTENT AND LONG-ACTING STEM CELL-BASED THERAPY FOR LIVER CIRRHOSIS

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Liver cirrhosis (LC) is a global health problem since the annual number of mortalities due to LC is approximately 50,000 in the USA, 25,000 in Japan, and more than one million worldwide. Liver transplantation is the only treatment for advanced LC, however donor shortage prevents sufficient liver transplantation. Here, we report the development of novel stem cell-based therapy based on stem cell biology and cell sheet engineering. We previously found that human mesenchymal stem cells (MSCs) can transdifferentiate into hepatocytes by downregulation of Wnt/b-catenin signal. To apply this knowledge to stable and safe treatment, we identified a chemical compound IC-2 suppressing Wnt/b-catenin signal in human MSCs. Temperature-sensitive cell sheet was also used. Briefly, human bone marrow-derived MSCs seeded on temperature-sensitive culture dish were incubated with IC-2 at 37 ºC for 9 days, and then were peeled off as IC-2 sheet at 20 ºC. Anti-fibrotic effects of IC-2 sheet were examined using CCl4-induced liver fibrosis. Eleven weeks after CCl4 administration, BALB/c-nu/nu mice which were equally divided into three groups, received transplantation of three layered IC-2 sheets or MSC sheets on liver surface. Sham-operated mice served as a control. Mice were sacrificed 9 days after transplantation. Azan staining and Sirius red staining showed that IC-2 group exhibited 40 % reduction in liver fibrosis. Surprisingly, 47 % reduction in hydroxyproline content was observed in IC-2 group. To our knowledge, IC-2 sheet has the greatest effect on reduction of liver fibrosis. We also confirm that IC-2 sheets can act at least for 4 months. Liver fibrosis is caused by balance between fibrogenesis and fibrolysis. Expression of a-smooth muscle actin, a marker of hepatic stellate cell activation, was markedly decreased in IC-2 group. As fibrolytic system, MMP-1 and MMP-14 levels were prominently increased in IC-2-treated cells. Notably, MMP-1 and MMP-14 activity in IC-2 sheets are negatively correlated with collagen contents in posttranslanted fibrotic livers. These data suggest IC-2 sheet exerts anti-fibrotic effect via both suppression of fibrogenesis and enhancement of fibrolysis. In conclusion, we developed potent and long-acting stem cell-based therapy for LC.

Funding Source
the project for realization of regenerative medicine from the Ministry of Education, Culture, Sports, Science and Technology in Japan

Keywords: stem cell biology, tissue engineering, liver cirrhosis

Poster Abstracts

720

INTEGRATION OF NEURAL PROGENITOR CELLS INTO MEMORY CIRCUIT DURING SLEEP IN THE ADULT BRAIN

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How neural progenitor cells integrate into the preexisting neural circuit in the adult brain is key for functional brain regeneration. As a model system, we study the adult-neurogenesis in the hippocampal dentate gyrus (DG), where adult-born neurons (ABNs) daily integrate into the memory circuit. Indeed, we showed that a sparse activity of young, but not old, ABNs during REM sleep is necessary for fear memory consolidation 1. The coordination of preexisting and ABN activities during sleep may play key roles in functional integration. During REM sleep, a prominent synchronous neural activity of preexisting neurons (i.e., theta rhythm) appears in the DG2. This theta rhythm coordinates both synaptic plasticity3 and memory consolidation4. Therefore, we examined the functional roles of ABN activity during theta rhythm in REM sleep by a close-feed-back optogenetic intervention. This study provides insight into how neural progenitor cells integrate into functional circuits in the adult brain.

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Keywords: adult-neurogenesis, sleep, memory

721

EFFICIENT INDUCTION AND EXPANSION OF ENDOTHELIAL CELLS INDUCED FROM HUMAN IPSC AND THE DIVERSITY OF THE GROWTH AMONG IPS CELL LINES

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Blood vessel is essential for most of the tissue structure. Endothelial cell (EC) line inner surface of the vessels, and directly interact with substances in the blood. Thus, stable production of human ECs is necessary for both regenerative medicine and disease modeling. Many groups reported EC induction method from human embryonic stem cells (hESs) and human induced pluripotent stem cells (hiPSCs) using cell sorting or genetic modification. Previously, we also reported an efficient EC induction from hiPSCs with endothelial progenitor cell (vascular endothelial growth factor (VEGF) receptor-2-positive cells) purification. High-purity ECs were obtained, however we had two problems, the great many of VEGFR2(+) cell (~80%) were lost by purification, and induced ECs became senescent in 2 weeks.Now, we modified this method to none-cell sorting method, just re-plating VEGFR2(+) cell. High-purity ECs were obtained from 4iPSCs which have some difference with iPSC establishment. Also, we cultured induced ECs in optimized medium, which contain 20% serum, ECs were continuously proliferated. Induction efficiencies of ECs were not largely different among 4 hiPSC lines, properties of ECs, proliferation capacity was different. Whereas ECs derived from 201B6 (EC-201B6) proliferated well in an optimized medium with serum approximately at 8.4 times/5days, other ECs, representatively EC-836B3, proliferated slower than EC-201B6 but continuously at 1.7 times/5days with no senescence for more than 3 months. In general, ECs cannot survive in serum-free condition. However, several proliferating ECs can survive and proliferate in serum-free condition. EC-836B3 were able to be maintained for more than 5 weeks even in the serum-free condition. Whereas EC-201B6 failed to be maintained, resulting in disappearance of cells within 10 days, indicating that serum dependency of growth and survival is much different among ECs from different hiPSC lines. Even among ECs induced with the identical method, properties of ECs are different depending on host hiPSC lines, suggesting that genetic and epigenetic varieties of host hiPSC lines still affect the derivative EC properties.This EC induction and expansion method has great advantage to regenerative medicine which needs many high-purity ECs.

Keywords: endothelial cells, proliferation, efficient induction

722

ROBUST AND SCALABLE TRANSPLANTATION OF HPSC-DERIVED MICROTISSUES COMPOSED OF DOPAMINERGIC NEURONS WITH BEST-IN-CLASS RESULTS IN PRECLINICAL MODEL OF PARKINSON S DISEASE

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Despite the development of efficient protocols to differentiate human pluripotent stem cells (hPSC) into dopaminergic neurons, the transplantation of mature DA neurons to treat Parkinson's disease is generally considered as a non-viable option. In mature neurons cultured in vitro, neurite outgrowth indeed greatly complexifies cell harvest and transplantation. To circumvent this issue, most cell replacement therapies in development for Parkinson's disease use dopaminergic progenitors. However, the clinical use of these multipotent cells remains challenging with regards to : i) the in situ control over the graft's cellular identity and ii) the management of cell proliferation risks. In addition, such progenitor-based cell therapies rely on differentiation protocols which do not meet industrial requirements for scalable manufacturing in bioreactors.Since 2018, we have been addressing some of these challenges with the C-Stem™ technology. Using high-speed cell encapsulation microfluidics, hiPSC were grown in alginate shells and differentiated into functional microtissues composed of dopaminergic neurons. Following capsule dissolution, mature neural microtissues were transplanted into hemiparkinsonian rats, leading to full motor recovery within 8 weeks (vs 16 weeks with progenitors). We believe the reduction of time-to-effect is partly linked with the transplantation of mature neurons, which eliminates the time lag required for progenitor in situ differentiation. Importantly, we replicated our results with 3 different cell lines differentiated "in capsulo", demonstrating the robustness of the process.Capable of scaling-up hPSC production in industrial bioreactors (single batch of 15 billion hiPSC recently delivered in a 10L bioreactor), the C-Stem™ technology open new avenues for the production of large-scale commercial batches of dopaminergic neurons to treat Parkinson's disease. First-in-human trial with this novel therapeutic format is tentatively scheduled for 2024.

Funding Source
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Keywords: hiPSC-derived cell therapy, Parkinson's disease, Dopaminergic neurons

723

RE-GENERATION OF CYTOTOXIC GAMMA DELTA T CELLS FROM HUMAN GAMMA DELTA DERVIVED IPSCS

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GAMMADELTAT cell-based immunotherapy is attractive, but patients' peripheral blood-derived GAMMADELTAT cells are difficult to expand to an adequate amount for clinical use in some cases. iPSCs, which have the infinite proliferative capacity, may solve the problem. The ALPHABETAT cell-derived iPSCs were reported to differentiate into T cells that retain the same TCR rearrangement as original ALPHABETAT cells. Previously, we successfully established iPS cell lines which harbor GAMMA9DELTA2 TCR (GAMMADELTAT-iPSC) and differentiated them into hematopoietic progenitor cells (HPC) with high efficacy in feeder-free and serum-free conditions. We aimed to regenerate GAMMADELTAT cells from two GAMMADELTAT-iPS cell lines and to investigate marker expressions and functions of the resultant cells.We successfully generated CD3+GAMMADELTATCR+ cells (iGAMMADELTATs) from GAMMADELTAT-iPSC, which retained single clonal TCR as the original cell. Also we revealed that HMBPP, a precursor of IPP, was useful to activate iGAMMADELTATs in our differentiation protocol. In an MHC-unrestricted manner, iGAMMADELTATs showed cytotoxicity against several kinds of cancer cell lines at a lower E: T ratio than that in the previously reported experiments using iPSC-derived ALPHABETAT cells. The granzyme and perforin were expressed in the iGAMMADELTATs. Clustering analysis of single-cell RNA-seq revealed that iGAMMADELTATs had closely similar gene expression patterns to a small subset of peripheral blood-derived GAMMADELTATs. The iGAMMADELTATs also expressed common NK cell markers. The limitation of this study is that we depended on the feeder cell co-culture system from HPCs to iGAMMADELTATs. If we succeed in differentiation from HPCs to iGAMMADELTATs under feeder-free and serum-free conditions, regenerated iGAMMADELTAT-based immunotherapy becomes more easy and safe for clinical use.
Keywords: hiPSC, gamma-delta T cells, immunotherapy

Poster Abstracts

725

DEVELOPMENT OF HYPOIMMUNOGENIC IPSC-DERIVED ALLOGENEIC T-CELLS FOR OFF-THE-SHELF IMMUNOTHERAPY AGAINST CANCER

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Since the first report of antigen-specific TCR-expressing T cell generation from iPSC in 2013, we have been improving a method to generate CD8ab T cells from CD4 and CD8ab positive precursor (DP) T cells induced by notch signal and lymphopoietic cytokines. These regenerated CD8ab T cells showed favorable gene expression profile to accelerate cancer-immunity cycle and showed significantly improved proliferation and therapeutic efficacy in vitro and in vivo against the antigen expressing target cells.To generate clinically relevant iPS-T cells by excluding non-human biological materials, the induction method of iPS-T cells was modified by the combinational use of p38 inhibitor and SDF-1a and it was found that they significantly contributed to effective generation of DP T cells by accelerating early expression of T-cell related master transcriptional factors. Then, to know potential of the iPS-T cells as a source of allogeneic immunotherapy, we transduced CD19-CAR and IL-15 genes to the iPS-T cells and evaluated therapeutic efficacy on human B cell leukemia cell line bearing NSG mice in comparison with healthy volunteer-derived CD19-CAR transduced primary T cells. Both NSG mice treated by the iPS-T cells or treated by the primary T cells showed similarly prolonged survival that indicated usefulness of iPS-T cells as cell source of allogeneic T cell immunotherapy.An obstacle of allogeneic iPS-T cells for clinical use is allo antigenicity that must shorten survival of iPS-T cells in patient, so, we edited iPS cells to reduce the antigenicity. HLA-A, B, and CIITA KO iPS cells or B2M, CIITA, PVR KO and single chain HLA-E transduced iPSC (tKO/E iPSC) were generated by genome edit and immune cells from those iPSC were confirmed to reduce allogeneic antigenicity. Especially tKO/E iPSC-derived iPS-T cells with CD20-CAR showed improved persistency in animal model reconstituted by allogeneic human immune cells and showed rapid control of CD20-
expressing EBV-immortalized B lymphocytes.Our first clinical trial of allogeneic immune cell has just started using CAR-expressing iPSC-derived NK cells to know safety of the iPS cell-derived immune cell product in cancer patients. Following the clinical trial, allogeneic iPS-T cells modified by combination of indicated technologies will be tested in near future.

Funding Source
AMED, KAKENHI, T-CiRA, KIRIN holdings, and Thyas
Keywords: cancer immunotherapy, clinical protocol for T cell differentiation, hypoimmunogenic iPSC

726

IN SITU MATURATED EARLY-STAGE HUMAN-INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES IMPROVE CARDIAC FUNCTION BY ENHANCING SEGMENTAL CONTRACTION IN INFARCTED RATS

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The scant ability of cardiomyocytes to proliferate makes heart regeneration one of the biggest challenges of science. Current therapies do not contemplate heart re-muscularization. In this scenario, stem cell-based approaches have been proposed to overcome this lack of regeneration. We hypothesize that early-stage hiPSC-derived cardiomyocytes (hiPSC-CMs) could enhance the cardiac function of rats after myocardial infarction (MI). Animals were subjected to the permanent occlusion of the left ventricle (LV) anterior descending coronary artery (LAD). Seven days after MI, early-stage hiPSC-CMs were injected intramyocardially. Rats were subjected to echocardiography pre-and post-treatment. Thirty days after the injections were administered, treated rats displayed 6.2% human cardiac grafts, which were characterized molecularly. Left ventricle ejection fraction (LVEF) was improved by 7.8% in cell-injected rats, while placebo controls showed an 18.2% deteri- oration. Additionally, cell-treated rats displayed a 92% and 56% increase in radial and circumferential strains, respectively. Human cardiac grafts maturate in situ, preserving proliferation with 10% Ki67 and 3% PHH3 positive nuclei. Grafts were perfused by host vasculature with no evidence for immune rejection nor ectopic tissue formations. Our findings support the use of early-stage hiPSC- CMs as an alternative therapy to treat MI. The next steps of preclinical development include efficacy studies in large animals on the path to clinical-grade regenerative therapy targeting human patients.

Funding Source
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Keywords: cardiomyocytes, iPSCs, heart failure

727

ESTABLISHMENT OF A CELL BANK FOR CELL THERAPY WITH HUC-MSCS: FROM THE UMBILICAL CORD TO THE PATIENT

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Umbilical cord (UC) has been proposed as a promising source of human mesenchymal stem cells (hMSCs) due to its plasticity, expandability and immunomodulatory action, even after prolonged cryopreservation. UC collection is easy and without ethical concerns, as it does not harm newborns or mothers. hUCMSCs can be expanded and do not elicit any strong immune responses. Our aim was to develop a MSCs bank that would ensure availability of high quality, reliable and well characterized hUCMSCs for clinical application.UC samples were collected with informed consent and validated according tissue bank criteria. hUCMSCs were isolated and cultured following good manufacturing practice (GMP) using a clinical grade human Platelet Lysate (hPL). The Master and Working Cell Banks (MCB and WCB) were tested after expansion and cryopreservation. Additionally, to study the potential effects of hLP on hUCMSCs growth and characteristics, we performed a microarray analysis to compare WCB hUCMSCs cultured with hPL and FBS.UC from 4 donors were successfully obtained after natural or cesarean deliveries. All samples tested negative for infectious disease markers. Data of MCB counts at passage 1 were about 15-45x10^6 cells. After thawing, each expanded MCB vial contributed between 150-250x10^6 cells to establish the WCB, in less than 4 passages. Our procedures allowed us to obtain a good cryopreserved cell reservoir available for immediate clinical use, without changes in viability or cell identity during expansion and cryopreservation. All MCB and WCB presented a typical MSC immunophenotype. Sterility tests, mycoplasma detection, endotoxin test and adventitious agents test were performed as quality control in all MCB and WCB. Regarding the hPL effect on hUCMSCs transcriptome, we observed 41 differentially expressed genes with respect to FBS, related, among others, to proliferation and inflammatory response pathways. Our experience demonstrates that appropriate validated protocols for the processing of UC within GMP facilities can aid in the development of a cryopreserved bank of clinical grade hUCMSCs, keeping quality and identity requisites necessary for subsequent therapeutic application in patients.

Funding Source
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Keywords: UCMSCs, Human platelet lysate, Advanced Therapy Medicinal Products

728

RELATIONSHIP BETWEEN THE EFFECT OF STEM CELL THERAPY AND THE PRETREATMENT STATE EVALUATED BY LIVE MRI FOR SPINAL CORD INJURY IN MICE

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After spinal cord injury (SCI), spontaneous recovery is limited, and the sequelae become serious depending on the degree of injury. Rehabilitation is the only treatment that has generally been shown to be effective for spinal cord injury, but in recent years various new treatments have been developed and are gradually being realized. The effect of new treatment for SCI generally depends on the timing of treatment and the degree of injury. Rare cases, however, even with complete symptoms in chronic phase have remarkable therapeutic effects, suggesting that the condition of the spinal cord is different even if the severity of the symptom is the same. Imaging technique is useful as a live method of evaluating the spinal cord condition, and MRI is especially superior because it can visualize and quantify the fiber-structure in the spinal cord by diffusion tensor tractgraphy (DTT). We created thoracic spinal cord

Poster Abstracts

injury model mice of different severity and performed live MRI in the chronic phase to quantify the number of fibers passing through the injured site. After that, human iPS-derived neural progenitor cells were transplanted, and motor function was observed over time. The fiber number yielded by the DTT was counted in all mice. We investigated the relationship between the MRI results and pretreatment motor function, as well as the relationship between the MRI results and therapeutic effect. While the strong relationship was demonstrated between the fiber number and the pretreatment motor function, we revealed the individual characteristics in which the transplantation treatment became effective even in severe state.

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Keywords: diffusion tensor tractgraphy, iPS, spinal cord injury

731

FUNCTIONAL MATURATION OF TRANSPLANTED HUMAN IPSC-DERIVED GABAERGIC INTERNEURON PROGENITORS RESTORES HIPPOCAMPAL NETWORK FUNCTION IN AN ALZHEIMER'S DISEASE MOUSE MODEL

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Alzheimer's disease (AD) is the most common form of dementia, characterized by significant neuronal loss and a progressive decline of memory and cognition. Apolipoprotein E4 (APOE4), the major genetic risk factor for AD, markedly increases AD risk compared with the APOE3 allele and is associated with 60-75% of all AD cases. Loss of hippocampal GABAergic interneurons, one of the mechanisms contributing to hippocampal network dysfunction in AD pathogenesis, is exacerbated by apoE4 expression. We proposed to develop a cell replacement therapy with human APOE3 iPSC-derived GABAergic interneuron progenitors to target the apoE4-positive AD population. Here, we developed an effective cell differentiation protocol in vitro to generate human iPSC-derived MGE lineage GABAergic interneuron progenitors with high purity. Transplantation of these APOE3 GABAergic progenitors into the dentate gyrus of 10-month-old APOE4 knock-in (apoE4-KI) mice robustly demonstrated long-term survival (22.6 ± 7.7% of transplanted cells) and exhibited neuritic projections throughout the entire hippocampus at 7 months post-transplantation. Furthermore, histological analysis revealed that the transplanted human GABAergic progenitors gave rise to cells expressing high levels of gamma aminobutyric acid (GABA), and maturation continuing into somatostatin (SST) and neuropeptide Y (NPY) subclasses. Moreover, half of the surviving human interneurons migrated out of the dentate gyrus, formed synapses, and incorporated into the CA1 and CA3 subfields of the host hippocampus, with undetected levels of proliferating cells in the long term. Importantly, in vivo electrophysiological recording revealed a significant rescue of apoE4-induced hippocampal network deficits with an increase in both sharp-wave ripple (SWR) rate and the associated slow gamma power in the transplanted apoE4 mice in a cell number-dependent manner. Together, these results imply that transplanted human iPSC-derived GABAergic interneuron progenitors can functionally mature and integrate into the hippocampal network and rescue AD-related phenotypes in aged apoE4-KI mice. This represents a critical step toward a potential cell-based therapy for apoE4-expressing AD patients.

Keywords: iPSC-derived GABAergic interneuron, Alzheimer's disease, cell-based therapy

732

MESENCHYMAL STEM CELLS EXERT RENOPROTECTION VIA EXTRACELLULAR VESICLE-MEDIATED MODULATION OF M2 MACROPHAGES AND SPLEEN-KIDNEY NETWORK

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The number of dialysis patients is increasing, and there is an urgent need to develop new treatments for nephritis, a major cause of renal failure. To date, the renoprotective effects of adipose-derived mesenchymal stem cells (ASCs) have been shown, and several clinical trials using ASCs to treat kidney diseases are underway. However, the detailed therapeutic mechanisms remain unclear. Here, we report the therapeutic potential of human ASCs for nephritis, focusing on in vivo cellular dynamics and multi-organ networks. Intravenously-administered ASCs accumulated in the spleen but not the kidneys. Nevertheless, ASCs increased M2 macrophages and Tregs in the damaged kidney, decreased neutrophils and M1 macrophages, and drove strong renoprotection. Splenectomy abolished these therapeutic effects. Flow cytometry analysis revealed that ASC-derived extracellular vesicles (EVs) were specifically transferred to M2 macrophages, and intravital microscopy imaging demonstrated that EV-transferred macrophages entered the bloodstream from the spleen. EVs induced the transcriptomic signatures of macrophage activation and PGE2 stimulation

in M2 macrophages and partially ameliorated glomerulonephritis. Furthermore, ASCs, ASC-derived EVs, and EV-transferred M2 macrophages enhanced Treg induction in T cells. These findings collectively suggest that specific EV transfer from spleen-accumulated ASCs to M2 macrophages and subsequent modulation of the kidney immune environment underlie the renoprotective effects of ASCs. Our results provide new insights into the therapeutic mechanisms of ASCs, focusing on EV-mediated modulation of macrophages and the spleen-kidney immune network, which may lead to maximized potential of cell therapies in clinical settings.

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Keywords: adipose-derived mesenchymal stem cells, extracellular vesicles, multi-organ networks

733

SECRETED FACTORS FROM DENTAL PULP STEM CELLS IMPROVE SJOGRENS SYNDROME VIA REGULATORY T CELL-MEDIATED IMMUNOSUPPRESSION

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Sjögren's syndrome (SS) is a chronic autoimmune disease primarily characterized by inflammation in the salivary and lacrimal glands. Activated T cells contribute to the pathogenesis of this disease by producing proinflammatory cytokines, which results in the establishment of a positive feedback loop. The aims of this study were to evaluate the effects of secreted factors derived from dental pulp stem cells (DPSCs) or bone marrow mesenchymal stem cells (BMMSCs) on hyposalivation in SS and to investigate the mechanism involved. Eighty percent confluent stem cells were replenished with serum-free Dulbecco's modified Eagle's medium and incubated for 48 h; following which, conditioned media from the DPSCs (DPSC-CM) and BMMSCs (BMMSC-CM) were collected. Cytokine array analysis was performed to assess the types of cytokines present in the media. Flow cytometric analysis was performed to evaluate the number of activated T cells cultured in the DPSC-CM or BMMSC-CM. Subsequently, DPSC-CM or BMMSC-CM was administered to an SS mouse model. The mice were categorized into the following groups (n = 6 each): non-treatment; Dulbecco's modified Eagle's medium (-); BMMSC-CM; and DPSC-CM. Histological analysis of the salivary glands was performed. The gene and protein expression levels of cytokines associated with T helper subsets in the submandibular glands (SMGs) were evaluated. DPSC-CM contained more secreted factors with tissue regenerating mechanisms such as cell proliferation, anti-inflammatory effects, and immunomodulatory effects. DPSC-CM was more effective in suppressing the activated T cells than other groups in the flow cytometric analysis. The stimulated salivary flow rate increased in the SS mice with DPSC-CM compared with that in the other groups. In addition, the number of inflammation sites in the SMGs of the mice administered with DPSC-CM was lower than that in the other groups. The expression levels of interleukin (Il)-10 and transforming growth factor-BETA1 were up-regulated in the DPSC-CM group, whereas those of Il-4 and Il-17a were down-regulated. The DPSC-CM-administered group presented with a significantly increased percentage of regulatory T (Treg) cells and a significantly decreased percentage of type 17 Th (Th17) cells compared with the other groups. These results indicated that DPSC-CM ameliorated SS by promoting Treg cell differentiation and inhibiting Th17 cell differentiation in the mouse spleen.

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Keywords: Secreted factors, Dental pulp stem cells, Sjogrens syndrome

734

ATTENUATION OF KNEE OSTEOARTHRITIS PROGRESSION IN MICE BY INTRA-ARTICULAR TRANSPLANTATION OF NON-CULTURED HUMAN ADIPOSE-DERIVED REGENERATIVE CELLS

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Adipose-derived regenerative cells (ADRCs) are non-cultured heterogeneous or mixed populations of cells obtained from adipose tissue by collagenase digestion, and injection of ADRCs have been tried clinically for the treatment of osteoarthritis (OA). However, the effects of ADRCs on OA progression have not been fully examined. The purpose of this study was to evaluate the effects of intra-articular transplantation of human ADRCs on OA progression in mice. Human ADRCs were obtained from patients who received intraarticular injection of ADRCs for treatment of knee OA and, a part of the remaining cells not used in the treatment was used for this study under the patient's consent. Mouse OA model was created by destabilizing the medial meniscus of 12-weeks-old BALB/c-nu mice on the same day of the injection of ADRCs. Mice were received intraarticular transplantation of either PBS (control group) or human ADRCs (ADRC group). OA progres-

Poster Abstracts

sion was evaluated 4 and 8 weeks after surgery using the OARSI score, the expression of OA-related proteins in the cartilage, and that of macrophage-associated markers in the synovium by immunohistochemistry. The mean OARSI score for the ADRC group was significantly lower than that for the control group. The immunohistochemical analysis showed that type 2 collagen was significantly more detected while ADAMTS-5, MMP-13, IL-6, and IL-1BETA were significantly decreased in the ADRC group compared with the control group. In the synovium, more iNOS-positive cells were detected in the control group than in the ADRC group, while more CD206-positive cells were detected in the ADRC group than in the control group. Human nuclear antigen was mainly observed in the synovial tissue of the supra-patellar pouch and infra-patellar fat pad area one week after transplantation. The intra-articular transplantation of human ADRCs attenuates OA progression in mice, possibly by reducing catabolic factors in chondrocytes and modulating macrophage polarization.

Keywords: adipose-derived regenerative cells, osteoarthritis, macrophage

735

ENHANCEMENT OF NEURITE EXTENSION FROM THE GRAFT BY EXPRESSION OF AXON GUIDANCE MOLECULES IN THE HOST BRAIN

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The corticospinal tract (CST) is the descending spinal tract system originating from the motor cortex and related to the motor function. Damage to the brain often destroys it and causes motor dysfunction. We are now trying to reconstruct the damaged CST using human induced pluripotent stem (iPS) cell-based neural replacement therapy. We previously reported that human embryonic stem cell-derived cerebral organoids transplanted into mouse cerebral cortices extended their axons along the CST and reached the spinal cord. However, it is still insufficient for the reconstruction of the CST, and it would be desirable that the host brain environment is more supportive. During neurogenesis, various axon guidance molecules play a role in the downward migration of neuronal axons from the cerebral cortex to the spinal cord. Among them, L1CAM, a cell adhesion molecule, is expressed on the CST from embryonic to neonatal stages and is thought to guide the axons in a haptotactic manner. Since the L1CAM expression decreases in adults, we hypothesized that forced expression of L1CAM on the CST would enhance the neurite extension from the graft. In this study, we injected L1CAM-targeted adeno-associated virus vector into the motor cortex of adult mice and transplanted mouse embryonic brain tissue. We compared the number of graft-derived axons along the CST 12 weeks after transplantation and found that the number of axons in the L1CAM group was significantly higher than that in the control group. This result suggests that the expression of axon guidance molecules in the host brain enhances the effects of cell replacement therapy.

Funding Source
Grants in research center network for realization of regenerative medicine, Japan Agency for Medical Research and Development (AMED)

Keywords: cell replacement therapy, L1CAM, axon guidance molecules

736

CHEMICAL CONTROL OF GRAFTED HIPSC DERIVED NEURONS DEMONSTRATED THE CONTRIBUTION OF GRAFT NEURONAL ACTIVITY TO THE RECOVERY FOLLOWING SPINAL CORD INJURY

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The therapeutic effect of human-induced pluripotent stem cell-derived neural stem/progenitor cell (hiPSC-NS/PC) transplantation to the subacute phase of spinal cord injury (SCI) animals has been previously reported, yet the mechanism of recovery has not been elucidated. Clarifying the therapeutic effect of engrafted cells indicates the necessity of NS/PC transplantation and could directly link to a further improvement of this therapy. The purpose of the present study was to evaluate the contribution of grafted neuronal function to the recovery of host locomotor activity. To determine the functional effect of grafted neuronal activity, transplanting NS/PCs were genetically modified by lentiviral vectors. First, NS/PCs coding TRE-GFP-2A-WGA/EF1-rtTA (WGA-NS/PCs), which express a trans-synaptic tracer wheat germ agglutinin (WGA) in control of doxycycline administration, was transplanted to Th10 contusion injury model mice. Ten weeks after transplantation, immunohistological analyses revealed a trans-synaptic migration of WGA to host neurons. WGA migrated not only to the neurons nearby the graft cells but also to the motor neurons in the caudal site; suggesting the integration of graft neurons into the host motor circuits. Second, NS/PCs coding hSyn-hM4Di-mCherry (hM4Di-NS/PCs), which express a chemogenetically-engineered receptor that permits temporal inhibition by synthetic ligand clozapine N-oxide (CNO), and NS/PCs coding hSyn-mCherry (mCherry-NS/PCs), as a control, were transplanted to SCI mice. By administrating CNO to hM4Di-NS/PCs and mCherry-NS/PCs transplanted mice, BMS score (pre-CNO 3.3, post-CNO 3.1; p=0.021) and parameters of treadmill gait analysis (stride length: pre-CNO 4.0cm, post-CNO 3.5cm; p=0.003, paw angle: pre-CNO 22.7°, post-CNO 31.0°; p=0.027) has signifi-

cantly deteriorated in hM4Di-NS/PCs transplanted mice. These results suggest that inhibiting the neuronal function of grafted neurons guide to a relapse of functional disorder by SCI. In conclusion, grafted neurons derived from hiPSC-NS/PCs transplanted to SCI animals integrate into host neuronal circuits and contribute to the functional recovery of host animals.

Funding Source
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Keywords: Spinal cord injury, Cell transplantation therapy, DREADD

737

DREADD INDUCED LONG-TERM SELECTIVE STIMULATION OF TRANSPLANTED NEURAL STEM/PROGENITOR CELLS FOR SPINAL CORD INJURY IMPROVES LOCOMOTOR FUNCTION

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Transplantation of human-induced pluripotent stem cell-derived neural stem/progenitor cells (hiPSC-NS/PCs) is effective for functional recovery after spinal cord injury (SCI). The main mechanism of this recovery is to make synaptic connection between the host and the graft and restore the disrupted neuronal circuits. In the field of embryology, the activity of immature neurons is important to make and maintain synaptic connections. This evidence provided a hypothesis that long-term selective stimulation of transplanted hiPSC-NS/PCs after SCI can enhance motor function recovery by enhancing synaptic connections. For selective stimulation of transplanted cells, we used hM3Dq, one of the designer receptors. First, hM3Dq was lentivirally transfected to hiPSC-NS/PCs. These hM3Dq-NS/PCs-induced neural cells (hM3Dq neural cells) were treated with Clozapine N-oxide (CNO). The intracellular response was investigated by RNA sequence and hM3Dq neural cells expressed significantly higher levels of immediate early genes. Second, the extracellular response from CNO administered hM3Dq neural cells to neighboring neurons was recorded using the GCaMP calcium indicator. CNO-administered hM3Dq neural cells activated neighboring neurons. Next, the hM3Dq-NS/PCs were transplanted into the lesion epicenter of mouse spinal cords nine days after contusive SCI. Then, CNO was intraperitoneally injected daily to the stimulation group, and saline was injected to the control group. RNA sequence and capillary electrophoresis revealed that expressions of synapse-related genes and proteins were significantly enhanced on day 14 after SCI in the stimulation group. On day 42, axial spinal cord areas were significantly larger in the stimulation group. Synapse formations between the transplanted cells and host neurons were detected in immunoelectron microscopic examination. Consequently, the stimulation group presented significant improvement of motor functions such as BMS scores, stride length, coordination, and captured overall walking motion. In conclusion, long-term repetitive and selective stimulations of transplanted hiPSC-NS/PCs after SCI enhanced synaptic transmission, increased synapse-related genes and proteins expressions, preserved host spinal cord tissue, and improved motor functions.

Funding Source
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Keywords: spinal cord injury, cell transplantation therapy, DREADD induced selective stimulation

738

POTENTIAL OF HEK293 CELLS AS POSITIVE CONTROL CELLS IN TUMORIGENICITY TESTS FOR HUMAN CELL-BASED THERAPEUTIC PRODUCTS

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As for tumorigenicity assessment of human cell-based therapeutic products (hCTPs), the amount of transformed cells as impurities in hCTPs or their intermediates can be estimated quantitatively, by assuming that the tumorigenicity of the transformed cellular impurities is comparable to that of the positive control cells. In the present study, the tumorigenic characteristics of HEK293 cells (passage number: <48) spiked into normal human cells were evaluated by various tumorigenicity-associated tests. Conducting in vivo tests using severely immunodeficient NOD/Shi-scid IL2RGAMMAnull (NOG) mice, the 50% tumor

Poster Abstracts

producing dose (TPD50) of HEK293 cells co-transplanted with human mesenchymal stem/stromal cells (hMSCs) was about 20 cells, which indicates high tumorigenicity of HEK293 cells comparable to that of HeLa cells (TPD50: about 10 cells). Next, we assessed the anchorage-independent growth potential of HEK293 cells based on their colony formation efficiency from single cells in three-dimensional culture. The colony formation efficiency of HEK293 cells co-cultured with hMSCs was about 8% in three-dimensional culture using conventional soft agar medium, but low molecular weight agar LA717 (SphereMax®) mixed in liquid medium enhanced it to about 30%. We also examined the growth rates of hMSCs contaminated with various doses of HEK293 cells, and compared them with that of hMSCs alone. The cell growth analysis detected a significant increase in the growth rate of the hMSCs spiked with 0.001% HEK293 within 30 days. These results indicated that HEK293 cells spiked into normal cells have a constant level of tumorigenicity, which can be detected and quantified by various tumorigenicity-related tests. Therefore, HEK293 cells are suggested to be useful as positive control reference cells in tumorigenicity tests for hCTPs.

Funding Source
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Keywords: HEK293 cells, Tumorigenicity test, Human cell-based therapeutic products

739

CLINICAL AND RADIOLOGICAL COMPARISON OF SINGLE AND TWICE INTRAARTICULAR INJECTION OF ADIPOSE DERIVED STROMAL VASCULAR FRACTION FOR KNEE OSTEOARTHRITIC PATIENTS

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Recently, there has been increasing attention in adipose-derived stromal vascular fractions (SVF) cells for the treatment of knee osteoarthritis (OA). This present study aimed to compare the clinical and radiological outcomes between the single and twice SVF injection for knee OA patients. Fifty-seven patients (60 knees) who had varus knee OA were treated with intraarticular injection of 2.5×10^7 SVF cells into the knee joint. One year after the first SVF injection, patients who want to receive additional treatment were secondly treated with SVF injection via the same procedure. In this retrospective study, 57 patients (60 knees) were divided into two groups: those who had good satisfaction following the first injection (group A; n = 31) and those who needed a second injection (group B; n = 29). Clinical evaluation including Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score was assessed preoperatively, 3, 6, 12, and 24 months following the first injection. Imaging evaluations including the hip-knee-ankle angle (HKA) with plain radiograph, and T2 mapping value with a magnetic resonance imaging unit were assessed preoperatively, at 12, and 24 months following the first injection. Additionally, both clinical and imaging evaluation were compared between the 2 groups. In group A, the total WOMAC score was significantly improved at 3, 6, 12, and 24 months. The T2 mapping values of the medial femur, lateral femur and lateral tibia were significantly improved at 12 and 24 months. The total WOMAC score was also significantly improved at 24 months, but not at 3, 6, 12 months after the injection. And, T2 mapping values of lateral femur and tibia in group B were significantly improved at 24 months, but not at 12 months after the injection. Preoperative HKA was significantly different between the 2 groups, 185.9° in group A and 188.1° in group B. The total WOMAC score and T2 mapping value of the medial femur in group B were significantly worse than group A before the second injection, and there was no significant difference between the 2 groups after the second injection. These findings suggested that the intraarticular SVF cell injection is a novel and innovative treatment for knee OA, but the clinical effects for severe varus knee could be achieved only with several injection.

Funding Source
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
Keywords: stromal vascular fraction, knee osteoarthritis, intraarticular injection

740

GENERATION OF CHEMICALLY-INDUCED LIVER PROGENITOR (CLIPS) FROM MATURE HEPATOCYTES OF PATIENTS WITH SEVERE LIVER CIRRHOSIS BY STIMULATION WITH SMALL MOLECULE COMPOUNDS

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Although liver transplantation is the only standard treatment for patients with end-stage cirrhosis, there is a serious shortage of donor livers at present. A few studies have reported that hepatocyte transplantation improved the liver function. But donor tissue for hepatocyte transplantation is also lacking, and the transplant efficiency is low. On the other hands, it is reported the chemical stimulation by the combination of three small molecules (Y-27632, A83-01, and CHIR99021: YAC) is possible to directly reprogram from mature hepatocyte to progenitor cell called Chemically-induced Liver Progenitor (CLiPs). Although CLiPs are successfully generated from mouse and human-derived hepatocytes, it has not yet been reported to produce from liver derived from cirrhosis. In this study, we attempted to produce CLiPs from hepatocytes isolated from cirrhotic patients. The isolated primary hepatocytes were cultured in YAC cocktail medium. Around 14 days, small cells with a high N/C ratio, which is not characteristics of hepatocytes, appeared and proliferated while mixed with fibroblasts. These small cells grew to 80-100% confluence when cultured for about 4 weeks, and the cells number became 5.5-6.6 times. The gene expression of hepatic progenitor markers (EpCAM and CD133) was greater than that of primary hepatocytes collected during hepatocyte isolation. In contrast, the gene expression of mature hepatocytes markers (ALB and TO) was lower than that of primary hepatocytes. Furthermore, in flow cytometry, about 60% of the cultured cells were CD133 positive. When cultured in hepatocytes isolated from cirrhotic liver, the fibroblast proliferation was faster than when cultured in hepatocytes isolated from normal liver. In addition, we also found that changes in the combination of small molecule compounds affect cell proliferation and pattern of gene expression in progenitor cells, for example for example combining HGF with small molecules. These results suggested that we can produce CLiPs from cirrhotic liver and that its properties depend on the combination of small molecule compounds.

Keywords: chemical reprogramming, hepatic progenitor cell, liver cirrhosis

741

DEVELOPMENT OF AN EFFICIENT EXPANSION CULTURE FOR HUMAN IPSC-DERIVED PANCREATIC PROGENITOR CELLS

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Type 1 diabetes (T1D) patients can achieve insulin independence from transplantation of cadaveric islets, but the scarcity of donor pancreatic organs limits the widespread application of this treatment. Recent efforts to differentiate human pluripotent stem cells (hiPSCs/ESCs) into islet cells have led to the development of protocols that generate glucose-responsive insulin-secreting BETA cells for transplantation. To meet clinical demand, further manufacturing refinement is required to prepare BETA cells in an efficacious and cost-effective manner. Therefore, the in vitro proliferation of human pancreatic progenitor cells (PPCs), an intermediate cell type differentiated during the course of the induced differentiation of hiPSCs/ESCs into BETA cells, is critical for developing cell therapies for diabetes. However, the mechanism of PPC growth during organogenesis or in vitro induction is incompletely understood. In this study, using transcriptome analysis combined with siRNA screening, we revealed that WNT7B is a downstream growth factor of AT7867, a compound we previously identified that promotes the proliferation of PPCs derived from hiPSCs/ESCs. Feeder cells stably expressing mouse/human Wnt7a or Wnt7b, but not other Wnts, enhanced proliferation of PPCs more than 107 times in 10 passages in the absence of AT7867. The expanded pancreatic progenitors cultured in the presence of Wnt7a/b ligands maintained the expression of progenitor markers including PDX1, NKX6.1, FOXA2, SOX9 and HNF1BETA. Importantly, these expanded PPCs also maintained differentiation potential into INSULIN-producing BETA cells. These results were reproduced using PPCs differentiated from three different hiPSC/ESC lines. We are currently examining the feeder-free expansion culture methods using Wnt7a/b for hiPSC-derived PPCs in view of clinical application. Our expansion culture method developed in this study will contribute to the stable supply of a cell source for pancreatic disease modeling and regenerative therapies for T1D.

Keywords: Diabetes, Expansion, Wnt

Poster Abstracts

742

A NONHUMAN PRIMATE LIVER FIBROSIS MODEL TOWARDS PLURIPOTENT STEM CELL-BASED CELL THERAPY FOR LIVER CIRRHOSIS

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Chronic liver diseases (i.e. hepatitis B and C virus infection, alcohol abuse, NASH, etc) develop into liver cirrhosis if left untreated. Liver cirrhosis is a major cause of mortality and leads to around 1 million deaths globally every year. Orthotopic liver transplantation (OLT) is the only curative treatment for refractory liver failure in liver cirrhosis. However, the supply of donated livers does not meet the demand for OLT due to donor organ shortage. Cell therapies using hepatocyte-like cells (HLCs) derived from human induced pluripotent stem cells (hiPSCs) in place of hepatocytes procured from donor livers are expected to improve the impaired liver function and postpone OLT. For the successful clinical translation of the cell therapies, realistic animal models are required. Here, we created a nonhuman primate (NHP) liver fibrosis model by repeated administrations of thioacetamide (TAA) and evaluated the short-term engraftment of hiPSC-HLCs in the fibrotic liver. The NHP liver fibrosis model reproduced well the pathophysiology of human liver cirrhosis including portal hypertension. Notably, portal venography revealed that one of these models has a portosystemic shunt which is unsuitable for a cell transplantation site. Under immunosuppressive treatment, we transplanted ALBUMIN-GFP reporter hiPSC-HLC aggregates into fibrotic livers of the NHP model via the portal vein. Fourteen days after the transplantation, GFP-expressing hiPSC-HLC clusters were detected in the portal areas of the fibrotic livers. Preclinical studies using our NHP liver fibrosis model will faciliate the development of iPSC-based cell therapies against liver cirrhosis.

Keywords: Liver cirrhosis, NHP model, iPSC

743

BLOOD OUTGROWTH ENDOTHELIAL CELL-BASED ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE THERAPY FOR THE PROTECTION FROM THE VASCULAR COMPLICATIONS OF DIABETES

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Enhancement of endothelial nitric oxide synthase (eNOS) expression and function in endothelial cells using gene therapy is a promising strategy for the prevention and treatment of diabetic vascular complications. Blood outgrowth endothelial cells (BOECs) expanded from blood progenitors are attractive carriers for the eNOS gene due to their high proliferative ability and ease of isolation. eNOS activity in BOECs from patients with type 2 diabetes (T2D) could be enhanced and then returned to the patient where they then may restore function. In this study we aimed to investigate the effectiveness of eNOS gene enhancement to rescue vascular dysfunction using minicircle (MC) DNA; a technology that allows the transfer of genes in a circular form (<10kb) without bacterial backbone. BOECs were isolated from healthy and T2D subjects using selective plating (n=7 and n=4 respectively), and were compared in terms of time of emergence, colony number, proliferation and eNOS mRNA expression. BOECs were transfected with MC eNOS (SBI) and angiogenesis was assessed using Matrigel assay. Data are represented as mean ± SEM of n=3-4 and $p<0.05$ was considered significant. Emergence of BOEC colonies (per 107 seeded PBMCs) was reduced in T2D when compared to healthy subjects (0.51± 0.09 and 0.24± 0.09, respectively, $p<0.05$, Mann-Whitney U test) as was time of colony emergence (13.00± 0.88 and 19.33± 3.27 days respectively, $p<0.05$, Mann-Whitney U test). BOECs from both groups showed typical cobblestone morphology and expressed VEGFR2 but not CD45. BOECs proliferation (CTRL: 0.39±0.03 and T2D: 0.29±0.03, $p<0.05$, Mann-Whitney U test) and eNOS expression (CTRL: 48.75± 15.19 and T2D: 14.61±3.81, $p<0.05$, Mann-Whitney U test, 2-DELTACt) were significantly reduced in T2D. Gene enhancement resulted in an increase of eNOS expression (T2D+ MC

eNOS: 2313±1588, p<0.05, 2-DELTADELTACt).
The eNOS-enhanced BOECs demonstrated restored ability to generate blood vessels as indicated by increased numbers of closed networks in transfected T2D BOECs (T2D: 1±0.51, T2D+MC eNOS: 1.66±0.23, p<0.05, relative to CTRL). These findings indicate the potential to utilize BOECs as carriers of the eNOS gene to restore vascular function in diabetes. The use of endogenous blood stem cells provides a non-invasive, targeted therapeutic option to reduce vascular dysfunction in T2D.

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Keywords: endothelial progenitor cells, endothelial nitric oxide synthase, Type 2 diabetes

744

BIOACTIVE SL-13R PEPTIDE EXPANDS HUMAN UMBILICAL CORD BLOOD AND PERIPHERAL BLOOD HEMATOPOIETIC STEM/PROGENITOR CELLS

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Hematopoietic stem cell (HSC) transplantation is a curative treatment of hematological disorders or ischemic disease. Umbilical cord blood (UCB) is one of the cell sources for HSC transplantation (HSC-T) and has several advantages, non-invasiveness collection procedure, better tolerated across the HLA barrier, and relatively low risk of GVHD. On the other hand, the presence of an insufficient number of HSCs in these preparations limits their use, prompting need for ex vivo HSC amplification. To establish a more efficient method to expand UCB HSCs, we developed the SL-13R peptide and cultured UCB CD34+ cells with peptide in serum-free medium containing a cytokine cocktail for 9 days. After culturing UCB CD34+ cells with the peptide for 9 days, the number of CD34+CD38- cells increased 1.5-fold compared to control. The effect was also confirmed in mobilized peripheral blood CD34+ cells cultured for 9 days using the peptide. Transplantation of UCB CD34+ cells cultured with SL-13R into immunodeficient NOD/Shi-scid/IL-2RGAMMA knockout mice confirmed that they possess long-term reconstitution ability. To understand the mechanisms of HSC expansion by SL-13R, we investigated peptide binding proteins using biotin-conjugated SL-13R and identified AHNAK proteins as interactors of SL-13R by LC-MS/MS and MASCOT analysis. AHNAK knockdown UCB CD34+ cells cultured with bioactive peptide showed a decreased number of hematopoietic colonies. These results suggest that AHNAK functions in HSC expansion promoted by SL-13R. In summary, we have identified a novel bioactive peptide promoting expansion of UCB and peripheral blood CD34+ cells with long-term reconstitution ability. Its use may facilitate clinical use of HSCs.

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Keywords: Hematopoietic stem cell, peptide, ex vivo

745

AMINO ACID BASED DIETARY PREVENTION OF TERATOMA DEVELOPMENT FROM PLURIPOTENCY

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Discovery of human pluripotent stem cell (hPSC) offer great promise for regenerating a diseased tissue and ultimately, providing a therapeutic mean. One major hurdles involves the risk of teratoma formation from remaining hPSC in vivo. Despite early safety proof in hPSC based therapy in humans, current therapy utilizes relatively smaller number of differentiated cells, thus there are some uncertainty as to teratoma production if numerous cells be transplanted. Recent studies indicate more than 1,000,000 of contaminated undifferentiated hPSC encompasses risks of teratoma formation in vivo after transplantation. Given that the other quality control analysis has lower limit of detection, it is vital to develop supportive means to prevent formation and/or proliferation of undesired cells derived from stem cells to alleviate the safety concerns. Herein, we hypothesized in vivo nutrient modulation can influence hPSC behaviors

Poster Abstracts

upon transplantation with a major focus on amino acid (AA) imbalance. We applied essential and non-essential amino acid deficient diets to test this hypothesis in 107 hPSC bearing mouse. In our in vivo screening study, we found that valine (68 days treatment), a branched chain AA, deficient diet (50 days treatment) has the greatest inhibitory effect on hPSC-derived teratoma formation of the tested several AA specimens. In line with this, the number of undifferentiated hPSCs cultured in the valine-deprived medium significantly decreased as compared with the valine-containing medium. Collectively, transient dietary valine deprivation has a great potential to augment the safety of hPSC use in clinical transplantation.

Keywords: human pluripotent stem cell (hPSC), teratoma formation, amino acid deficient diets

746

DEVELOPMENT OF ALLOGENEIC CELL BANK USING POLYDACTYLY-DERIVED CHONDROCYTES FOR CHONDROCYTE SHEET TRANSPLANTATION TO TREAT OSTEOARTHRITIS

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Allogeneic chondrocyte transplantation is a novel and promising therapy for cartilage defects associated with osteoarthritis. Dr. Masato Sato at Tokai University established sheet fabrication method using polydactyly-derived chondrocytes on temperature-responsive culture inserts. We are currently developing allogeneic cell therapy product (CLS2901C) based on this technology. To develop this product, it requires allogeneic cell source that is available for commercial use. Therefore, we established a sustainable tissue procurement system from patients with polydactyly in collaboration with National Center for Child Health and Development. Using this system, we obtained 19 tissue samples from 14 donors and optimized manufacturing processes including tissue transportation, chondrocytes isolation, cell expansion, cryopreservation, sheet fabrication, and quality testing. Then we evaluated characteristics of the cell sheet manufactured from the polydactyly-derived cells. We confirmed that the cell sheet highly expressed surface antigen of mesenchymal stem cells (CD44, CD 81, and CD 90) and secreted TGF-BETA. With gene expression analysis of type 1/ type 2 collagen and histopathological analysis, the cell sheet showed chondrogenic potential when cultured in 3D culture system established by Dr. Sato. The sheet did not show any severe toxicity in nude rat osteochondral defect model nor showed tumorigenicity in soft agar colony formation assay. We completed fundamental assessment of characteristics and safety of the chondrocyte sheet. Based on the results, we plan to establish a cell bank for large-scale production of CLS2901C, and to ensure the quality and the safety by validated testing methods. We also plan to submit its clinical trial notification in Japan in the near future.

Funding Source
Japan Agency for Medical Research and Development
Keywords: tissue procurement, chondrocyte sheet, allogeneic transplantation

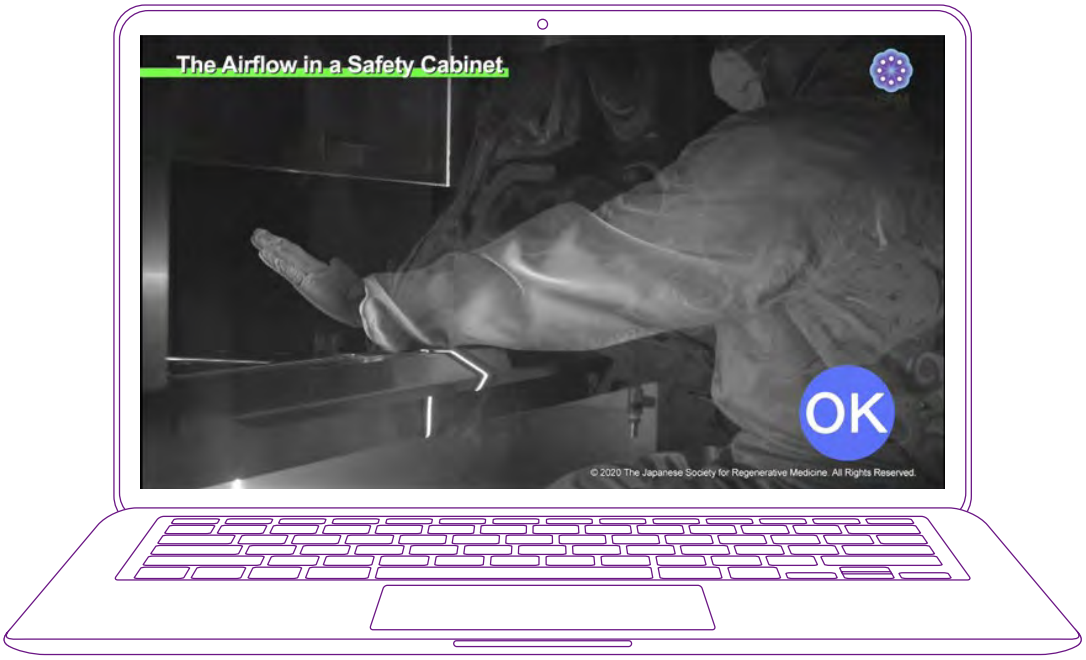
Author Index

PRESENTER NAME	PAGE NUMBER
Akutsu, Hidenori	77
Alvarez Fallas, Mario Enrique	76
Ando, Miki	32
Antos, Christopher L.	51
Aoki, Takahiro	119
Asakura, Atsushi	21
Baba, Koichi	78
Baric-Parker, Jean B	110
Becklin, Kelsie L	90
Bernardes De Jesus, Bruno	103
Chan, Hon Fai	104
Chanthra, Nawin	68
Chetty, Sundari	82
Cleitman Blackstien, Gal	49
Conesa-Buendía, Francisco M	123
Cong, Le	32
Currie, Joshua D.	34
Daghero, Hellen	77
De Jong, Simone	105
Eguchi, Susumu	98
Elfadil Ahmed, Razan	52
Enomoto, Hideki	53
Eto, Koji	37
Fan, Jing	37
Fujioka, Masato	118
Fujita, Masahiro	55, 128
Fujita, Misao	26
Funakoshi, Shunsuke	68
Furuhashi, Kazuhiro	124
Furukawa, Satomi	114
Futatsugi, Yoko N	81
Gage, H. Fred	27
Gotoh, Yukiko	22
Guo, Ge	29
Haim, Guy	50
Hara, Susumu	59
Harada, Yujin	54
Hatakeyama, Jun	49
High, A. Katherine	31
Hirai, Takamasa	57
Homma, Kohei	86
Horie, Kazumasa	74
Hotta, Akitsu	110
Hsieh, Wan-Ying	124
Ikeo, Satoshi	115
Imaizumi, Kent	25
Imamura, Keiko	94
Imamura, Satoshi	51
Inagaki, Emi	89
Ishikawa, Mitsuru	66
Ito, Takuji	95
Jonlin, Erica	33
June, Carl	31
Kajihara, Ryota	109
Kamada, Kohei	125
Kamon, Masayoshi	50
Kanda, Yutaro	103
Kaneda, Hayato	65
Kaneko, Naoko	48
Kaneko, Shin	122
Kase, Yoshitaka	48
Katagiri, Naoko	63
Kataoka, Takeshi	106
Katsuma, Asako	43
Kawai, Momotaro	127
Kawai, Shunsuke	58
Kawasaki, Hiroshi	40
Kawasaki, Satoshi	93
Kawatou, Masahide	99
Kikuchi, Tetsutaro	65
Kim, Mee-hae	57
Kimura, Azuma	129
Kishimoto, Keiko	56
Kitagawa, Takahiro	126
Kobayashi, Jun	101
Kobayashi, Yoshiomi	112
Kobayashi-Ishihara, Mie	46
Komura, Makoto	46
Konno, Katsuhiro	131
Krol, Rafal P.	72, 111
Kume, Shoen	98

Author Index

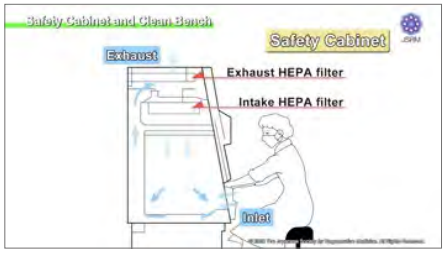
PRESENTER NAME	PAGE NUMBER
Kusakawa, Shinji	127
Kuzumaki, Naoko	93
Lan, Kuan Chun	56
Leventoux, Nicolas	90
Lewis Israeli, Yonatan R.	25
Li, Menglu	61
Liput, Michal	81
Lutolf, Matthias P.	31
Mabuchi, Yo	39
Maeda, Sumihiro	74, 87
Manley, William	65
Marei, Isra	130
Maruyama, Yusuke	55
Masumoto, Hidetoshi	99
Matsumoto, Mami	43
Matsumoto, Ryusaku	80
Matsuno, Kumiko	100, 106
Matsuo, Junko	132
Matsuura, Ryohei	118
Méndez-Ferrer, Simón	36
Minakawa, Tomohiro	58
Minobe, Toshiki	45
Mitsuzawa, Shio	91
Miura, Kyoko	34
Miura, Yasutomo	108
Miyabe, Yoei	102
Miyamoto, Daisuke	129
Miyawaki, Atsushi	22
Miyawaki, Shingo	61
Morikawa, Takayuki	71
Morimoto, Satoru	83
Morita, Ritsuko	21
Murai, Nobuyuki	121
Murakami, Rei	96
Nakagawa, Masato	44, 67
Nakanishi, Anna	73
Nakauchi, Hiromitsu	29
Nemoto, Akisa	96
Niikura, Takahiro	114
Nio, Yasunori	131
Nishida, Hidetaka	116
Nishimura, Toshiya	53
Ogata, Kenichi	125
Ohshima, Hayato	42
Ohta, Kunimasa	83
Okada, Kiyoshi	52
Okano, Hideyuki	28
Okui, Go	47
Oliva, Joan	68
Omiya, Hanae	63
Onodera, Kazunari	88
Osafune, Kenji	117
Ouchi, Rie	27
Ouchi, Takehito	41
Parmar, Malin	38
Pasca, P. Sergiu	25
Pellegrini, Laura	35
Plummer, Richie T.	80
Pode Shakked, Naomi P.	40
Prudon, Nicolas	121
Qian, Emi	69
Rashid, Muhammad I	89
Renault-Mihara, Francois	67
Sada, Aiko	33
Sadahiro, Taketaro	109
Saegusa, Chika	91
Saiki, Norikazu	76
Saito, Yoichi	70
Sakaguchi, Masanori	120
Sakashita, Akihiko	41
Sakimoto, Susumu	60
Sakuma, Kensuke	117
Sarel Gallily, Roni Z.	28
Sato, Katsuya	113
Sato, Tsukika	92
Sekiya, Sachiko	105
Seo, Ha-rim	78
Shaji, Maneesha	79
Shibuya, Soichi	102
Shibuya, Yoichiro	116
Shiga, Takahiro	84
Shimada, Hiroko	79
Shimizu, Reina	107
Shimizu, Tatsuya	88
Shimmura, Shigeto	113
Shinozaki, Munehisa	123
Shiota, Goshi	119

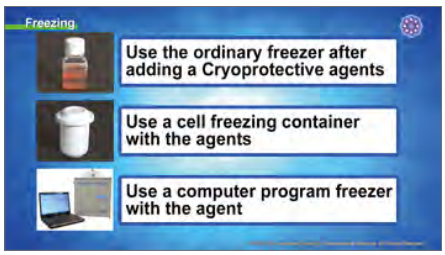
PRESENTER NAME	PAGE NUMBER
Skoniecka, Aneta	66
Snyder, Kimberly A.	70
Sone, Naoyuki	95
Song, Sun	67
Suda, Yukari	94
Sugawara, Tohru	62
Supakul, Sopak	97
Suzuki, Naoki	72
Suzuki, Takahiro	45
Tabuchi, Ayumu	75
Takada, Takuma	100
Takafuji, Yoshimasa	104
Takahashi, Katsu	101
Takahashi, Masayo	36
Takahashi, Takumi	115
Takanori, Takebe	26
Takemori, Chihiro	87
Takimoto, Kurumi	107
Tanabe, Shihori	85
Tani, Shoichiro	47
Taniguchi-Ikeda, Mariko	82
Thanuthanakhun, Naruchit	44
Togo, Kazuyuki	64
Tsai, Li-kuang	39
Tsang, Suk Ying	53
Tsuchimochi, Ryosuke	126
Tsuchiya, Atsunori	113
Treutlein, Barbara	24
Uehara, Keiichiro	73
Uyama, Hirofumi	112
Valadares, Marcos C	122
Van Eyk, Jessica	59
Van Spranh, Johnick	75
Vassilev, Svetlan	30
Wada, Masanori	70
Wada, Takumi	69
Watanabe, Hirotaka	84
Watanuki, Shintaro	23
Yakushiji Kaminatsui, Nayuta	60
Yamaguchi, Akihiro	85
Yamamura, Yuki	54
Yamanaka, Shinya	24
Yamane, Junko	71
Yamazaki, Rika	97
Yang, Zhennan	60
Yano, Masato	86
Yashiro, Yoshimi	30
Yasuda, Katsutaro	130
Yasukawa, Tsutomu	108
Yilmaz, Atilgan	49
Yoneyama, Yosuke	23
Yoshimatsu, Sho	31
Yoshioka, Miki	120
Yui, Shiro	35
Yuizumi, Naoya	62
Zernicka-Goetz, Magdalena	21
Zheng, Chenhuang	111
Zhou, Baoyi	42



Cell Processing Operations Model Video

The Japanese Society for Regenerative Medicine has produced a comprehensive video resource for cell processing operators.
These videos show a series of model operations from entering to exiting the clean room, as well as mistakes that can easily be made in culture operations.
We have already completed the Japanese and English versions of these contents and are expecting to translate them into various languages. We are willing to provide the translated versions to organizations that wish to use them under a paid license.
(General inquiries: hrd@jsrm.jp)





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