WELCOME

Dear Colleagues:

We would like to welcome you on behalf of the Stem Cell Society of Singapore (SCSS) and International Society for Stem Cell Research (ISSCR). The SCSS and ISSCR are proud to partner together to bring you *Global Controls in Stem Cells*, a joint-conference of the ISSCR Regional Forum series and the SCSS 6th Annual Symposium.

We have created a diverse, engaging programme that will explore the fundamental molecular mechanisms controlling cell identity. Throughout the next few days, you will hear from leaders in embryonic stem cell pluripotency, development and cellular reprogramming.

Our supporters and exhibitors help make this meeting possible. We ask that you join us in thanking them for their support by exploring the newest tools on exhibit during the meeting and recognizing the supporters listed in our acknowledgements.

As always, we are grateful for your support – thank you for joining us. The measure of our meeting's success is not only the information you gain from listening to presentations, but also the connection and future collaborations that can be initiated. Singapore is recognized as a global leader in increasing international and academic-corporate collaboration, and we hope to facilitate even more growth in these efforts. Our meeting is small enough to allow access to our speakers and leaders, so we encourage you to engage in conversation during tea breaks, lunches or poster receptions.

On behalf of all of the organizers and people who made this event possible, we hope you find great value during the scientific sessions and in the new connections you are able to cultivate.

Sincerely,
Members of the Organizing Committee

Huck-Hui NG

Richard A. YOUNG

Ron MCKAY

Gerald UDOLPH

TABLE OF CONTENTS

GLOBAL CONTROLS IN
STEM CELLS

ACKNOWLEDGEMENTS

The ISSCR and SCSS would like to thank our supporters for making this Forum possible.

PLATINUM

Fluidigm®

GOLD

BECKMAN COULTER
Life Sciences

SILVER

abcam® discover more
GE Healthcare
life technologies™
Lonza
STEMCELL™ TECHNOLOGIES

BRONZE

apceth applied cell therapy
BD
MACS Miltenyi Biotec
PAKAIR
RAJAH & TANN ASIA LAWYERS WHO KNOW ASIA
STEMGENT®
Takeda

ACADEMIC

Agency for Science, Technology and Research (A*STAR)
Bioinformatics Institute
Department of Biological Sciences
Duke-NUS Graduate Medical School Singapore
Genome Institute of Singapore
Institute of Medical Biology
Institute of Molecular and Cell Biology
School of Biological Sciences, Nanyang Technological University

CONTRIBUTORS

Bio-techne
Eupheria Biotech GmbH
SPD Scientific
UBS AG, Singapore

LEADERSHIP

EVENT ORGANIZING COMMITTEE

Ronald D. MCKAY
Lieber Institute for Brain Development, USA

Huck-Hui NG
Genome Institute of Singapore, Singapore

Richard A. YOUNG
Whitehead Institute for Biomedical Research, USA

Gerald UDOLPH
Stem Cell Society Singapore, Singapore

LOCAL ORGANIZING COMMITTEE

Leah A. VARDY
Institute of Medical Biology, Singapore

Jonathan LOH
Institute of Molecular and Cell Biology, Singapore

Winnie LIM
Genome Institute of Singapore, Singapore

Vivek TANAVDE
Bioinformatics Institute, Singapore

Susan LIM
Visiting Scholar, University of California, Berkeley and Co-chair, Global Advisory Council, ISSCR

Indira RAMASAMY
Genome Institute of Singapore, Singapore

STEM CELL SOCIETY SINGAPORE BOARD OF DIRECTORS

SOCIETY OFFICE BEARERS

Huck Hui NG, President
Genome Institute of Singapore, Singapore

Paul ROBSON, Vice-President
Genome Institute of Singapore, Singapore

Vivek TANAVDE, Secretary
Bioinformatics Institute of Singapore, Singapore

Ray DUNN, Vice-Secretary
Institute of Medical Biology, Singapore

Jonathan LOH, Treasurer
Institute of Molecular and Cell Biology, Singapore

Shigeki SUGII, Vice-Treasurer
Singapore Bioimaging Consortium, Singapore

SOCIETY EXECUTIVE COMMITTEE MEMBERS

Larry STANTON
Genome Institute of Singapore, Singapore

Hongyan WANG
Duke-NUS, Singapore

Evelyn YIM
NUS, Department of Bioengineering, Singapore

Hannes HENTZE
Nanyang Technological University, Singapore

Steve OH
Bioprocessing Technology Institute, Singapore

Karl TRYGVASSON
Duke-NUS, Singapore

Peter DROEGE
Nanyang Technological University, Singapore

Leah A. VARDY
Institute of Medical Biology, Singapore

Wei Seong TOH
National University Singapore, Singapore

LEADERSHIP

INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH BOARD OF DIRECTORS

OFFICERS

Rudolf JAENISCH, President
Whitehead Institute for Biomedical Research, USA

Janet ROSSANT, Past President
The Hospital for Sick Children Research Institute, Canada

Sean J. MORRISON, President Elect
UT Southwestern Medical Center, USA

George Q. DALEY, Clerk
Boston Children's Hospital, USA

Sally TEMPLE, Vice President
Neural Stem Cell Institute, USA

Haifan LIN, Treasurer
Yale University School of Medicine, USA

DIRECTORS

Timothy ALLSOPP
Neusentis Regenerative Medicine, UK

Arturo ALVAREZ-BUYLLA
University of California, San Francisco, USA

Nissim BENVENISTY
Hebrew University, Israel

Hans C. CLEVERS
Hubrecht Institute, Netherlands

Hongkui DENG
College of Life Sciences at Peking University, China

Connie J. EAVES
Terry Fox Lab, BC Cancer Agency, Canada

Urban LENDAHL
Karolinska Institute, Sweden

Christine L. MUMMERY
Leiden University Medical Center, Netherlands

Hideyuki OKANO
Keio University, School of Medicine, Japan

Kathrin PLATH
University of California, Los Angeles, USA

David T. SCADDEN
Massachusetts General Hospital and Harvard University, USA

Austin G. SMITH
Wellcome Trust-Medical Research Council Cambridge Stem Cell Institute, UK

Deepak SRIVASTAVA
Gladstone Institutes, USA

Elly TANAKA
DFG Research Center for Regenerative Therapies, Technische Universitaet Dresden, Germany

Amy WAGERS
Joslin Diabetes Center, USA

Shinya YAMANAKA
Center for iPS Cell Research and Application, Japan

EX OFFICIO MEMBERS

Elaine FUCHS
Rockefeller University, USA

Fred H. GAGE
Salk Institute for Biological Studies, USA

Leonard I. ZON
Boston Children's Hospital, USA

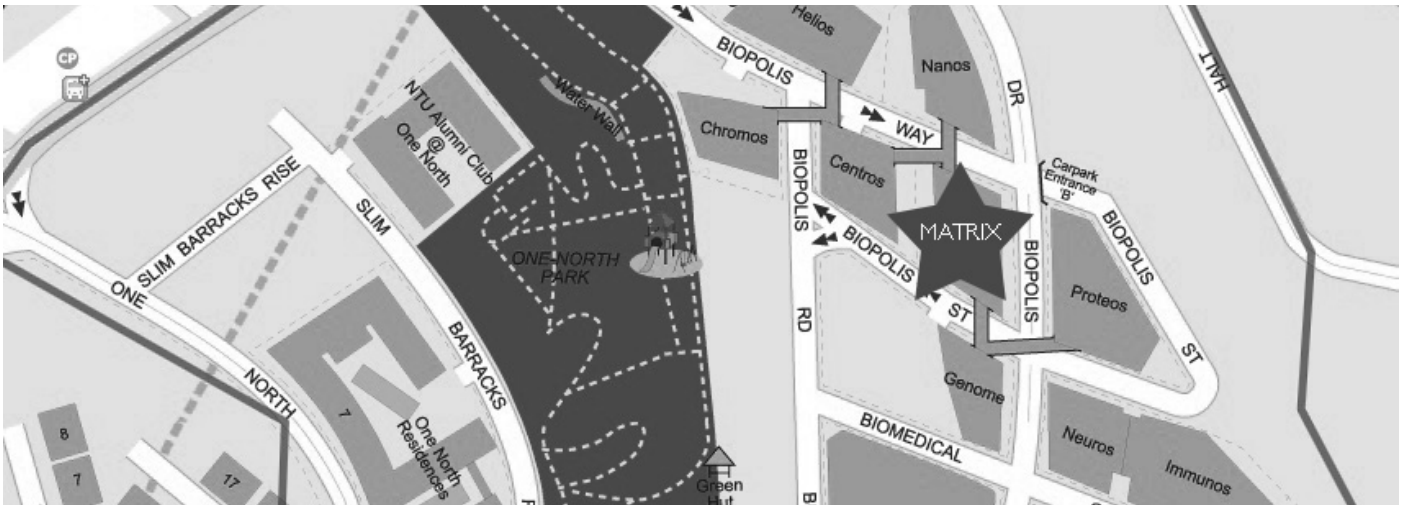
GENERAL INFORMATION

NAVIGATING THE BIOPOLIS

The Forum is located at the Matrix Building, 30 Biopolis Street, Biopolis, Singapore. All sessions will be in The Auditorium, Matrix Building which can be accessed in Levels 2 and 4.

Registration, lunches, tea breaks, and Platinum, Gold and Silver exhibitors are located in Level 2, Matrix Building outside the main entrance to the Auditorium. Additional lunch and tea break stations as well as Bronze exhibitors will be located in Level 4, Matrix Building. The poster sessions and receptions are all located at Level 4, Matrix Building.

Attendees registered for the Networking Safari Night Tour on Thursday 6 November will be picked up at the Biopolis at 19.00 sharp.



LEVEL 2

- Registration Desk
- Access to the Auditorium
- Morning and Tea Break stations
- Lunch stations
- Exhibition area for these exhibitors:

Fluidigm
BECKMAN COULTER Life Sciences
abcam discover more
GE Healthcare
life technologies
Lonza
STEMCELL TECHNOLOGIES
MACS Miltenyi Biotec

GENERAL INFORMATION

LEVEL 2M

- Access to Auditorium

LEVEL 4

- Morning and Tea Break stations
- Lunch stations
- Poster Hall
- Poster Receptions
- Exhibition area for these exhibitors:

REGISTRATION AND BADGE PICKUP

Pick up your attendee name badge at the registration desk in Level 2, Matrix Building, Biopolis during posted hours. Bring your meeting confirmation receipt and a photo ID. Name badges are required for admission to all sessions, poster presentations and the exhibition areas. Since the meeting badge serves as proof of participation, all attendees, speakers and exhibitors are required to wear their badges at all times during the Forum as well as the Networking Tour and all other special events. Access to events may be refused if the meeting badge is not displayed.

An administration charge of $20 SGD will be imposed for any replacement badge. A drop box for badge recycling will be provided at the registration counter in Level 2, Matrix Building on Friday 7 November.

INTERNET ACCESS

Complimentary access to the internet is available within the Matrix Building during the Forum. To access the internet, select the *Biopolis Shared Facilities* network; no password is required. Please note that the bandwidth of this connection might be limited.

As a courtesy to speakers, please be sure to silence any mobile phones and devices and refrain from using the internet during sessions.

GENERAL INFORMATION

HELP DESK

Please visit the registration counter in Level 2, Matrix Building with any questions you may have.

EXHIBITION HALL

The exhibition areas feature 14 leading suppliers and vendors. Walk through the hall in Levels 2 and 4, Matrix Building and support the Exhibitors who help make this Forum possible.

COMMERCIAL TUTORIALS

Attendees are encouraged to attend the industry session's commercial talks. Sit in on Translating Stem Cells from Bench to Bedside industry session Wednesday 7 November, as well as the daily Tools and Technologies industry talks. All of these sessions will be held in the Auditorium, Level 2, Matrix Building.

RECORDING POLICY

Still photography, video and/or audio taping of the sessions, presentations and posters at the Forum is strictly prohibited. Additionally, blogging, tweeting and other intent to communicate or disseminate results or discussion presented at the meeting is prohibited until the start of each individual presentation. Thank you for your cooperation.

SPECIAL EVENTS

MORNING BREAKS

Complimentary coffee and tea will be served during the following days and times:

Wednesday 5 November, 8.15 – 9.00

Thursday 6 November, 8.15 – 9.00

Friday 7 November, 8.15 – 9.00

TEA BREAKS HOURS

Complimentary coffee, tea and light snacks will be served during the following days and times:

Wednesday 5 November, 10.00 – 10.30 and 16.00 – 16.30

Thursday 6 November, 10.15 – 10.45 and 15.30 – 16.00

Friday 7 November, 10.15 – 10.45 and 15.30 – 16.00

GENERAL INFORMATION

LUNCH HOURS

Complimentary lunch buffet will be served during the following days and times:

Wednesday 5 November, 12.30 – 14.00

Thursday 6 November, 12.15 – 14.00

Friday 7 November, 12.30 – 14.00

POSTER PRESENTATIONS AND RECEPTIONS HOURS

Poster receptions will provide complimentary beer and wine as well as hors d'oeuvres during the following times:

Wednesday 5 November, 18.30 – 20.00

Thursday 6 November, 17.00 – 18.30

NETWORKING TOUR

Attendees who registered for the Networking Safari Night Tour on Thursday, 6 November 2014 will have transportation provided to and from the Biopolis and the Safari.

Date: Thursday 6 November

Departure from Biopolis: 19.00 (after the Poster Reception)

Arrival Biopolis: 22.30 (speakers and guests of the Shangri-La Hotel will be dropped off at the hotel)

NOTE: The Networking Tour is non-refundable. Tour will take place rain or shine.

PROGRAMME SCHEDULE

WEDNESDAY 5 NOVEMBER

08.00 – 18.30	Forum Registration
08.00 – 09.15	Morning Coffee
09.00 – 09.10	Welcome Remarks & Opening Address **Ronald D. MCKAY**, *Lieber Institute for Brain Development, USA*
09.10 – 09.15	Welcome Remarks & Opening Address **Nancy WITTY**, *CEO, ISSCR*

SESSION I: REPROGRAMMING

Chair: **V. Narry KIM**, *Seoul National University, South Korea*

09.15 – 10.00	Keynote Address IPS CELL TECHNOLOGY, GENE EDITING AND DISEASE RESEARCH **Rudolf JAENISCH**, *Whitehead Institute for Biomedical Research, USA (Abstract S1)*
10.00 – 10.30	Tea Break
10.30 – 11.00	VITAMIN C REGULATES SOMATIC CELL REPROGRAMMING THROUGH HISTONE/DNA DEMETHYLASES **Duanqing PEI**, *Guangzhou Institute of Biomedicine & Health CAS, China (Abstract S2)*
11.00 – 11.30	DNA METHYLTRANSFERASE 3-LIKE IN GONOCYTES AND POSTNATAL SPERMATOGONIAL STEM/PROGENITOR CELLS **Bing LIM**, *Genome Institute of Singapore, Singapore (Abstract S3)*
11.30 – 11.45	MOLECULAR CONTROLS IN CELLULAR REPROGRAMMING **Jonathan LOH**, *Institute of Molecular and Cell Biology, Singapore (Abstract S4)*
11.45 – 12.00	THE RB TUMOR SUPPRESSOR RESTRICTS REPROGRAMMING BY DIRECTLY SILENCING PLURIPOTENCY NETWORKS **Michael KARETA**, *Stanford University, United States (Abstract S5)*
12.00 – 12.30	HUMAN HEPATOCYTES WITH DRUG METABOLIC FUNCTION INDUCED FROM FIBROBLASTS BY LINEAGE REPROGRAMMING **Hongkui DENG**, *College of Life Sciences, Peking University, China (Abstract S6)*
12.30 – 14.00	Lunch Break and Poster Viewing

SESSION II: CHROMATIN DYNAMICS

Chair: **Leah A. VARDY**, *Institute of Medical Biology, Singapore*

PROGRAMME SCHEDULE

14.00 – 14.30	GLOBAL REORGANIZATION OF CHROMATIN ARCHITECTURE DURING EMBRYONIC STEM CELL DIFFERENTIATION **Bing REN**, *University of California, San Diego, USA (Abstract S7)*
14.30 – 14.45	DISTINCT METHYLATION STATES OF H3K27 INFLUENCE CELL LINEAGE PREFERENCE IN EMBRYONIC STEM CELLS **Stan WANG**, *National Institutes of Health, United States (Abstract S8)*
14.45 – 15.00	DNA METHYLTRANSFERASE 3-LIKE IN GONOCYTES AND POSTNATAL SPERMATOGONIAL STEM/PROGENITOR CELLS **Shau-Ping LIN**, *National Taiwan University, Taiwan (Abstract S9)*
15.00 – 15.30	DNA OXIDATION TOWARDS PLURIPOTENCY IN MAMMALIAN DEVELOPMENT AND CELL REPROGRAMMING **Guoliang XU**, *Institute of Biochemistry & Cell Biology, Shanghai Institute for Biological Sciences, CAS, China (Abstract S10)*
15.30 – 16.00	CHROMATIN MODIFIERS IN EPIGENETIC CONTROL OF CANCER AND STEM CELLS **Sung Hee BAEK**, *Seoul National University, South Korea (Abstract S11)*
16.00 – 16.30	Tea Break
16.30 – 17.00	PIONEER FACTORS AND IMPEDIMENTS TO CELLULAR REPROGRAMMING **Ken ZARET**, *University of Pennsylvania School of Medicine, USA (Abstract S12)*

TRANSLATING STEM CELLS FROM BENCH TO BEDSIDE

17.00 – 17.30	DISSECTING CANCER HETEROGENEITY AND THERAPEUTIC RESPONSES AT SINGLE CELL RESOLUTION **Qiang TIAN**, *Institute for Systems Biology, USA (Abstract S13)* *Organized and supported by Fluidigm, USA*
17.30 – 18.00	CLINICAL DEVELOPMENT AND MANUFACTURING OF CELL-BASED BIOPHARMACEUTICALS FOR TARGETED DELIVERY OF THERAPEUTIC GENES IN AN ONCOLOGY TRIAL FOR ADVANCED CANCER **Ralf HUSS**, *apceth, Germany (Abstract S14)* *Organized and supported by apceth, Germany*

EVENING INDUSTRY SESSION: TOOLS AND TECHNOLOGIES

18.00 – 18.30	LONZA L7™ HIPSC REPROGRAMMING AND HPSC CULTURE SYSTEM - THE ROBUST GENERATION AND MAINTENANCE OF HUMAN PLURIPOTENT STEM CELLS UNDER DEFINED AND XENO-FREE CONDITIONS **Scott D'ANDREA**, *Lonza Pharma Bioscience Solutions, USA (Abstract S15)*
18.30 – 20.00	Opening Reception/Poster Presentations *(odd numbered posters)* *Supported by Bioinformatics Institute*

PROGRAMME SCHEDULE

THURSDAY 6 NOVEMBER

08.00 – 17.00	Forum Registration
08.15 – 09.00	Morning Coffee

SESSION III: DEVELOPMENTAL BIOLOGY OF PLURIPOTENCY

Chair: **Ronald D. MCKAY**, *Lieber Institute for Brain Development, USA*

9.00 – 9.30	GERMLINE: SPECIFICATION AND REPROGRAMMING FOR TOTIPOTENCY AND DEVELOPMENT **Azim SURANI**, *Wellcome Trust/Cancer Research UK Gurdon Institute/ University of Cambridge, UK (Abstract S16)*
9.30 – 9.45	UNCOVERING THE PLURIPOTENCY PROGRAM IN EMBRYONIC STEM CELLS **Sara-Jane DUNN**, *Microsoft Research, United Kingdom (Abstract S17)*
9.45 – 10.15	A ROLE FOR POLYAMINE REGULATORS IN EMBRYONIC STEM CELL SELF-RENEWAL AND DIFFERENTIATION **Leah A. VARDY**, *Institute of Medical Biology, Singapore (Abstract S18)*
10.15 – 10.45	Tea Break *Supported by GE Healthcare*
10.45 – 11.15	LINEAGE PROPENSITY OF THE MOUSE EPIBLAST STEM CELLS. ENDODERM DIFFERENTIATION IN RESPONSE TO TGF-BETA SIGNALLING ACTIVITY **Patrick P.L. TAM**, *Children's Medical Research Institute, Australia (Abstract S19)*
11.15 – 11.45	MOLECULAR MECHANISM TO MAINTAIN PLURIPOTENCY OF MOUSE ES CELLS **Hitoshi NIWA**, *RIKEN Center for Developmental Biology, Japan (Abstract S20)*

LUNCHTIME INDUSTRY SESSION: TOOLS AND TECHNOLOGIES

11.45 – 12.15	AN INTEGRATED SYSTEM FOR HUMAN BLOOD CELL REPROGRAMMING AND IPS CELL LINE SELECTION AND MAINTENANCE **Arwen HUNTER**, *STEMCELL Technologies Inc., Canada (Abstract S21)*
12.15 – 14.00	Lunch Break and Poster Viewing

SESSION IV: TELOMERE AND RNA BIOLOGY

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

PROGRAMME SCHEDULE

14.00 – 14.30	IDENTIFICATION AND FUNCTIONAL STUDIES OF RNA-BINDING PROTEINS IN EMBRYONIC STEM CELLS **V. Narry KIM**, *Seoul National University, South Korea (Abstract S22)*
14.30 – 15.00	Institute of Medical Biology (IMB) Lecture TRANSPOSABLE ELEMENTS AND THEIR EPIGENETIC CONTROL MECHANISMS ARE KEY REGULATORS OF TRANSCRIPTIONAL NETWORKS IN PLURIPOTENT STEM CELLS **Didier TRONO**, *EPFL, Switzerland (Abstract S23)*
15.00 – 15.30	NOVEL EPIGENETIC PATHWAYS IN PLURIPOTENT STEM CELLS **Andrew XIAO**, *Yale Stem Cell Center, USA (Abstract S24)*
15.30 – 16.00	Tea Break
16.00 – 16.30	LANDSCAPE AND VARIATION OF RNA SECONDARY STRUCTURES IN THE HUMAN TRANSCRIPTOME **Yue WAN**, *Genome Institute of Singapore, Singapore (Abstract S25)*
16.30 – 17.00	AGING OF HEMATOPOIETIC STEM CELLS **Toshio SUDA**, *Keio University, Japan (Abstract S26)*
17.00 – 18.30	Reception/Poster Presentations *(even numbered posters)* *Supported by Bioinformatics Institute*
19.00 – 22.30	NIGHT SAFARI NETWORKING TOUR **Registration required**

PROGRAMME SCHEDULE

FRIDAY 7 NOVEMBER

08.15 – 09.00	Morning Coffee

SESSION V: FUNCTIONAL GENOMICS

Chair: **Hongkui DENG**, *Peking University, China*

9.00 – 9.30	SYSTEMS BIOLOGY OF STEM CELLS **Huck Hui NG**, *Genome Institute Singapore, Singapore (Abstract S27)*
9.30 – 9.45	ROLE OF A REST REGULATED MICRORNA IN HUMAN MOTOR NEURON DEVELOPMENT AND DISEASE **Akshay BHINGE**, *Genome Institute of Singapore, Singapore (Abstract S28)*
9.45 – 10.15	PLURIPOTENCY IN THE ARTIFICIAL CELL SPACE **Andras NAGY**, *Mount Sinai Hospital, Lunenfeld-Tanenbaum Research Institute, Canada (Abstract S29)*
10.15 – 10.45	Tea Break
10.45 – 11.15	SYSTEMS DISSECTION OF PLURIPOTENT STEM CELLS **Frank BUCHHOLTZ**, *Max Planck Institute of Molecular Cell Biology and Genetics, Germany (Abstract S30)*
11.15 – 11.45	EPIGENETIC AND TRANSCRIPTIONAL DYNAMICS OF GERMLINE STEM CELLS **Bradley CAIRNS**, *HHMI and Huntsman Cancer Institute, University of Utah, USA (Abstract S31)*
11.45 – 12.00	INHIBITION OF THE UPR PATHWAY RESCUES MOTOR NEURONS DERIVED FROM PATIENTS WITH SPINAL MUSCULAR ATROPHY **Shi Yan NG**, *Harvard University, USA (Abstract S32)*

LUNCHTIME INDUSTRY SESSION: TOOLS AND TECHNOLOGIES

12.00 – 12.30	TAL-BASED EDITING OF iPSCS FOR THE STUDY OF NEURODEGENERATIVE DISEASES **Mark FEDERICI**, *Thermo Fisher Scientific (Abstract S33)*
12.30 – 14.00	Lunch Break and Poster Viewing

PROGRAMME SCHEDULE

SESSION VI: CANCER

Chair: **Andras NAGY**, Mount Sinai Hospital, Canada

14.00 – 14.30	ROLES FOR TRANSCRIPTIONAL SUPER-ENHANCERS IN CELL IDENTITY AND CANCER **Richard YOUNG**, *Whitehead Institute for Biomedical Research, USA (Abstract S34)*
14.30 – 14.45	A GENOME-WIDE RNAI SCREEN IDENTIFIES OPPOSING FUNCTIONS OF SNAI1 AND SNAI2 ON THE NANOG DEPENDENCY OF ESTABLISHING PLURIPOTENCY **Jianlong WANG**, *Icahn School of Medicine at Mount Sinai, USA (Abstract S35)*
14.45 – 15.00	TRANSCRIPTION DRIVEN BY PRIMATE-SPECIFIC ENDOGENOUS RETROVIRUS HERVH DEFINES NAIVE STEM CELLS **Zsuzsanna IZSVAK**, *Max-Delbrück-Center for Molecular Medicine, Germany (Abstract S36)*
15.00 – 15.30	MODELING MYELODYSPLASTIC SYNDROMES IN MICE BY ALTERED HOXA1 SPLICEFORM EXPRESSION **Louise PURTON**, *St Vincent´s Institute of Medical Research, Australia (Abstract S37)*
15.30 – 16.00	Tea Break
16.00 – 16.30	EZH2 INHIBITORS AS POTENTIAL THERAPEUTICS IN GERMINAL CENTER B CELL LYMPHOMAS **Jesse SMITH**, *Epizyme Inc. USA (Abstract S38)*
16.30 – 17.00	REGULATION OF NEURAL STEM/PROGENITOR CELL FATE IN THE EMBRYONIC AND ADULT MOUSE BRAINS **Yukiko GOTOH**, *University of Tokyo, Japan (Abstract S39)*
17.00 – 17.45	CLOSING KEYNOTE STEM CELLS FROM THE MAMMALIAN BLASTOCYST **Janet ROSSANT**, *Hospital for Sick Children Research Institute, Canada (Abstract S40)*
17.45	CLOSING REMARKS **Huck Hui NG**, *Genome Institute of Singapore, Singapore* **Richard A. YOUNG**, *Whitehead Institute for Biomedical Research and MIT, USA*

Discover more in epigenetics research

Find the full range of tools for all your epigenetics research including:

> ChIP kits

> Histone & chromatin preparation kits

> Enzyme activity assay kits

> A wide range of agonists, activators, antagonists & inhibitors

abcam.com/epigenetics

abcam®
discover more

SUPPORTER DIRECTORY

PLATINUM

FLUIDIGM

7000 Shoreline Ct. Suite #100
South San Francisco, CA 94080 USA
Phone: +1-650-269-3170
Fax: +1-650-871-7152
www.fluidigm.com

Fluidigm (NASDAQ:FLDM) develops, manufactures, and markets life science analytical and preparatory systems for growth markets such as single-cell biology and production genomics. Our systems include the BioMark™ HD System which is a multi-application platform, the C_1™ Single-Cell Auto Prep System which delivers an easy, complete workflow that captures and prepares individual cells for gene expression and sequencing, and the CyTOF® 2 which is a time-of-flight mass spectrometer for high-speed acquisition of highly multi-parametric single-cell proteomic data. We sell to leading academic institutions, clinical laboratories, and pharmaceutical, biotechnology, and agricultural biotechnology companies worldwide. Our systems are based on proprietary microfluidics and multi-parameter mass cytometry technology, and are designed to significantly simplify experimental workflow, increase throughput, and reduce costs, while providing excellent data quality. Fluidigm products are provided for Research Use Only. Not for use in diagnostic procedures.

GOLD

BECKMAN COULTER LIFE SCIENCES

Beckman Coulter Life Sciences
5350 Lakeview Parkway South Drive
Indianapolis, IN 46268 USA
Phone: +1-800-742-2345
www.beckmancoulter.com

As a market leader, Beckman Coulter Life Sciences provides tools for the next generation of biomedical breakthroughs. From pharmaceutical research to cell biology; genomics to proteomics; and sophisticated sample analysis and preparation, our systems deliver comprehensive system solutions to researchers and commercial laboratories worldwide.

SUPPORTER DIRECTORY

SILVER

ABCAM (HONG KONG) LTD.

Unit 712, 7th Floor, Lakeside 1, No. 8 Science Park West Avenue, Hong Kong Science Park
Pak Shek Kok, New Territories, Hong Kong
Phone: +852-26036823
Fax: +852-30161888
www.abcam.com

Abcam is a producer and distributor of high quality protein research tools. These tools enable life scientists to analyse components of living cells at the molecular level which is essential in understanding health and disease. We are headquartered in Cambridge UK, with an office in Hong Kong serving the rest of South East Asia. We have R&D and production facilities in Eugene Oregon, Cambridge UK, Bristol UK and Hangzhou China.

Our vision is to create the world's leading lifescience reagents company. Discover more with our high-quality protein research tools, expert technical support, fast global delivery, and up-to-date and honest data. Our growing range of products include primary and secondary antibodies, biochemicals, proteins, peptides, lysates, immunoassays and other kits. Products are made in-house, as well as sourced externally, and are extensively tested.

GE HEALTHCARE

GE Healthcare Pte Ltd
1 Maritime Square, #13-01
Harbourfront Centre
Singapore 099253
Phone: +65-6773-7303
Fax: +65-6773-7302
www.gelifesciences.com/

GE Healthcare provides transformational medical technologies and services to meet the demand for increased access, enhanced quality and more affordable healthcare around the world. GE (NYSE: GE) works on things that matter - great people and technologies taking on tough challenges. From medical imaging, software & IT, patient monitoring and diagnostics to drug discovery, biopharmaceutical manufacturing technologies and performance improvement solutions, GE Healthcare helps medical professionals deliver great healthcare to their patients.

LIFE TECHNOLOGIES

United States:
5791 Van Allen Way
Carlsbad, CA 92008 USA
Phone: 760-603-7200
Fax: 760-602-6500

Singapore:
33 Marsiling Industrial Estate Road 3, #07-06,
Singapore 739256
Phone: +65-6861-8638
Fax: +65-6861-9391
www.lifetechnologies.com

The Life Technologies™ family of instruments, everyday tools, and services offers high-quality, innovative life science solutions for every lab and every application. As a premier brand of Thermo Fisher Scientific, we believe in the power of science to transform lives.

LONZA

8830 Biggs Ford Rd.
Walkersville, MD 21793 USA
Phone: +1-301-898-7025
www.lonza.com

Lonza supports stem cell research with novel technology and services from the research laboratory to clinical application. Visit our booth to learn more about Lonza products, services and our new L7™ hPSC Reprogramming and Culture

SUPPORTER DIRECTORY

System. L7™ includes seven key components that function as a robust system:

1. Primary cells, including disease phentoypes
2. Nucleofector™ Technology
3. hPSC Reprogramming Kits
4. hPSC Medium
5. hPSC Matrix
6. hPC Passaging Solution
7. hPSC Cryosolution

This novel stem cell approach is a complete solution for the generation of induced pluripotent stem-cells (iPSCs) as well as the maintenance of human embryonic stem-cells (hESCs) and iPSCs under defined, xeno-free conditions. The L7™ culture system supports every-other-day feeding and long term culture and maintenance of hPSCs.

STEMCELL TECHNOLOGIES INC.

570 West Seventh Avenue, Suite 400
Vancouver, BC, V5Z 1B3, Canada
Phone: +1-604-877-0713
Fax: +1-604-877-0704
www.stemcell.com

STEMCELL Technologies Inc is a privately-owned biotechnology company that develops specialty cell culture media, cell separation products and ancillary reagents for life science research. Driven by science and a passion for quality, STEMCELL delivers over 1500 products to more than 70 countries worldwide. STEMCELL has been dedicated to providing standardized reagents and tools for all areas of stem cell research, and has extended this expertise to many other sectors of life science. The importance of quality control processes and raw material selection is critical to the production and optimal performance of STEMCELL's entire portfolio of products, ensuring that batch-to-batch variability is minimized. To learn more, please visit www.stemcell.com.

BRONZE

APCETH® GMBH & CO. KG

Max-Lebsche-Platz 30
D-81377 Munich
Germany
Phone: +49 (0)89-7009608 - 0
Fax: +49 (0)89-7009608 - 79
www.apceth.com/home/

apceth® is one of the leading European pioneers in the development, manufacturing and clinical implementation of innovative cell therapeutics. apceth's® unique platform technology employs human adult stem cells in order to produce cell products of the highest quality according to the EU regulations for Advanced Therapy Medicinal Products (ATMPs). The growing portfolio of apceth's® cell therapeutics aims for the treatment of advanced cancer and various nonmalignant diseases where standard medicine fails to achieve satisfactory results.

BD BIOSCIENCES

3A International Business Park
#12-10/18, ICON @ IBP
Singapore 609935
Phone: +65-6432-2034
www.bdbiosciences.com/sg/

BD Biosciences provides flow cytometers, reagents, tools and a wide range of services to support the work of researchers and clinicians who understand disease and improve care.

SUPPORTER DIRECTORY

MILTENYI BIOTEC GMBH

Friedrich-Ebert-Strasse 68
Bergisch Gladbach 51429 Germany
Phone: +49-2204-83060
Fax: +49-2204-85197
www.miltenyibiotec.com

With over 1400 employees worldwide, Miltenyi Biotec helps move basic research to the clinic. Innovative tools for stem cell research and clinical applications support biomedical scientists in their studies of adult stem cells, as well as ES and iPS cells.

PAKAIR

59-61 Leveson Street
North Melbourne
VIC 3051 Australia
Phone: +61-3-5825-5310
www.pakair.com.au

PAKair Cargo Specialists is a premier Time and Temperature sensitive courier & cargo solution service provider. Our services are available 24 hours a day, 7 days a week. PAKair offers solutions to meet your specialized requirements. If security, timeliness and efficiency is an issue, our network is built on direct single consignment movements, eliminating the use of a "hub and spoke" system or a slow, unreliable consolidation system. We have the capacity and capability to achieve the desired result each and every time.

RAJAH & TANN SINGAPORE LLP

9 Battery Road
#25-01 Straits Trading Building
Singapore 049910
Republic of Singapore
www.rajahtann.com/

Rajah & Tann has grown to be one of the largest full service law firms in Singapore, providing full service and high quality advice to an impressive list of clients. We have more than 300 lawyers, many ranked among the very best in their specialist practice areas.

STEMGENT

51 Moulton St
Cambridge, MA 02138 USA
Phone: +1-617-245-0000
www.stemgent.com

Stemgent, a leading supplier of cellular reprogramming products and services and tissue-based research, provides a comprehensive portfolio of tools for cell research, and is recognized as expert in stem cell reprogramming, provide mRNA reprogramming systems, cell culture media, antibodies, small molecules, and iPS reprogramming services.

TAKEDA PHARMACEUTICAL COMPANY LIMITED

1-1, Doshomachi 4-chome, Chuo-ku
Osaka 540-8645 Japan
Phone: +81-6-6204-2111
Fax: +81-6-6204-2880
www.takeda.com

Our mission is simple and compelling: we are committed to strive toward better health for people worldwide through leading innovation in medicine. Our commitment to improving health and supporting our employees, partners and the larger community gives us the purpose to build on the tremendous success we enjoy as an emerging global leader in the pharmaceutical industry.

SUPPORTER DIRECTORY

ACADEMIC

AGENCY FOR SCIENCE, TECHNOLOGY AND RESEARCH (A*STAR)

1 Fusionopolis Way, #20-10
Connexis North Tower, Singapore 138632
Phone: (65)-6826-6111
Fax: (65)-6777-1711
www.a-star.edu.sg

The Agency for Science, Technology and Research (A*STAR) is Singapore's lead public sector agency that fosters world-class scientific research and talent to drive economic growth and transform Singapore into a vibrant knowledge-based and innovation driven economy.

In line with its mission-oriented mandate, A*STAR spearheads R&D in fields that are essential to grow Singapore's manufacturing sector and catalyse new growth industries. A*STAR supports these economic clusters by providing intellectual, human, and industrial capital to its partners in industry.

A*STAR oversees 18 biomedical sciences as well as physical sciences and engineering research entities, located in Biopolis and Fusionopolis, as well as their vicinity. These two R&D hubs house a bustling and diverse community of local and international research scientists and engineers from A*STAR's research entities as well as a growing number of corporate laboratories.

BIOINFORMATICS INSTITUTE

30 Biopolis Street
#07-01 Matrix, Singapore 138671
Phone: +65-6478-8298
www.bii.a-star.edu.sg/index.php

Located in the Biopolis, Bioinformatics Institute (BII) is conceived as the computational biology research and postgraduate training institute as well as a national resource centre in bioinformatics within the Biomedical Research Council (BMRC) of A*STAR. The BII focuses on theoretical approaches aimed at understanding biomolecular mechanisms that underlie biological phenomena, the development of computational methods to support this discovery process, and experimental verification of predicted molecular and cellular functions of genes and proteins with biochemical methods.

DEPARTMENT OF BIOLOGICAL SCIENCES

National University of Singapore
14 Science Drive 4
Singapore 117543
Phone: +65-162692
Fax: +67-792486
www.dbs.nus.edu.sg/

The mission of the Department of Biological Sciences is education, research, and outreach. DBS is an international department with an international faculty and strong international ties in research and teaching with MIT through the Singapore-MIT Alliance (SMA) and the MIT SMART research groups, with labs at Tsinghua and Xiamen University through the Trilateral Research Collaboration, and with top Indian research institutes and universities such as the Indian Institute of Science, Anna University, and the University of Madras.

DUKE-NUS GRADUATE MEDICAL SCHOOL

8 College Road, Singapore 169857
Phone: +65-6516-7666
www.duke-nus.edu.sg/

Duke-NUS was established in 2005 as a strategic collaboration between Duke University, N. Carolina, USA and National University of Singapore (NUS). The Duke-NUS Graduate Medical School is one of Singapore's leading medical schools and a prolific contributor to world-class scientific research. It has pioneered a graduate-entry, US-styled medical training program that is based on Duke University School of Medicine's curriculum and enrolls over

SUPPORTER DIRECTORY

60 medical students annually. A number of medical graduates will go on to develop careers as clinicians and clinician scientists. Together with their PhD counterparts, Duke-NUS graduates play increasingly critical roles in translating meaningful scientific discoveries into quality innovations in patient care.

GENOME INSTITUTE OF SINGAPORE

60 Biopolis Street, Genome, #02-01
Singapore 138672
Phone: +65-6808-8000
Fax : +65-6808-8292
www.gis.a-star.edu.sg/internet/site/

The Genome Institute of Singapore is a unique place for scientific discovery. We believe that a future in biology lies in the fusion of highly comprehensive and massively parallel genomic and computational approaches with cell and medical biology. Therefore, we seek the integration of technology and biology towards answering questions of medical importance. We support this vision by creating a social culture that encourages open communication, fluid organizational structures and teamwork but without diminishing individual effort. Our approach is strategic and is based on long-term scientific and social objectives.

INSTITUTE OF MEDICAL BIOLOGY

8A Biomedical Grove, #06-06 Immunos
Singapore 138648
www.imb.a-star.edu.sg/php/main.php

Research activities in IMB today range across stem cells, development and differentiation, skin biology, cancer and genetic diseases. By studying how molecular changes lead to increasing cell specialization and complexity in the context of human tissues and diseases, new knowledge gained will lead us to novel therapeutic strategies for improved quality of life. From Singapore and around the world, our scientists are bringing together their pioneering spirit and creative research talent to form a research institute of international excellence. IMB's research will help to build the bridges between basic science and clinical medicine that are so necessary to nurture an innovative and productive biomedical industry here in Singapore.

INSTITUTE OF MOLECULAR AND CELL BIOLOGY

61 Biopolis Drive
Proteos
Singapore 138673
Fax: +65-6779-1117
www.imcb.a-star.edu.sg/php/main.php

The Institute of Molecular and Cell Biology (IMCB) has established itself as a premier molecular and cell biology institute. Our vision is to remain a premier molecular biology and cell institute with increasing focus on addressing the mechanistic basis of human diseases. Our mission is to conduct cutting-edge discovery research in disease pathways; groom early career researchers to be future leaders in their fields; and collaborate with medical & industry communities for research impact. IMCB's research focuses on Discovery research and Translational research.

SCHOOL OF BIOLOGICAL SCIENCES, NANYANG TECHNOLOGICAL UNIVERSITY

60 Nanyang Drive, Singapore 637551
Phone: +65-6316-2800
Fax: +65-6791-3856
www.sbs.ntu.edu.sg

The field of Life Sciences has been widely acknowledged as the next major technological revolution after the Internet. New knowledge and discoveries generated as a result of genome and molecular biology research will have major impact on the Life Sciences and pharmaceutical industries, healthcare, food and nutrition, people and the environment. It is therefore not surprising that the Singapore government has committed

SUPPORTER DIRECTORY

itself to developing Life Sciences to be the forth pillar of Singapore's economy. The NTU School of Biological Sciences was established in July 2001 to meet this great demand for the training of high quality research scientists and executives in this field. We aim to provide students with a high quality university education and training in the life sciences. Graduates from its undergraduate B.Sc.(Honours) course as well as from its graduate research programmes are expected to fill the rapidly increasing demand for a talent pool of high-quality workforce and researchers for the life sciences industry.

CONTRIBUTORS

BIO-TECHNE

614 McKinley Place NE
Minneapolis, MN 55413 USA
Phone: +1-612-379-2956
www.RnDSystems.com

Expanding coordination and experience within our organization brings together expertise in a variety of disciplines and functional areas. By combining our best-in-class brands, Bio-Techne will leverage our resources and capabilities to create solutions that will deepen our relationships with our customers and allow us to become even better strategic partners.

SPD SCIENTIFIC

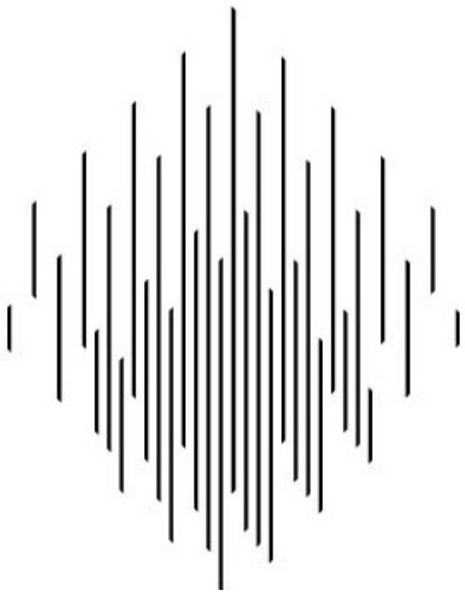
192 Pandan Loop #06-20
Pantech Business Hub 128381 Singapore
Phone: 6303-9877
Fax: 6775-0995
www.biomediaholdings.com

SPD Scientific provides a wide range of biomedical and laboratory equipment, reagents, test-kits, laboratory consumables, as well as microbiology culture media and diagnostic products. The company plays a key role for customers in industries such as healthcare, academic and government research institutions, pharmaceutical and food and beverage, by providing them with the appropriate tools that aid them in their research and medical discoveries.

EUPHERIA BIOTECH GMBH

Tatzberg 47-51
01307 Dresden, Germany
www.eupheria.com/home/

Our mission is dedication to our customer's research needs delivering high quality RNAi products combined with aligned services. We develop and produce RNAi reagents using cutting-edge technology, such as endoribonuclease prepared siRNAs (esiRNAs). Our company is continuously aiming for innovations to provide the most efficient silencing triggers available in the market with substantially less off-target effects guaranteeing best results for our customers.



GE Healthcare
Life Sciences

Xuri™. Believe in better futures.

Finding solutions.
Advancing breakthrough cell therapies.

Xuri products deliver solutions to accelerate access to safe and ground-breaking treatments, supporting the transition from research to clinic with confidence.
Making cellular therapies a clinical reality.

www.gelifesciences.com/xuri

imagination at work

GE, imagination at work, and GE monogram are trademarks of General Electric Company. Xuri is a trademark of GE Healthcare companies.
© 2014 General Electric Company – All rights reserved. GE Healthcare Life Sciences, Amersham Place, Little Chalfont, Bucks, HP7 9NA, UK. First published Mar. 2014.

SPEAKER ABSTRACTS

S1
IPS CELL TECHNOLOGY, GENE EDITING AND DISEASE RESEARCH

JAENISCH, Rudolf
Whitehead Institute for Biomedical Research, Cambridge, MA, USA

The recent demonstration of ***in vitro*** reprogramming using transduction of 4 transcription factors by Yamanaka and colleagues represents a major advance in the field. However, major questions regarding the mechanism of ***in vitro*** reprogramming need to be understood and will be one focus of the talk. A major impediment in realizing the potential of ES and iPS cells to study human diseases is the inefficiency of gene targeting. Methods based on Zn finger or TALEN mediated genome editing have allowed us to overcome the inefficiency of homologous recombination in human pluripotent cells. Using these genome editing approaches we have established efficient protocols to target expressed and silent genes in human ES and iPS cells. The most recent advance comes from the use of the CRISPR/Cas9 system to engineer ES cells and mice. This technology allows the simultaneous editing of multiple genes and will facilitate establishing relevant models to study human disease. We have used this technology to generate isogenic pairs of cells that differ exclusively at a disease causing mutation. The talk will describe the use of isogenic pairs of mutant and control iPS cells to establish in vitro systems for the study of diseases such as Parkinson's and Rett syndrome.

Biography

Rudolf Jaenisch is a Founding Member of the Whitehead Institute for Biomedical Research and a Professor of Biology at MIT. He generated the first transgenic mice carrying exogenous DNA in the germ line and was the first to use insertional mutagenesis for identifying genes crucial for embryonic development. Perhaps his most fundamental contributions have been in the study of epigenetic processes during development. His work has focused on mammalian cloning and has defined some of the molecular mechanisms that are crucial for nuclear reprogramming. In particular he showed that methylation of DNA plays important roles in gene expression, imprinting and X-inactivation as well as in neurological disorders and cancer.

S2
VITAMIN C REGULATES SOMATIC CELL REPROGRAMMING THROUGH HISTONE/DNA DEMETHYLASES

PEI, Duanqing
Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China

Vitamin C (Vc) is known for its anti-scurvy activity and is required for human health. We showed previously that Vc promotes the generation of mouse and human iPSCs by the Yamanaka factors. Vc promotes reprogramming in part by suppressing the ROS generated during reprogramming and protecting the cells from senescence in culture. However, the main function of Vc in reprogramming is to promote cellular demethylations at both H3K36 and H3K9 through histone demethylases Jhdm1a/1b (Kdm2a/2b) and Kdm3/4 . Dramatically, Jhdm1b appears to be able to replace three Yamanaka factors except Oct4 to mediate robust reprogramming, suggesting that H3k36 might be

SPEAKER ABSTRACTS

a major barrier for reprogramming . Recently, we and others have began to investigate the role of Vc in stimulating DNA demethylases Tet1/2/3 in the context of cell fate switching. We will discuss the role of Vc in regulating the activities of Tet enzymes during reprogramming.

Biography

Duanqing Pei, Ph.D, is Professor of Stem Cell Biology and also serves as the Director General (President) at the Guangzhou Institutes of Biomedicine and Health (GIBH), CAS, in Guangzhou, China. The Pei lab was the first in China to create mouse iPSCs using a non-selective system, and then improved the iPS process systematically. Recent publications from the Pei lab includes the discovery of vitamin C as a potent booster for iPSC generation and the histone demethylases Jhdm1a/1b are key effectors of somatic cell reprogramming downstream of vitamin C, as well as a mesenchymal to epithelial transition initiates the reprogramming process of mouse fibroblasts. Now, his lab continues to explore new ways to improve iPS technology, dissect the reprogramming mechanisms driven by Oct4/Sox2/Klf4 or fewer factors, and employ iPSCs to model human diseases in vitro.

BD Horizon™ Brilliant Violet™ Polymer Conjugates
Bright dyes help resolve rare and dim cell populations

BD Helping all people live healthy lives

Helping nature reveal itself

Now you can resolve rare or dim cell populations with BD Horizon™ Brilliant Violet™ polymer conjugates from BD Biosciences.

Developed from pioneering polymer dye technology acquired from Sirigen Ltd., BD Horizon Brilliant Violet dyes are brighter than conventional dyes. Improved brightness enables you to identify cell populations with lower receptor density than was previously possible, resolving cell populations previously obscured.

The complete portfolio of BD conjugated antibodies can be used to explore cellular features and characterize cells through surface, intracellular, or secreted markers. To ensure you can use BD reagents across your entire multicolor panel, our portfolio contains a broad selection of fluorochrome-conjugated antibodies.

Find out how you can use BD Horizon Brilliant Violet polymer conjugates with our expanded set of tools and information at **bdbiosciences.com/go/brilliant**.

BD Biosciences
30 Tuas Avenue 2
Singapore 639461
bdbiosciences.com

For Research Use Only. Not for use in diagnostic or therapeutic procedures.
BD, BD Logo and all other trademarks are property of Becton, Dickinson and Company. © 2014 BD
23-14929-00

SPEAKER ABSTRACTS

S3

DNA METHYLTRANSFERASE 3-LIKE IN GONOCYTES AND POSTNATAL SPERMATOGONIAL STEM/ PROGENITOR CELLS

Cheng, Hui, Ang, Heather Yin-Kuan, Li, Pin, Fang, Haitong, Farran, Chadi A. El, Liu, Tongming, Kong, Say Li, Chin, Lingzi, Lim, Kok Hao, Li, Hu, Huber, Tarah, Loh, Yuin-Han, **LIM, Bing**
Genome Institute of Singapore and Institute of Molecular and Cell Biology, Singapore, Singapore

The transdifferentiation of one cell type to another is an amazing biological phenomenon that speaks to the malleability of the mammalian genome. The basic Yamanaka technical strategy underpinning the generation of Induced pluripotent stem (iPS) cells paved the way for the generation of several "induced" lineages such as neuronal and hepatic cells. More recently hematopoietic stem cells have been induced from either hematopoietic cells such as B lymphocytes, hemogenic-related cell types such as endothelial cells or from completely unrelated cells such as fibroblasts. Each of the results use quite different combination of reprogramming factors, suggesting either the relevance of the starting epigenome or the possible multiple alternate Waddington routes to the same lineage. We attempted to reprogram fibroblasts to hematopoietic stem cells by testing a list of transcriptional factors implicated in hematopoietic development. Our results suggest that a basic minimal combination of key hematopoietic transcriptional factors is critical and sufficient to rewrite the hematopoietic program. We propose that these factors may be the equivalent of the "lineage commitment " factors that write and orchestrate lineage specification during embryonic hematopoiesis. ChipSeq analysis of chromatin markers and transcriptional factor binding sites at different time points further revealed the transition of lineages during reprogramming and provide insights into what are some of the molecular pathways recruited for the rewiring of the epigenome.

Biography

Dr Lim's research interest has been focused in stem cell biology, beginning with his Ph.D training under Dr Ernest McCulloch at the Ontario Cancer Institute, Toronto and as a post-doctoral fellow under Dr Stuart Orkin at the Children's Hospital, Boston. He started his lab at Beth Israel Hospital, Harvard Medical School then in 2002 he took on a joint appointment as Senior Group Leader at the newly founded Genome Institute of Singapore to start a Stem Cell and Regenerative Biology program. The team contributed significant discoveries about the genetic regulatory circuits in ES cells and genetic factors that impact on iPS reprogramming efficiency and quality. More recently they identified cancer stem cells in different cancers and their transcriptional signature that revealed novel classes of potential therapeutic targets.Dr Lim has recently taken on a new position as Lead for External Collaborations and Research Integration, Merck Research Laboratories, Translational Medical Research Center, Singapore.

SPEAKER ABSTRACTS

S4
MOLECULAR CONTROLS IN CELLULAR REPROGRAMMING

Toh, Cheng-Xu Delon, Chong, Zheng Shan, Maury, Julien J.P., Farran, Chadi EL, **LOH, Yuin-Han Jonathan**
Epigenetics and Cell Fates Laboratory, Institute of Molecular and Cell Biology, Singapore, Singapore

The ectopic expression of transcription factors can reprogram somatic cell fate into pluripotency. Although several factors that enhance or inhibit iPSC formation have been discovered, incomplete knowledge of the molecular mechanisms at work continues to hamper efforts to maximise iPSC yield and efficiency. Here, we present a novel systematic genome-wide interference screen to identify the molecular controls during the early stages of the reprogramming process. Our screen elicits key "Initiating" repressors and effectors which exert controlling mechanisms resulting in refractory or enhancement of reprogramming. We observed three striking aspects from our study. Firstly, repressors and effectors of reprogramming form close interacting network in novel pathways including RNA biosynthesis, G-protein signalling and Chromatin modification. Secondly, ChIP-seq analyses reveal tight relationships between the "Initiating factors" with OCT4, SOX2, KLF4 and c-MYC (OSKM). The 4 factors co-localized extensively at the repressor and effector gene loci. In particularly, c-Myc binds selectively at the promoters of the repressor genes, while OSK preferentially bind at the enhancers. A novel Smad protein which is a downstream target of the 4 factors is found to be repressed at the early stages of reprogramming. ChIP-seq analysis further revealed the role of the Smad protein in hindering the access of OSKM to its early pioneering target loci. Thirdly, the "Initial factors" act synergistically in controlling reprogramming. Simultaneous removal of two or more repressors greatly enhanced reprogramming. To this end, combinatorial knockdown of 5 factors resulted in an efficiency of 75% TRA-1-60 positive reprogrammed cells. Collectively, our integrative analysis offer new mechanistic insights into the controlling events during defined factor cellular reprogramming.

Biography

Jonathan did his Ph.D research (2003-2007) in the lab of Dr Ng Huck-Hui at the Genome Institute of Singapore where he elucidated the link between the genetic and epigenetic regulation mechanisms controlling embryonic stem cells (ESCs). He completed his Postdoctoral fellowship (2008-2011) with Dr George Daley at the Children's Hospital Boston, Harvard Medical School. Jonathan showed that terminally differentiated human blood cells can be epigenetically reprogrammed to pluripotent stem cells. Jonathan is currently a Principal Investigator at the Institute of Molecular and Cell Biology (IMCB). His laboratory is interested in dissecting the regulatory mechanisms regulating cell fate changes, and developing novel tools in deriving reprogrammed and differentiated cell types. He is a recipient of the Singapore Youth award, A*STAR Investigatorship research award and the MIT TR35 Regional award (Asia Pacific). He serves as the treasurer for the Singapore Association for the Advancement of Science (SAAS) and the Stem Cell Society Singapore.

SPEAKER ABSTRACTS

S5

THE RB TUMOR SUPPRESSOR RESTRICTS REPROGRAMMING BY DIRECTLY SILENCING PLURIPOTENCY NETWORKS

KARETA, Michael S.[1], Gorges, Laura[1], Hafeez, Sana[1], Benayoun, Bérénice A.[1], Marro, Samuele[1], Zmoos, Anne-Flore[1], Cecchini, Matthew J.[2], Spacek, Damek[1], Batista, Luis FZ[1], O'Brien, Megan[1], Ng, Yi-Han[1], Ang, Cheen E.[1], Vaka, Dedeepya[1], Artandi, Steven E.[1], Dick, Frederick A.[2], Brunet, Anne[1], Sage, Julien[1], Wernig, Marius[1]

[1]Stanford University, Stanford, CA, USA, [2]Western University, London, ON, Canada

Reprogramming of differentiated cells to induced pluripotent stem (iPS) cells is a fascinating yet incompletely understood process. Based on the similarities between reprogramming and cancer, we investigated the tumor suppressor Rb in the induction of pluripotency. Here, we show that loss of Rb increases the efficiency of iPS cell reprogramming. Surprisingly, Rb inactivation does not enhance reprogramming by accelerating the cell cycle. Instead, Rb directly binds regulatory regions of many pluripotency genes, including Sox2 and Oct4, and controls the chromatin state of these factors and their targets. Consequently, loss of Rb leads to a slight but widespread de-repression of the pluripotency program, rendering Rb-deficient cells reprogrammable without exogenous Sox2. Moreover, Sox2 was found to critically mediate Rb-induced tumorigenesis. These results identify Rb as a global transcriptional repressor of the pluripotency network, explaining previous reports about Rb's involvement in cell fate pliability. Our observations also implicate factors involved in pluripotency such as Sox2 in cancers driven by loss of Rb.

Biography

In the laboratories of Marius Wernig and Julien Sage I have combined their two specialties, cell fate reprogramming and mouse modeling of cancer respectively, to study the role of tumor suppressors in reprogramming to iPS cells. In particular I studied the Rb tumor suppressor which is functionally inactivated in nearly every tumor type. Rb normally promotes differentiation, yet I found that it also serves to block cellular dedifferentiation in part by repressing Sox2 as well as other members of the greater pluripotency network. Additionally, I showed that Sox2 genetically interacts with Rb to block tumor initiation in a mouse model of an Rb-loss initiated tumor. This work demonstrates an intersection between tumor formation and pluripotency pathways.

S6

HUMAN HEPATOCYTES WITH DRUG METABOLIC FUNCTION INDUCED FROM FIBROBLASTS BY LINEAGE REPROGRAMMING

DENG, Hongkui

Stem Cell Research Center, Peking-Tsinghua Center for Life Sciences, Peking University, Beijing, China

Obtaining fully functional cell types is a major challenge for drug discovery and regenerative medicine. Currently, a fundamental solution to this key problem is still lacking. Recently, we demonstrated that functional human induced hepatocytes (hiHeps) can be generated from fibroblasts by overexpressing the hepatic fate conversion factors along with the maturation factors. hiHeps express a spectrum of phase

SPEAKER ABSTRACTS

I and II drug-metabolizing enzymes and phase III drug transporters. Importantly, the metabolic activities are comparable between hiHeps and freshly isolated primary human hepatocytes. Transplanted hiHeps repopulate up to 30% of the livers of Tet-uPA/Rag2(-/-)/yc(-/-) mice. Our data demonstrate that human hepatocytes with drug metabolic function can be generated by lineage reprogramming.

Biography

Hongkui Deng, Ph.D is a Professor of Cell Biology and Principal Investigators of Center for Life Sciences at Peking University. He is also Director of Peking University Stem Cell Research Center. Dr. Deng's laboratory works on cellular reprogramming and generation of functional human cells from pluripotent stem cells. Dr. Deng has published over 100 research articles, reviews and position papers in the arena of Stem Cell Biology and Viral Immunology. He has served as a member of the International Society for Stem Cell Research Board of Directors, and as an Editorial Board Member of Cell and Cell Stem Cell.

S7

GLOBAL REORGANIZATION OF CHROMATIN ARCHITECTURE DURING EMBRYONIC STEM CELL DIFFERENTIATION

REN, Bing
University of California, San Diego, San Diego, CA, USA

Higher order chromatin structure is emerging as an important regulator of gene expression. Although dynamic chromatin structures have been identified in the genome, the full scope of chromatin dynamics during mammalian development and lineage specification remains obscure. By mapping genome-wide chromatin interactions in human embryonic stem cells (hESC) and four hESC-derived lineages, we uncover extensive chromatin reorganization during lineage specification. We observe that while topological domain boundaries remain intact during differentiation, interactions both within and between domains change dramatically, altering 36% of chromosomal "compartments" throughout the genome. By integrating chromatin interaction maps with haplotype resolved epigenome and transcriptome datasets, we find widespread allelic bias in gene expression correlated with allele-biased chromatin states of linked promoters and distal enhancers. Our results therefore provide a global view of chromatin dynamics and a resource for studying long-range control of gene expression in human cells.

Biography

Dr. Ren is currently Member of the Ludwig Institute for Cancer Research (LICR) and Professor of Cellular and Molecular Medicine at the University of California, San Diego (UCSD) School of Medicine. He obtained his Ph.D from Harvard University in 1998, and subsequently conducted postdoc research at the Whitehead Institute till 2001, when he became a faculty member at LICR and UCSD. Dr. Ren is an

SPEAKER ABSTRACTS

internationally recognized investigator in the study of genome regulation in mammalian cells. His research has been focused on genomic and epigenomic analyses of human embryonic stem cell differentiation and mammalian development. He has made contributions to the annotation of transcriptional regulatory sequences in the human and mouse genomes, characterization of epigenomes of the human cell types, and understanding of the chromatin architecture in mammalian cells. He has been a participant of the ENCODE consortium since 2003, and a member of the NIH Roadmap Epigenomic Project since 2008. He is a recipient of the Kimmel Scholar award, Young Investigator Award of the Chinese Biological Investigator Society and a fellow of the American Association for the Advancement of Science.

S8

DISTINCT METHYLATION STATES OF H3K27 INFLUENCE CELL LINEAGE PREFERENCE IN EMBRYONIC STEM CELLS

WANG, Stan[1], Juan, Aster[1], Vivanco, Karinna[1], Feng, Xuesong[1], Gutierrez-Cruz, Gustavo[1], Zare, Hossein[1], Zhou, Jizhong[1], Pedersen, Roger[2], Gurdon, John[2], Sartorelli, Vittorio[1]
[1]National Institutes of Health, Bethesda, MD, USA, [2]University of Cambridge, Cambridge, United Kingdom

The dynamic state of H3K27 methylation controls either transcriptional activation or repression to influence fate determination in embryonic stem (ES) cells. More than 50% of histone H3 is dimethylated (H3K27me2) in ES cells, while 10-20% is trimethylated (H3K27me3). Polycomb repressive complex 2 (PRC2) deposits H3K27me2/3 to regulate development by preventing non-cell-type-specific expression through transcriptional repression. H3K27me2 serves as an important intermediary PRC2 product by being the substrate for H3K27me3 formation. However, it is currently unclear whether H3K27me2 and H3K27me3 identify different chromatin and transcriptional states. In order to reveal whether the balance between H3K27me2 and me3 affects the global control of ES cell lineage preference, we introduced a point mutation via genome editing in the catalytic domain of Ezh2--the enzymatic component of PRC2. This Ezh2 mutation (hyperactive Ezh2) altered H3K27 substrate specificity by increasing overall formation of H3K27me3 while decreasing global H3K27me2. ES cells with a single hyperactive Ezh2 allele differentiated earlier and proliferated faster, forming both larger embryoid bodies (EB) in vitro and mouse teratomas in vivo. During late differentiation, adherent EBs surprisingly saw preferential formation of neural-like cells instead of expected cardiac cells that represent default meso-endoderm formation. Accordingly, RNA-seq transcriptome analysis uncovered activation of an already established preference for neural lineage development in pluripotent ES cells with hyperactive Ezh2. Specifically, genes of the TGF-beta pathway--known to inhibit neurodevelopment--were preferentially repressed in hyperactive Ezh2 ES cells. Unbiased ChIP-seq further identified increased H3K27me3 on Tgfb1 regulatory regions, concomitant with its decreased transcription. Therefore, our findings indicate that a fundamental shift in balance between H3K27me2 and me3 influences lineage preference in ES cells by selectively inhibiting specific master regulators of cell fate commitment.

Biography

Stan Wang is pursuing his Ph.D in stem cell biology and regenerative medicine at the University of Cambridge as both an NIH-Cambridge MD/Ph.D and Gates Scholar. Stan works with

SPEAKER ABSTRACTS

Sir John Gurdon and Professor Roger Pedersen at Cambridge (UK), and Dr. Vittorio Sartorelli at the NIH (USA). Stan studies the genetics and epigenetics controlling reprogramming of stem cells and their ability to differentiate into all tissue types. Through understanding these fundamental mechanisms, he is working towards ultimately bioengineering customized cells capable of any task: from synthesizing new materials and adaptive drugs to directly acting on targets in the body. After completing his research at the NIH in the coming year, he will finish the remainder of his MD at Columbia University as he continues to pioneer at the intersection of medicine, science, and entrepreneurship. In his spare time, Stan has given a TEDx talk on the future of medicine (http://goo.gl/tnUh74).

S9

DNA METHYLTRANSFERASE 3-LIKE IN GONOCYTES AND POSTNATAL SPERMATOGONIAL STEM/PROGENITOR CELLS

Liao, Hung-Fu[1], Kao, Tzu-Hao[1], Chang, Kai-Wei[2], Tseng, Yen-Tzu[1], Yen, Pauline[3], **LIN, Shau-Ping[1]**
[1]Institute of Biotechnology, National Taiwan University, Taipei, Taiwan, [2]Graduate Program for Genomics and Systems Biology, National Taiwan University/Academia Sinica, Taipei, Taiwan, [3]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan

DNA methyltransferase 3-like (DNMT3L) is an important epigenetic regulator known to be important for mouse fertility. Our recent studies suggested that ectopic DNMT3L can induce chromatin modifying complex (DNMT3L-DNMT3A-TRIM28-HDAC1-SETDB1) formation in mouse embryonic fibroblasts and introduce repressive chromatin modifying marks to newly infected Mo-MuLV as well as endogenous retroviruses (J. Virology, 2014). Embryonic17.5 Dnmt3l-/- fetal testes also have reduced interactions among TRIM28, SETDB1, HDAC1 and DNMT3A compared to their counterparts in the fetal testes of wild type littermates. The significance of the DNMT3L induced repressive chromatin modifying activities in epigenetic profiling establishment in gonocytes will be discussed. Apart from the important role in facilitating epigenetic landscape shaping in gonocytes, we have also recently demonstrated that DNMT3L also expressed in spermatogonia stem cell enriched population and is involved in regulating the balance between quiescence and proliferation of this cell type. DNMT3L influenced the stability of promyelocytic leukemia zinc finger (PLZF), potentially by downregulating Cdk2/CDK2 expression, which sequestered CDK2-mediated PLZF degradation. Reduced PLZF in Dnmt3l KO THY1+ cells released its antagonist, Sal-like protein 4A (SALL4A), which is associated with overactivated ERK and AKT signaling cascades. Furthermore, DNMT3L was required to suppress the cell proliferation-promoting factor SALL4B in THY1+ SPCs and to prevent premature stem cell exhaustion (Development, 2014). Our recent progress on how DNMT3L directly or indirectly regulate PiRNA composition of the spermatogonia progenitor cell enriched THY1+ population will be presented in this meeting.

Biography

Associate Professor Shau-Ping Lin from National Taiwan University has over 20 years of research experience in reproductive biology, embryo development and epigenetic reprogramming in germ cells and stem cells. She identified the genomic imprinting control center (Nat Genet, 2003) for a developmentally necessary (Development, 2007), tumor suppression associated,

SPEAKER ABSTRACTS

microRNA containing Dlk1-Dio3 imprinted locus, since her Ph.D study in University of Cambridge, UK. In order to further tackle the reprogramming events during germ cell development, her lab further characterize how a germ cell differentiation essential epigenetic regulator, DNA methyltransfearse 3-like (DNMT3L), attract repressive chromatin modifying complex (J Virology, 2014) and interpret chromatin modifications (Nature, 2007) in order to maintain the quiescent status in spermatogonial stem/progenitor cells (Development, 2014) and in shaping epigenetic landscape essential for storing full germ cell function including proper meiosis ability (Biol Cell, 2012). http://www.iob.ntu.edu.tw/people/bio.php?PID=6

S10

DNA OXIDATION TOWARDS PLURIPOTENCY IN MAMMALIAN DEVELOPMENT AND CELL REPROGRAMMING

XU, Guo-Liang
Institute of Biochemistry & Cell Biology, Chinese Academy of Sciences, Shanghai, China

Mammalian development begins with a single cell resulted from the fertilization of a sperm and an oocyte. The early embryonic genome undergoes profound epigenetic reprogramming to prepare for development. The biological significance and mechanisms of epigenetic reprogramming are poorly understood. We find that 5-methylcytosine (5mC), the most abundant type of base modification in DNA, can be oxidized to 5-carboxylcytosine (5caC). The Tet family of dioxygenases catalyzes the 5mC oxidation and oxidized bases are excised by DNA glycosylase TDG to complete the demethylation process. In zygotes, Tet3 is responsible for the genome-wide oxidation of 5mC in both the male and female pronuclear DNA. Deficiency of zygotic Tet3 impedes genome-wide demethylation including demethylation at the paternal *Oct4* and *Nanog* loci and delays the reactivation of the paternal *Oct4* in early embryos. The heterozygous mutant embryos lacking maternal Tet3 suffer increased developmental failures. Importantly, oocytes lacking Tet3 also show impaired reprogramming of injected somatic cell nuclei in cloning experiment. In addition, MEFs deficient in all Tet genes were unable to be reprogrammed into iPSCs by Yamanaka factors. We conclude that Tet-mediated oxidation is important for DNA demethylation and gene activation in the early embryo following fertilization, as well as in cell reprogramming such as somatic cell nuclear transfer and factor-based iPSC generation.

Biography

Dr. Xu is a principal investigator at the Institute of Biochemistry and Cell Biology (IBCB), Chinese Academy of Sciences, Shanghai. He trained for Ph.D at the Max Planck Institute for Molecular Genetics, Berlin. He conducted his postdoctoral training with Prof. Timothy Bestor, Columbia University, New York. Dr. Xu established the DNA metabolism research group in 2001 under the auspices of Max Planck research group at Shanghai IBCB, where his team investigates the importance of DNA methylation (5-methylcytosine) in stem cells and development. In 2011 his team described a role for Tet DNA dioxygenases in oxidation of 5-methylcytosine to 5-carboxylcytosine and the involvement of a DNA glycosylase -TDG in active demethylation, a critical step for the epigenetic reprogramming of early embryos and throughout development.

SPEAKER ABSTRACTS

S11
CHROMATIN MODIFIERS IN EPIGENETIC CONTROL OF CANCER AND STEM CELLS

BAEK, Sung Hee
Seoul National University, Seoul, South Korea

Epigenetics encompass inheritable changes in gene expression profiles that occur without alterations to the genomic DNA sequence. Epigenetic modifications conduct important roles in diverse biological processes. Histones and nonhistone proteins including chromatin modifiers and transcriptional coregulators are subject to diverse epigenetic modifications including phosphorylation, acetylation, methylation, ubiquitination, and SUMOylation. These diverse post-translational modifications can modulate the activity of chromatin modifiers altering the chromatin state for either activation or repression. In this presentation, I will discuss our continued efforts to decipher epigenetic regulatory mechanisms of cancer and stem cells. I will introduce newly identified signaling crosstalk regulated by protein modification and address its biological meaning in cancer and stem cells.

Biography

Dr. Baek's work is focused on the chromatin dynamics and epigenetic regulatory mechanism in stem cells and cancer. She received her BS, MS, and Ph.D degrees from Seoul National University. Following her postdoctoral research in Michael Rosenfeld's lab at HHMI and following research professor at HHMI, she joined the faculty of Seoul National University in 2003, and now works as a professor. She has been selected as a distinguished fellow of Seoul National University since 2012. She received numerous awards and honors, including the L'OREAL-UNESCO for Women in Science Award. She has been invited as a managing editor for BBA Molecular Basis of Disease and a guest Editor for Annual Review of Physiology. She is serving on a review committee for the Human Frontier Science Program.

S12
PIONEER FACTORS AND IMPEDIMENTS TO CELLULAR REPROGRAMMING

ZARET, Kenneth S.
University of Pennsylvania, Philadelphia, PA, USA

The ability to change cell fate at will is an ultimate goal of the regenerative medicine field. If we can direct one type of cell to become another type of cell, we could generate new cells and tissues to repair diseased organs in the clinic. The primary means of cell type control is by regulatory proteins (transcription factors) that bind to sites on chromosomes and govern the expression of genes specific for a given cell type. Yet chromosomes consist of DNA packaged into nucleosomes and higher-order protein complexes which create physical barriers to transcription factor binding and cellular reprogramming. We previously showed that a subset of transcription factors called "pioneer factors" can function in early development and are useful in cell reprogramming because they have the special ability to bind their DNA target sites on nucleosomes. We recently discovered that this feature is dictated by the ability of the DNA binding domain of pioneer factors to flexibly adapt to DNA on the nucleosome surface. We further find that there are higher-order chromosomal structures that block diverse transcription factors, including pioneer factors, from accessing nucleosomal DNA and thereby impede cellular reprogramming. We established

SPEAKER ABSTRACTS

conditions to biochemically isolate different kinds of resistant chromosome structures and analyze their respective protein content by mass spectroscopy. Using such information, we are learning to break down different kinds of chromosome structures to facilitate cellular reprogramming. Overall, we seek to understand the means by which pioneer factors and resistant chromosomal states can be manipulated to control cell fate.

Biography

Kenneth S. Zaret, Ph.D is the Director of the Institute for Regenerative Medicine and the Joseph Leidy Professor in the Department of Cell and Developmental Biology at the Perelman School of Medicine, University of Pennsylvania. His laboratory discovered "pioneer factors" that endow the competence for cell differentiation and promote cellular reprogramming. His laboratory also identified a dynamic signaling network that coordinately induces liver and pancreas cell fates in the mammalian embryo. His group used stem cell technology to reprogram human pancreatic cancer cells and found that the reprogrammed cells can model for pancreatic cancer progression. Dr. Zaret received a Searle Scholar faculty award (1986), the Hans Popper Basic Science Award from the AASLD and American Liver Foundation (2002), a MERIT award from the National Institutes of Health (2006-), and in 2007 he was elected as a Fellow of the American Association for the Advancement of Science.

S13

DISSECTING CANCER HETEROGENEITY AND THERAPEUTIC RESPONSES AT SINGLE CELL RESOLUTION

Organized and supported by Fluidigm, Inc

TIAN, Qiang
Institute for Systems Biology, Seattle, WA, USA

Cancers are highly complex diseases characterized by intricate intertumoral and intratumoral heterogeneity. Comprehensive genomic, transcriptomic, and epigenomic profilings of tumor specimens have unveiled a variety of genetic aberrations, gene expression and epigenetic signatures for molecular stratification of cancer. However, few of these studies were conducted at the clonal population and/or single cell resolution. These molecular measurements examine averaged signals of the mixed tumor cell populations. We have established an integrated strategy to interrogate tumor heterogeneity by combining several state-of-the-art technologies, including single cell sorting/dispensing, microfluidics qPCR, and next-generation sequencing (NGS). I will discuss our experience applying these technologies to the studies of brain tumor glioblastoma (GBM) and colorectal cancer (CRC), in search of cancer-initiating population and for understanding the cell state transition in response to treatment.

Biography

With a career path traversing clinical medicine, molecular genetics and systems biology, Dr. Tian is primarily interested in applying the powerful systems approach with the enabling genomics, proteomics, and single cell analysis technologies to address some of the most pressing issues pertaining to human health: cancer and stem cell biology, immunity, and disease biomarker discovery. He directs the interdisciplinary cancer and stem cell group striving to dissect the clonal origin of cancer heterogeneity, aiming to identify the tumor-

SPEAKER ABSTRACTS

initiating cells and the underlying genomic landscape leading to their tumorigenic properties. Dr. Tian collegially reaches out to local and national medical research communities (UW-Medicine, Swedish Hospital, FHCRC, MD Anderson) to disseminate ISB strategy and technologies. He is on faculty of the UW-Institute for Stem Cell & Regenerative Medicine, and an affiliate professor of Chinese Academy of Sciences.

S14

CLINICAL DEVELOPMENT AND MANUFACTURING OF CELL-BASED BIOPHARMACEUTICALS FOR TARGETED DELIVERY OF THERAPEUTIC GENES IN AN ONCOLOGY TRIAL FOR ADVANCED CANCER

Organized and supported by apceth GMBH & CO. KG

HUSS, Ralf
Apceth GmbH & Co.KG, München, Germany

Cell-based biopharmaceuticals will change clinical medicine in the same way as biologics did in the recent past. The clinical application of cellular therapeutics represents also a new challenge to meet the regulatory requirements for manufacturing, quality control and approval. Based on a vast number of preclinical data regarding safety and efficacy but also the increasing understanding of the underlying biology, we have initiated a phase I/II clinical trial for the treatment of advanced adenocarcinoma of the gastrointestinal tract applying genetically-modified mesenchymal stem cells (MSC) from the patients' own bone marrow (TREAT-ME 1). Autologous MSC have the capability to home to (inflammatory) tumor sites or metastasis to deliver therapeutic gene products or other secreted substances in a highly effective way. Our proprietary pharmaceutical product is a genetically modified somatic cell suspension. The stable integration of a therapeutic gene under the control of a tumor specific promoter (based on the molecular tumor tissue profile) is accomplished through the use of a retroviral, replication incompetent and self-inactivating (SIN) vector system. The MSC cell-suspension is applied intravenously for three consecutive weeks, followed by the administration of the prodrug, which is exclusively activated only in the context of the tumor environment to avoid unwanted off-target toxicity. The study is designed to define the optimal cell dose and feasibility. In this trial we have already demonstrated that the genetic modification of autologous MSCs from cancer patients and their manufacturing is feasible according to GMP standards and does not alter the typical biological properties of MSCs. The therapeutic administration of genetically modified MSCs as a principle mechanism of a cell-based gene therapy respectively a gene product delivery tool holds promise for the clinical application of a patient-tailored combination of cell- and gene therapy not only for autologous, but also allogeneic cellular biopharmaceuticals.

Biography

Dr. Ralf Huss joined the apceth® management from Roche in October 2011, where he was the global head of the Therapeutic Cell Initiative and VP in Pharma Research & Early Development. There he was also involved in identifying tissue-based biomarker to stratify cancer patients as part of a global personalized healthcare strategy. At apceth®, Dr. Huss is responsible for research and pre-clinical development of innovative cell-based biopharmaceuticals for the targeted delivery of therapeutics cells and genes. Professor Huss is a physician and practicing surgical / molecular pathologist with various international academic appointments in regenerative and molecular medicine. He has more than 20 years training and experience in immunology, transplantation biology and stem cell research in Switzerland, USA and Germany. Prof. Huss has authored more than hundred papers, book chapters and texts in this field and is a co-founder of apceth®.

SPEAKER ABSTRACTS

S15: EVENING INDUSTRY SESSION

LONZA L7™ HIPSC REPROGRAMMING AND HPSC CULTURE SYSTEM - THE ROBUST GENERATION AND MAINTENANCE OF HUMAN PLURIPOTENT STEM CELLS UNDER DEFINED AND XENO-FREE CONDITIONS

D'ANDREA, Scott
Lonza Pharma Bioscience Solutions, USA

In 2007, Dr. Shinya Yamanaka successfully generated human induced pluripotent stem cells (hiPSCs) from adult somatic cells. Stem cell research continues to fuel new discoveries with the underlining potential of novel clinical applications. Like human embryonic stem cells (hESCs), hiPSCs are able to indefinitely self-renew and differentiate into multiple human cell types. These abilities form the basic scientific tools for various applications like disease modeling, drug development, and regenerative medicine that may be easily translated into clinical applications. However, clinical applications require defined xeno-free and up-scalable culture conditions for maintenance as well as robust and efficient non-integrative and non-viral methods for reprogramming somatic cells. Given this scientific demand for a simpler approach, Lonza has developed a complete and robust system with easy to use protocols appropriate for human pluripotent stem cell (hPSC) research and easily translated for GMP studies. Lonza's new L7™ hiPSC Reprogramming and hPSC Culture System is a complete system comprised of a transfection system with priming and recovery medium, hPSC culture medium, maintenance matrix, passaging and cryopreservation solutions. The hPSC culture maintenance system supports every-other-day feeding and is xeno-free and defined. Our in-house results demonstrate that multiple hESC and hiPSC lines could be expanded for over 40 passages while maintaining normal morphology, high pluripotency marker expression and normal karyotype. In addition, the lines maintained the ability to efficiently differentiate into cell types of the three germ layers. Thus, L7™ products and protocols provide a systematic approach for non-viral hiPSC generation and efficient maintenance of hiPSCs for various research applications.

Biography

Scott D'Andrea is the Head of Global Market Development for Lonza Bioscience Solutions with over twenty-seven years of clinical diagnostic and life science industry experience from Abbott Laboratories, Chiron Corporation, Nanogen Inc. and Amaxa Biosystems. He initially joined Lonza following the acquisition of Amaxa, developers of the Nucleofector™ technology. Mr. D'Andrea helped drive Nucleofection™ adoption by optimizing difficult transfect applications with leading researchers worldwide. He currently has an active role in Lonza's portfolio expansion development and will introduce Lonza's new L7™ hiPSC Reprogramming and hPSC Culture System - The Robust Generation and Maintenance of Human Pluripotent Stem Cells under Defined and Xeno-Free Conditions during the Singapore ISSCR evening Industry Tools and Technologies session. Lonza is a leading supplier of innovative services and products with trusted brands such as Clonetics, Poietics, BioWhittaker, and Amaxa Nucleofector™ used in pharmaceutical, biotechnology, academic, and government research around the world.

SPEAKER ABSTRACTS

S16
GERMLINE: SPECIFICATION AND REPROGRAMMING FOR TOTIPOTENCY AND DEVELOPMENT

SURANI, Azim
Wellcome Trust/Cancer Research UK Gurdon Institute/ University of Cambridge, Cambridge, United Kingdom

Specification of primordial germ cells (PGCs) in mammals occurs during development of postimplantation epiblast cells, which also give rise to all somatic tissues. We now have wide-ranging knowledge of the mechanism of PGC specification in mice, which is linked to extensive epigenetic reprogramming. PGC specification is followed by sequential, orderly and dynamic epigenetic changes in histone modifications, reactivation of the X chromosome and comprehensive global DNA demethylation. These epigenetic changes are essential towards the establishment of the totipotent state, which follows after fertilisation and establishment of the zygote. Pluripotent state is established subsequently following genetic and epigenetic changes in the zygote and in the course of preimplantation development.

Biography

Azim Surani, born in Kenya received a Ph.D in 1975 at Cambridge University under Professor Sir Robert Edwards FRS (Nobel Laureate, 2010). Surani joined the Babraham Institute in 1979, and discovered 'Genomic Imprinting' in 1984, and subsequently, novel imprinted genes and their functions, with contributions to its mechanism through establishment and erasure of DNA methylation. In 1992, he was elected the Marshall-Walton Professor and subsequently, a Director of Research at Cambridge University. His recent work established the genetic basis for germ cell specification and epigenetic programming. He was elected a Fellow of the Royal Society in 1990, he was awarded a Royal Medal in 2010 and in January 2014 the ISSCR McEwen Award for Innovation.

S17
UNCOVERING THE PLURIPOTENCY PROGRAM IN EMBRYONIC STEM CELLS

DUNN, Sara-Jane[1], Martello, Graziano[2], Yordanov, Boyan[1], Emmott, Stephen[1], Smith, Austin[3]
[1]Microsoft Research, Cambridge, United Kingdom, [2]University of Padua, Padua, Italy, [3]University of Cambridge, Cambridge, United Kingdom

Pluripotency in embryonic stem (ES) cells is typically characterised by both the presence and expression level of key transcription factors, which are regulated upstream by external signals that have been found to sustain ES cells in vitro. A homogeneous population of ES cells can be established solely under '2i' (CH and PD) conditions, but the addition of the cytokine leukaemia inhibitory factor (LIF) is optimal. Moreover, it is possible to maintain and propagate ES cells using any two of these three components, suggesting a flexible transcriptional program exists. But how does an ES cell process the information that it receives from these input signals? Fundamentally, this amounts to the question: what is the biological computation that determines pluripotency? To address this question, we began by examining whether there is a transcriptional program for pluripotency, and if so, how complex it must be. We adapted formal methods traditionally applied to probe and verify safety-critical engineered systems, to design a methodological approach that enables 'automatic reductionism' of gene interaction networks constrained by experimental data. Inferring an interaction network is not hindered by the amount of data available, but rather by the non-trivial issue

SPEAKER ABSTRACTS

of considering all the information collectively. Our computational approach allowed us to tackle and reduce the complexity of a potentially vast interactome to derive a set of combinations of functionally validated components, gene interactions and regulation conditions sufficient not only to explain observed, but also to predict unobserved, ES cell behaviour. The resulting minimal network, which comprises only 23 interactions, 12 components, and three inputs, satisfies all prior specifications for self-renewal, and furthermore predicts unknown and non-intuitive responses to compound genetic perturbations. Utilising this pluripotency program, we ask whether it is possible to predict conditions under which naïve pluripotency is self-sustaining, or can be induced, and interrogate the efficiency of this process - the results of which could be applied both to understand and derive protocols for cellular reprogramming.

Biography

I am a researcher working in the Biological Computation group, which is half of the Computational Science Laboratory at Microsoft Research (MSR), Cambridge. My undergraduate degree was in Mathematics, following which I completed a DPhil in Computational Biology in 2011, before starting as a postdoc with MSR. I have recently transitioned to a permanent Scientist position within the lab. In this role, my research is focused on how and why cells perform computation. As part of this, I am interested in the decisions that cells make throughout development, and in the design of theoretical and computational approaches to uncover the information-processing that is carried out by cells.

WELCOME TO THE ISSCR-SCSS REGIONAL FORUM

COME VISIT US ON MATRIX LEVEL 4

SPEND SOME TIME LEARNING MORE ABOUT

STEMGENT®

A REPROCELL GROUP COMPANY

THE GLOBAL SUPPLIER IN STEM CELL REAGENTS

SPEAKER ABSTRACTS

S18

A ROLE FOR POLYAMINE REGULATORS IN EMBRYONIC STEM CELL SELF-RENEWAL AND DIFFERENTIATION

VARDY, Leah A.[1], Zhao, Tian Yun[1], Yang, Henry[2]
[1]Institute of Medical Biology, Singapore, Singapore, [2]NUS, Cancer Science Institute, Singapore, Singapore

The successful manipulation of embryonic stem cells (ESC) for therapeutic application requires a detailed understanding of the molecular pathways underlying gene expression on all levels. We have recently identified the polyamine pathway as being essential for the maintenance of ESC self-renewal. The polyamines, spermine and spermidine, and the diamine putrescine, are organic cations that have been implicated in a wide range of cellular processes. We show that knock-down of polyamine regulator, Amd1, results in differentiation of mouse ESCs and this can be rescued by the addition of the polyamine spermine. Over-expression of Amd1 or Odc1 promotes self-renewal in the absence of the cytokine LIF. Our data suggests the polyamine pathway works in part to promote high MYC protein levels which in turn drives LIF independent self-renewal. We have identified additional targets of the polyamine pathway in ESCs that are previously un-described self-renewal factors. These proteins are both required for self-renewal and can promote self-renewal in the absence of LIF. We are seeking to define the targets of the polyamines in ESC and determine their role in the promotion of self-renewal.

Biography

Leah Vardy is a Principal Investigator at the Institute of Medical Biology in Singapore. She completed her Ph.D at Cancer Research UK and her postdoctoral training at the Whitehead Institute in Cambridge, MA. Leah has a long running interest in stem cells and in understanding the molecular mechanisms underlying the decision to self-renew or differentiate. Her lab currently focuses on defining the role of polyamines in ESC self-renewal and differentiation.

S19

LINEAGE PROPENSITY OF THE MOUSE EPIBLAST STEM CELLS: ENDODERM DIFFERENTIATION IN RESPONSE TO TGF-BETA SIGNALLING ACTIVITY

TAM, Patrick P.L.
Children's Medical Research Institute, Westmead, Australia

Analysis of cell fates and gene expression patterns in the epiblast of the early postimplantation mouse embryo have uncovered a regionalized pattern of signalling activity can be superimposed on the prospective fates of cells, highlighting that the differentiation of epiblast cells may be influenced by the nature and level of signalling activity experienced by the cells and that the signalling activity may intersect with the function of the transcription factors in lineage allocation and differentiation. For the multipotent epiblast stem cells (EpiSC, which were derived from the epiblast of postimplantation embryos), the propensity of endoderm differentiation does not correlate with the developmental stage of embryo from which they were derived, but to the readiness to up-regulate Mixl1 at the initial step of differentiation. EpiSCs that up-regulated Mixl1 rapidly responded more robustly to both Activin A and Nodal in generating foregut endoderm and

SPEAKER ABSTRACTS

precursors of pancreatic and hepatic tissues. In contrast, EpiSCs in which Mixl1 up-regulation was delayed responded less effectively to Nodal and showed a suboptimal outcome of directed differentiation. The enhancement in endoderm potency in Mixl1-early cells may be accounted for by a rapid exit from the progenitor state and the efficient response to the induction of differentiation by Nodal. Nodal appeared to elicit responses that were associated with transition to a mesenchyme phenotype, whereas Activin A promotes gene expression associated with maintenance of an epithelial phenotype. We postulate that the formation of definitive endoderm in the embryoid bodies follows a similar process to germ layer formation from the epiblast, requiring a forward followed by a reversed epithelial-mesenchymal transition. Our results also show that priming EpiSCs by Nodal signalling at the initial phase of directed differentiation impacts on the progression to the generation of the definitive endoderm progenitor.

Biography

Patrick Tam is the Deputy Director and Head of the Embryology Research Unit at the Children's Medical Research Institute. His research focuses on the elucidation of the cellular and molecular mechanisms of body patterning during gastrulation, the integration of gene transcription and the signaling activity in the coordination of cell differentiation in the formation of organs and major body parts. He co-edited, with James Nelson and Janet Rossant, the book "Mammalian Development" published by Cold Spring Harbor Laboratory Press and is an Editor of Development. Patrick Tam is the Mok Hing-Yiu Distinguished Visiting Professor at the University of Hong Kong, and Fellow of the Australian Academy of Science, the Institute of Biology UK, the Society of Biology UK and the Royal Society of London.

S20

MOLECULAR MECHANISM TO MAINTAIN PLURIPOTENCY OF MOUSE ES CELLS

NIWA, Hitoshi
CDB RIKEN Laboratory for Pluripotent Studies, Kobe, Japan

The pivotal role of transcription factors to determine cellular pluripotency is elegantly highlighted by the generation of iPS cells, but their modes of action remain unclear. Since the requirement of the exogenous transgene expression is limited in induction but not maintenance of pluripotency in generation of iPS cells, the endogenous genes encoding the counterparts of transcription factors establish an autonomous transcription factor network to maintain their own expression in the exogenous signal-dependent manner in established iPS cells as found in ES cells. We recently reported that the function of the transcription factors is determined by the context of the transcription factor networks. I will talk about recent progress of our research to dissect the functions of pluripotency-associated transcription factors in the context of the transcription factor network under the control of the exogenous signal input.

Biography

I graduated Nara Medical University in 1989 and obtained my Ph.D degree at Kumamoto University in 1993. I worked as a postdoc at the University of Edinburgh from 1994 to 1996 under the supervision of Dr

SPEAKER ABSTRACTS

Austin Smith. Then I stayed at Osaka University Medical School until 2001 and joined RIKEN Center for Developmental Biology (CDB) as PI of Laboratory for Pluripotent Stem Cell Studies. I have been studying the molecular mechanisms governing cellular pluripotency of mouse ES cells, especially focusing on the function of the transcription factor network.

S21: LUNCHTIME INDUSTRY SESSION

AN INTEGRATED SYSTEM FOR HUMAN BLOOD CELL REPROGRAMMING AND IPS CELL LINE SELECTION AND MAINTENANCE

HUNTER, Arwen
STEMCELL Technologies Inc, Vancouver, BC, Canada

This tutorial will highlight two new tools for pluripotent stem cell research from STEMCELL Technologies. These tools are aimed at improving the efficiency of feeder-free reprogramming of peripheral blood (PB)-derived cells, and at the non-mechanical and non-enzymatic selection of undifferentiated cells as aggregates for passaging.ReproTeSR™ Medium is a xeno-free reprogramming medium for the efficient generation of human induced pluripotent stem cells (hiPSCs) from PB in feeder-free conditions. PB-derived cells are commonly used for generating hiPSCs due to the minimally invasive procedure for sample collection. This presentation will describe an integrated workflow for the isolation and expansion of human PB-derived CD34+ or erythroid cells, and methods for subsequent reprogramming of these cells to hiPSCs using defined culture conditions. Colonies arising in ReproTeSR™ Medium have defined borders and no overgrowth of non-reprogrammed cells allowing for easy colony identification. ReLeSR™ is an enzyme-free passaging reagent that eliminates manual removal of differentiated cells, colony scraping and techniques to obtain uniform cell aggregates. This presentation will demonstrate that ReLeSR™ can be used to passage cells maintained in mTeSR™1 or TeSR™-E8™, and that it enables the use of closed vessels, thus facilitating scale-up and automation. Applicability of ReLeSR™ in selection of reprogrammed cells will also be demonstrated using fibroblast reprogramming at clonal density. Together with our existing products, these new tools provide a complete workflow for the isolation and expansion of cells prior to reprogramming, efficient feeder-free reprogramming, selection of hiPSC colonies, and maintenance of hiPSCs in defined, serum-free and feeder-free conditions.

Biography

Dr. Arwen Hunter is a Scientist in the Pluripotent Stem Cell Group of the Research and Development Department at STEMCELL Technologies Inc. She received her Ph.D from the University of British Columbia, Canada and then performed postdoctoral training at Stanford University, USA where she generated patient specific induced pluripotent stem cells (iPSCs) and iPSC-derived endothelial cells to model vascular disease. In addition, she examined novel strategies for nuclear reprogramming. At STEMCELL Technologies, Dr. Hunter is currently working on developing products for the generation and maintenance of induced pluripotent stem cells.

SPEAKER ABSTRACTS

S22
IDENTIFICATION AND FUNCTIONAL STUDIES OF RNA-BINDING PROTEINS IN EMBRYONIC STEM CELLS

KIM, V. Narry
Seoul National University, Seoul, South Korea

RNA-binding proteins (RBPs) play essential roles in RNA-mediated gene regulation, and yet the current annotation of RBP is largely limited to those with known RNA-binding domains. To systematically identify the RBPs of embryonic stem cells (ESCs), we here employ "interactome capture", which combines UV-crosslinking of RBP to RNA in living cells, oligo(dT) capture, and mass spectrometry. From mouse ESCs, 555 proteins are defined here to constitute the mESC mRNA-interactome, which includes 283 proteins not previously known to bind RNA. Interestingly, 68 novel RBP candidates are highly expressed in ESCs when compared to differentiated cells, suggesting that they may play important roles in stem cell physiology. As only a small fraction of RBPs has been functionally characterized, we delineated biological roles of RBPs in pluripotency maintenance by performing RNAi screen in mESCs. From the screen, we identified a subset of RBPs including exoribonucleases and the components of small subunit processome (SSUP) as factors important for maintaining pluripotency. Our new data suggest that a high level of ribosome biogenesis is required to maintain self-renewal and pluripotency in mESCs. Our mESC mRNA-interactome expands the atlas of RBPs with novel candidates, and provides a valuable resource for the study of RNA-RBP networks in stem cells.

Biography

V. Narry Kim is a Professor at Seoul National University, Korea. She also serves as the founding director of RNA Research Center at Institute for Basic Science (IBS). Kim received her Ph.D in 1998 from Oxford University where she studied retroviruses. She then carried out her postdoctoral research on mRNA surveillance in the laboratory of Gideon Dreyfuss at the University of Pennsylvania. She set up her research group in 2001 at Seoul National University and has been investigating how microRNAs are made and regulated, and what microRNAs do to modulate cell signaling and cell fate. Her group identified several key factors in the microRNA pathway including Drosha and TUTases, and studies mechanisms underlying microRNA regulation in stem cells and cancer. Narry Kim is a recipient of L'Oreal-UNESCO Women in Science (2008) and the Ho-Am Prize in medicine (2009).

S23: INSTITUTE OF MEDICAL BIOLOGY LECTURE
TRANSPOSABLE ELEMENTS AND THEIR EPIGENETIC CONTROL MECHANISMS ARE KEY REGULATORS OF TRANSCRIPTIONAL NETWORKS IN PLURIPOTENT STEM CELLS

TRONO, Didier
EPFL, Lausanne, Switzerland

Endogenous retroelements (EREs) account for more than half of the human genome. These mutagens constitute essential motors of evolution, which are subjected to tight epigenetic control notably during the waves of genome reprogramming that take place during early embryogenesis. We demonstrated that this process is in part mediated by a large family of

SPEAKER ABSTRACTS

transcriptional repressors, the tetrapod-specific KRAB-containing zinc finger proteins (KRAB-ZFPs), and by their cofactor KAP1 (KRAB-associated protein 1). KRAB/KAP1-mediated regulation is responsible for silencing a very broad range of EREs in human embryonic stem (ES) cells, in an evolutionally dynamic fashion. As a consequence, it exerts a marked influence on the transcriptional landscape of these cells. During the reprogramming of somatic cells to induced pluripotent stem cells (iPSCs), we observed that the transcriptional activity of EREs is altered, which likely both perturbs and facilitates the establishment of pluripotency. New light is shed on these events by our determination of the genomic binding sites of hundreds of human KRAB-ZFPs, which opens the door to deciphering stem cell transcriptional networks and, more generally, to cracking the code of DNA recognition by poly-zinc finger proteins.

Biography

Didier Trono, M.D., completed a clinical training in pathology, internal medicine and infectious diseases in Geneva and at Massachusetts General Hospital before performing post-doctoral work with David Baltimore at the Whitehead Institute. In 1990, he moved to the Salk Institute to launch a center for AIDS research. From 1997 to 2004, he was at the University of Geneva as professor and then head of the Department of Genetics and Microbiology. He then joined the EPFL as dean of its newly launched School of Life Sciences, a position he held for eight years. Didier Trono's research has long focused on interactions between viruses and their hosts and on developing tools for gene therapy. This led him to epigenetics, the current topic of his investigations.

S24

NOVEL EPIGENETIC PATHWAYS IN PLURIPOTENT STEM CELLS

XIAO, Andrew
Yale Stem Cell Center, New Haven, CT, USA

My laboratory focuses on epigenetic regulation in pluripotent stem cells. We have recently discovered novel epigenetic pathways in ESCs and iPSCs. (1) Intermittent expression of a group of early embryonic genes that are "wired" by endogenous transposons is implicated in ESC self-renewal. However, their functions and regulatory mechanisms remain elusive. We have recently identified Rif1 as a novel regulatory factor for these genes (Dan et al., 2014). A major function of Rif1 is to control telomere homoeostasis in ESCs via the regulation of a key telomere recombination factor, Zscan4. Our current studies aim to elucidate Rif1-regulated epigenetic pathways and their functions in pluripotency. (2) For future application of iPSCs technology, the ability to assess the overall quality of iPSC clones will be an important issue. We have recently demonstrated that the deposition pattern of histone variant H2A.X is a functional marker that can distinguish the developmental potentials of mouse iPSC lines (Wu et al. 2014). ESC-specific H2A.X deposition patterns are faithfully recapitulated in iPSCs that support the development of ''all-iPS'' animals via tetraploid complementation. In contrast, iPSCs that fail to support all-iPS embryonic development show aberrant H2A.X deposition, upregulation of extraembryonic lineage genes, and a predisposition to extraembryonic differentiation, all of which are reminiscent of H2A.X deficient ESCs. Our ongoing work aims to delineate the genetic pathways regulating H2A.X deposition during reprogramming.

SPEAKER ABSTRACTS

Biography

Dr. Andrew Xiao is an assistant professor in the Department of Genetics at the Yale University School of Medicine. He is a member of the Yale Stem Cell Center. Dr. Xiao's laboratory focuses on epigenetic regulation in pluripotent stem cells, including embryonic stem cells and induced pluripotenct stem cells (iPSC), as well as epigenetic regulation in neural development and aging. Dr. Xiao received his Ph.D degree from Terry Van Dyke's lab at UNC-Chapel Hill and postdoctoral training from David Allis' lab at Rockefeller University. Since 2009, Andrew Xiao is a recipient of the NCI Howard Temin Award in Cancer Research (K99/R00) and in 2012, he received the New Scholar Award from the Ellison Medical Foundation.

S25

LANDSCAPE AND VARIATION OF RNA SECONDARY STRUCTURES IN THE HUMAN TRANSCRIPTOME

WAN, Yue
Genome Institute of Singapore, Singapore

In parallel to the genetic code for protein synthesis, a second layer of information is embedded in all RNA transcripts in the form of RNA structure. The ability of RNA to base pair with itself and other nucleic acids endow RNA with the capacity to form extensive structures, which are known to influence practically every step in the gene expression program. Yet the nature of most RNA structures or effects of sequence variation on structure are not known. We applied a genome-wide RNA structure probing method (Parallel Analysis of RNA Structures-PARS) to study RNA secondary structures (RSS) in a human family Trio, providing a comprehensive RSS map of human coding and noncoding RNAs. We identify unique RSS signatures that demarcate open reading frames, splicing junctions, and define authentic microRNA binding sites. Over 1900 transcribed single nucleotide variants (~15% of all transcribed SNVs) alter local RNA structure; these "RiboSNitches" occur in disease-associated variants. We discover simple sequence and spacing rules that determine the ability of point mutations to impact RSS. Selective depletion of RiboSNitches versus structurally similar variants at precise locations suggests selection for specific RNA shapes at thousands of sites, including 3'UTRs, binding sites of miRNAs and RNA binding proteins genome-wide. These results highlight the potentially broad contribution of RNA structure and its variation to gene regulation.

Biography

Yue Wan was awarded the NSS-PhD scholarship from A*STAR in 2003. She obtained her B.Sc in Cell Biology and Biochemistry from the University of California, San Diego in 2006. She obtained her Ph.D in Cancer Biology at Stanford University, California, USA, under the mentorship of Howard Y. Chang. During her Ph.D, she developed a high-throughput method for probing RNA structures genome-wide. She is currently a GIS fellow in the Genome Institute of Singapore and she is interested in identifying functional RNA structures and understanding their roles in regulating cellular biology.

SPEAKER ABSTRACTS

S26
AGING OF HEMATOPOIETIC STEM CELLS

SUDA, Toshio
Keio University, Tokyo, Japan

Stem cell maintenance in a haltered cell-cycle state (i.e., quiescence) has been proposed as a fundamental property of HSCs. Maintenance of quiescence protects HSCs from functional exhaustion and naturally producing extrinsic cellular insults to enable lifelong hematopoietic cell production. HSC quiescence is regulated through a complex network of cell intrinsic regulations along with extrinsic influences from the microenvironment. Normal HSCs maintain intracellular hypoxia, stabilize the hypoxia-inducible factor-1α(HIF-1α) protein and generate ATP by anaerobic metabolism. In HIF-1α-deficiency, HSCs became metabolically aerobic, lost cell cycle quiescence, and finally exhausted. Due to the longevity of stem cells, HSCs are a useful target to study the mechanism of aging from the aspects of DNA damage response (DDR). We have focused on the HSCs and elucidate the mechanism of how HSC stemness is maintained in the niche, and how HSCs defend themselves against stress. We have recently demonstrated that expression of protection of telomeres 1a (Pot1a), a component of shelterin, is crucial for the prevention of telomeric DDR and maintenance of HSCs activity during aging. Pot1a is expressed at high levels in HSCs during development, yet this expression declines with age. Overexpression of Pot1a or treatment with exogenous Pot1a protein prevented telomeric DDR and rejuvenated HSC activity. Similar rejuvenation was observed upon treatment of human cord blood HSCs with recombinant human POT1 protein. These results highlight a general, reversible mechanism by which aging compromises mammalian stem cell activity, with widespread implications for regenerative medicine.

Biography

Toshio Suda is an MD from Yokohama City University School of Medicine. He was Professor of the Department of Cell Differentiation at Kumamoto University School of Medicine, now he is Professor of CSI, NUS and Developmental Biology at Keio University. Toshio Suda identified the niche for hematopoietic stem cells and subsequently established the new field of oxidative stress and stem cell aging. The interaction of stem cells and niche is one of the hot topics for the stem cell and cancer biology fields.

S27
SYSTEMS BIOLOGY OF STEM CELLS

NG, Huck Hui
Genome Institute of Singapore, Singapore

Embryonic stem (ES) cells are characterized by their ability to self-renew and remain pluripotent. Transcription factors have critical roles in the maintenance of ES cells through specifying an ES-cell-specific gene expression program. Deciphering the transcriptional regulatory network that describes the specific interactions of these transcription factors with the genomic template is crucial for understanding the design and key components of this network. To gain insights into the transcriptional regulatory networks in ES cells, we use chromatin immunoprecipitation coupled to ultra-high-throughput DNA sequencing

SPEAKER ABSTRACTS

(ChIP-seq) to map the locations of sequence specific transcription factors. These factors are known to play different roles in ES cell biology. Our study provides new insights into the integration of these regulators to the ES cell-specific transcription circuitries. Collectively, the mapping of transcription factor binding sites identifies new features of the transcriptional regulatory networks that define ES cell identity. Using this knowledge, we investigate nodes in the network which when activated, will jump-start the ES cell-specific expression program in somatic cells.

Biography

Huck-Hui Ng is the Executive Director of the Genome Institute of Singapore. Huck-Hui Ng graduated from the National University of Singapore with a first class Honor degree in Molecular and Cell Biology and obtained his Ph.D from the University of Edinburgh. He spent the next few years working at the Harvard Medical School as a Damon Runyon-Walter Winchell research fellow. His lab works on different aspects of Systems Biology of Stem Cells. Specifically, his group uses genome wide approaches to dissect the transcriptional regulatory networks in embryonic stem cells with the aim to identify key nodes in this network. This had led to the first paper on the whole genome and unbiased mapping of key transcription factors in mouse embryonic stem cells. His group also conducted the whole genome genetic screen for human embryonic stem cells. More recently, his lab has begun to investigate the reprogramming code behind the induction of pluripotency in somatic cells. His research work has earned him several prestigious national and international accolades including the Singapore Youth Award (2005 and 2010), the National Science Award 2007, the HUGO Chen's New Investigator Award 2010 and the President's Science Award 2011.

S28

ROLE OF A REST REGULATED MICRORNA IN HUMAN MOTOR NEURON DEVELOPMENT AND DISEASE

BHINGE, Akshay[1], Namboori, Seema C.[1], Buckley, Noel[2], Stanton, Lawrence W.[1]
[1]Genome Institute of Singapore, Singapore, [2]Department of Neuroscience, King's College London, London, United Kingdom

Development of the central nervous system requires temporally controlled transition of proliferating neural progenitors into post-mitotic differentiated neurons. However, the exact mechanisms underlying this transition are poorly understood. During embryonic development, RE1-silencing transcription factor (REST) inhibits neuronal genes and downregulation of REST is required for neuronal differentiation. REST mediated suppression of the neuronal program is mediated not only by direct suppression of pro-neural genes but also by inhibiting microRNAs (miRNAs) that promote neurogenesis. By combining genome-wide REST binding sites with small RNA-sequencing data from REST-/- neural progenitors, we identified hundreds of miRNAs that are potentially direct targets of REST. One of the identified miRNAs was found to be specifically activated during spinal motor neuron development. Overexpression and knockdown experiments confirmed that this miRNA is not only required but also sufficient for motor neurogenesis and possibly acts via targeting a key cell-cycle gene and neural transcription factor. Importantly, our data suggests that the identified miRNA may also be involved in motor neuron degeneration observed in spinal muscular atrophy.

SPEAKER ABSTRACTS

Biography

Akshay Bhinge obtained his degree in clinical medicine and surgery from Sir JJ group of hospitals, India and his Masters in Biomedical Engineering from the Indian Institute of Technology, Bombay, India. He joined Vishwanath Iyer's lab at the University of Texas at Austin, USA for his doctoral work graduating with a Ph.D in 2009. In 2010, he joined Dr. Lawrence Stanton's group as a postdoc at the Genome Institute of Singapore. His postdoctoral work includes understanding non-coding regulators of human neural development and disease with a primary focus on motor neuron degeneration.

S29
PLURIPOTENCY IN THE ARTIFICIAL CELL SPACE

NAGY, Andras for the PG Consortium
Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada

The ability to reprogram somatic cells to a pluripotent state is paradigm shifting for both biology and medical research. Reprogramming continues to challenge many of our assumptions about the specification of cellular phenotypes and yet, despite major efforts, we still lack a complete molecular characterization of the process. To address this gap we generated a comprehensive molecular description of the reprogramming cascade toward two distinct pluripotent states. We explored alternative outcomes of somatic reprogramming by fully characterizing reprogrammed cells independent of preconceived definitions of reprogrammed iPSC states. We demonstrate that manipulating the expression level of the reprogramming factor influences cells arrival to a non-ES cell-like or ES cell like pluripotent state. This bifurcated process has been characterized with multiple "omic" platforms, consisting of the transcriptome (microRNA, lncRNA and mRNA), CpG methylation, ChIP-sequencing (for chromatin marks: H3K4me3, H3K27me3 and H3K36me3), in addition to quantitative mass spectrometry profiling of the global and cell surface proteome. This dataset enables cross-referencing between "omic" platforms, which facilitates deeper understanding of the cascade of molecular events driving the generation of pluripotent cells.

Biography

Dr. Nagy is currently a Senior Scientist at the Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Professor in the Department of Molecular Genetics at the University of Toronto, and Investigator at the McEwen Centre for Regenerative Medicine, Canada. He also holds a Tier I Canada Research Chair in Stem Cells and Regeneration. His research focuses on several areas of interest, which include 1) Functional studies of genes belonging to families with known roles in vessel formation; 2) Development of sophisticated genetic manipulation tools in the mouse model; 3) Applying genetics to cancer research; 4) Derivation, differentiation and genetic modification of both mouse and human Embryonic Stem cells; 5) Reprogramming of somatic cells to pluripotent stem cells. 5) Reprogramming of somatic cells to pluripotent stem cells using a novel method based on the piggyBac transposon system and understanding the key molecular players in the reprogramming process.

SPEAKER ABSTRACTS

S30
SYSTEMS DISSECTION OF PLURIPOTENT STEM CELLS

BUCHHOLZ, Frank[1,2,3]
[1]Medical Faculty of the TU Dresden, UCC, Medical Systems Biology, [2]Max Planck Institute of Molecular Cell Biology and Genetics, and 3Center for Regenerative Therapies, Dresden, Germany

Pluripotent stem cells are important to understand early mammalian development and hold great promise for future regenerative therapies. The identification of factors required to maintain pluripotency is a pivotal step to fully develop these cells for applied purposes and to decipher embryonic differentiation. I will describe the combined use of genome-scale RNAi screens with genetic interaction, protein localization and protein-level dependency studies to delineate connectivity between factors that control ES and EpiSC identity. Examples of newly identified protein coding, and long non-coding RNAs that impact on pluripotency will be presented.

Biography

Frank Buchholz is full Professor for Medical Systems Biology at the University Hospital and Medical Faculty Carl Gustav Carus of the University of Technology Dresden. He studied biology at the University Göttingen, received his Diploma in 1992 from the University in Göttingen, and worked from 1994 - 1998 on his Ph.D thesis at the EMBL in Heidelberg. From 1998 to 2002, he was a Postdoc at UC San Francisco in the Laboratory of Prof. J. Michael Bishop, before he started his own group at the Max-Planck-Institute for Molecular Cell Biology and Genetics in Dresden were he performed research from 2002 until 2010. In his current position Frank applies state of the art genomics technologies to unravel the biology of cancer and stem cells.

S31
EPIGENETIC AND TRANSCRIPTIONAL DYNAMICS OF GERMLINE STEM CELLS

Hammoud, Sue S.[1], Low, Diana H.p[2], Yi, Chongil[1], Carrell, Douglas T.[3], Guccione, Ernesto[4],
CAIRNS, Bradley R.[1]
[1]Oncological Sciences, HHMI and Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA, [2]Institute of Molecular and Cell Biology, ASTAR, Singapore, [3]Department of Surgery, University of Utah, Salt Lake City, UT, USA, [4]Department of Biochemistry, Division of Cancer Genetics Therapeutics, Institute of Molecular and Cell Biology, ASTAR, Singapore

In male mice, spermatogonial stem cells (SSCs) of the neonate and juvenile proliferate to populate the seminiferous tubule, and during/following puberty develop into adult germline stem cells (AGSCs) - which either self-renew (Thy1+ enriched) or commit to spermatogenesis (Kit+ enriched). To better understand how chromatin regulates SSCs, AGSCs and

SPEAKER ABSTRACTS

spermatogenesis we derived stage-specific high-resolution profiles of DNA methylation, histone modifications, and RNA-seq in SSCs, AGSCs and during spermatogenesis. First, we define striking signaling and transcriptional differences between SSCs and AGSC types that appear to underlie their differences in proliferation, stage in development, and interaction with their niche/environment. Second, we find key pluripotency factors in ES cells (e.g. Nanog, Prdm14 and Sox2) silent in SSCs and AGSCs, and find that their promoters/enhancers bear particular chromatin/DNAme attributes that may "poise" them for reactivation after fertilization, which may underlie their spontaneous ability to form ES-like cells in vitro. Third, AGSCs display chromatin "poising/bivalency" of enhancers and promoters for embryonic transcription factors. Remarkably, spermatogenesis occurs without significant changes in DNAme and instead involves transcription of DNA-methylated promoters bearing high RNAPol2, H3K9ac, H3K4me3, low CG content, and (often) 5hmC.

Biography

Bradley Cairns is interested in how chromatin structure helps regulate gene transcription. His lab purifies and characterizes large protein complexes that remodel and modify chromosomal structure. The lab also investigates how chromatin regulates RNA Pol III transcription, and noncoding RNAs methylation. A major emerging interest is germline chromatin–how genes are marked (by DNA methylation) and packaged by chromatin in sperm and eggs–to promote proper gene expression in the embryo. Dr. Cairns received his Ph.D from Stanford in Cell Biology and conducted postdoctoral studies at Harvard Medical School. He is now an HHMI Investigator and Chair of the Department of Oncological Sciences at the Huntsman Cancer Institute, University of Utah School of Medicine.

S32

INHIBITION OF THE UPR PATHWAY RESCUES MOTOR NEURONS DERIVED FROM PATIENTS WITH SPINAL MUSCULAR ATROPHY

NG, Shi Yan, Soh, Boon Seng, Rodriguez-Muela, Natalia, Hendrickson, David, Price, Feodor, Rinn, John, Rubin, Lee
Harvard University, Cambridge, MA, USA

Although the Survival of Motor Neuron (SMN) protein is ubiquitously expressed, it remains poorly understood why motor neurons are one of the most affected cell types in Spinal Muscular Atrophy (SMA), where patients have reduced SMN expression. In this study, we used induced pluripotent stem cells (iPSCs) from SMA patients to model the disease in vitro and combined it with a novel motor neuron purification method that we used for RNA-sequencing. We found SMA-specific changes in the iPSC-derived motor neurons, including hyper-activation of the endoplasmic reticulum (ER) stress pathway and enhanced apoptosis. Functional studies further demonstrated that inhibition of ER stress improves overall motor neuron health and survival in vitro even in motor neurons with low SMN levels. In SMA mice, we show that systemic delivery of an ER stress inhibitor that crosses the blood-brain barrier led to preservation of motor neurons in the spinal cord. Therefore, our study implies that ER stress is an important component of SMA, and can be explored as a therapeutic target.

SPEAKER ABSTRACTS

Biography

I received my Ph.D in 2012 from the National University of Singapore/ Genome Institute of Singapore for my work on long non-coding RNAs in neuronal differentiation in the lab of A/Prof Lawrence Stanton. Currently a postdoctoral fellow in Lee Rubin's lab at Harvard University, my research focuses on elucidating aberrant pathways in diseased neurons derived from patient induced pluripotent stem cells, and targeting these pathways for therapeutic purposes.

S33: LUNCHTIME INDUSTRY SESSION

TAL-BASED EDITING OF IPSCS FOR THE STUDY OF NEURODEGENERATIVE DISEASES

Federici, Mark

Due to the inherent biological variability between cell lines of different backgrounds, the study of subtle phenotypic changes associated with disease states when using iPSC derived materials requires isogenic control lines for proper comparison-- that is, cell lines that differ in a defined location but that are otherwise identical at the genetic level. This presentation will describe the approaches we've taken to modifying genes involved in Parkinson's Disease and related disorders. For example, modifying the glucocerebrosidase gene in iPSCs using TAL-based engineering technology presented several challenges, including the presence of an adjacent and highly homologous pseudogene. Initial phenotypic comparisons between the patient derived, TAL-edited lines that we have generated will be presented.

Biography

Mark Federici joined Life Technologies in 2006 and is Associate Director, Strategic Alliances. After graduating with a Biology degree from the St. Joseph's University, PA, he earned his Masters in Biotechnology/ Chemical Engineering from Manhattan College, NY. His career spans various positions including field applications scientist, technical sales specialist and collaborations manager. Prior to joining Life Technologies, Mark worked as Lab Manager at the Memorial Sloan Kettering Cancer Center, Department of Surgery, Breast and Ovarian Cancer Laboratory in 1987-2000, and was subsequently managing the Cell Biology department at Cell and Molecular Technologies/ Sentigen from 2000-2006. Mark has authored/ co-authored more than 35 peer-reviewed journal articles, and carries two patents under his belt - Division Arrested Cells for HTS and Tango Technology for Beta Arrestin Recruitment in GPCRs. Mark is a current member of the LRRK2 Consortium, at the Michael J. Fox Foundation for Parkinson's Disease Research.

SPEAKER ABSTRACTS

S34

ROLES FOR TRANSCRIPTIONAL SUPER-ENHANCERS IN CELL IDENTITY AND CANCER

YOUNG, Richard A.
Whitehead Institute for Biomedical Research and MIT, Cambridge, MA, USA

Super-enhancers are large clusters of transcriptional enhancers that drive high-level expression of key cell identity genes. I will describe new insights into the functions of these unusual domains in control of cell-type specific gene expression programs. Cancer cells acquire super-enhancers at oncogenes and other genes important to tumor pathogenesis. In these cells, super-enhancer-driven oncogene expression can be exceptionally vulnerable to drugs that target transcriptional cofactors, suggesting new approaches to cancer therapy. I will describe recent results that further illuminate the roles of super-enhancers in control of cell identity and disease.

Biography

Richard Young is a molecular biologist recognized for his work on gene regulation in health and disease. He is known particularly for development of genome analysis technologies and their use to identify the core regulatory circuitry of human cells. Dr. Young received his B.S. degree in Biological Sciences at Indiana University, his Ph.D in Molecular Biophysics and Biochemistry at Yale University, and conducted postdoctoral research at Stanford University. He has served as an advisor to the National Institutes of Health, the World Health Organization, the Vatican and numerous scientific societies and journals. Dr. Young has founded and advised companies in the biotechnology and pharmaceutical industry, and is currently a member of the Board of Directors of Syros Pharmaceuticals. His honors include Membership in the National Academy of Sciences, the Chiron Corporation Biotechnology Research Award, Yale's Wilbur Cross Medal, and in 2006 Scientific American recognized him as one of the top 50 leaders in science, technology and business.

S35

A GENOME-WIDE RNAI SCREEN IDENTIFIES OPPOSING FUNCTIONS OF SNAI1 AND SNAI2 ON THE NANOG DEPENDENCY OF ESTABLISHING PLURIPOTENCY

WANG, Jianlong, Gingold, Julian A., Fidalgo, Miguel, Guallar, Diana, Lau, Zerlina, Sun, Zhen, Zhou, Hongwei, Faiola, Francesco, Huang, Xin, Lee, Dung-Fang, Waghray, Avinash, Schaniel, Christoph, Felsenfeld, Dan P., Lemischka, Ihor R.
Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Nanog is a critical factor for optimal ESC self-renewal and efficient iPSC generation during the final stage of reprogramming. From a genome-wide siRNA screen using a Nanog-GFP reporter line we discovered surprisingly opposing effects of Snai1 and Snai2 depletion on Nanog promoter activity. When tested under the reprogramming setting, Snai1 and Snai2 mutually repress each other's expression and have opposing roles during the final stage of reprogramming. We found that Snai1, but not Snai2, is both a transcriptional target and partner protein of Nanog in promoting the transition of partially reprogrammed

SPEAKER ABSTRACTS

cells to ground state pluripotency. Ectopic expression of Snai1 or depletion of Snai2 greatly facilitate the Nanog-driven final stage of reprogramming. Snai1 (but not Snai2) and Nanog co-bind to and transcriptionally activate the pluripotency-associated genes including Lin28 and miRNA-290-295. Ectopic expression of individual or the whole miRNA-290-295 cluster genes partially rescues reprogramming inefficiency caused by the Snai1 depletion. Our studies thus uncover novel roles of Snai1 and Snai2 in reprogramming and highlight the complexity of molecular interplay between Nanog and mesenchymal transcription factors Snai1 and Snai2 in transcriptional regulation of pluripotency-associated genes and miRNAs during the critical phase of reprogramming.

Biography

Jianlong Wang is an Associate Professor in the Black Family Stem Cell Institute at Mount Sinai School of Medicine, New York. He received his Ph.D in 2000 from the University of Massachusetts and carried out his first postdoctoral research in UNC Lineberger Comprehensive Cancer Center at North Carolina. He then pursued further postdoctoral training in stem cell biology with Dr. Stuart Orkin in Children's Hospital Boston, where he explored proteomic approaches to understand pluripotency and constructed the first protein interaction network governing pluripotency of embryonic stem cells. He set up his research group in 2009 at Mount Sinai School of Medicine and has been investigating the molecular mechanisms of stem cell pluripotency and somatic cell reprogramming. His group established the extended Oct4/Nanog interactomes and identified important factors that contribute to Nanog/Oct4 functions in pluripotency and reprogramming. Dr. Wang is a recipient of the Hirschl Trust Career Scientist Award and the Dr. Harold and Golden Lamport Research Award.

S36

TRANSCRIPTION DRIVEN BY PRIMATE-SPECIFIC ENDOGENOUS RETROVIRUS HERVH DEFINES NAÏVE STEM CELLS

IZSVAK, Zsuzsanna
Max-Delbrück-Center for Molecular Medicine, Berlin, Germany

Primate-specific endogenous retrovirus driven transcription defines naïve-like stem cells. Naïve embryonic stem cells (ESCs) hold great potential for research and therapeutics. While such cells are readily derived from mouse blastocysts, to date it has been impossible to easily isolate human equivalents. Here we show that a sub-population within cultures of human ESCs (hESCs) and induced pluripotent stem cells (hiPSCs) manifest key properties of naïve state cells. These naïve cells can be genetically tagged, and are associated with elevated transcription of HERVH, a primate specific endogenous retrovirus. HERVH elements provide functional binding sites for a combination of naïve-specific pluripotency transcription factors, including LBP9, recently recognized as relevant to naivety in mice. LBP9/HERVH drives hESC and hiPSC specific alternative and chimeric transcripts, including pluripotency modulating long non-coding RNAs. Disruption of LBP9, HERVH and HERVH-derived transcripts compromise self-renewal. These observations define HERVH expression as a hallmark of naïve hESCs and establishes novel primate-specific transcriptional circuitry regulating pluripotency.

SPEAKER ABSTRACTS

Biography

Zsuzsanna Izsvák received her Ph.D from the Hungarian Academy of Sciences in Budapest, Hungary. After a postdoctoral period at the University of Minnesota, USA, she has returned to Europe with an EMBO fellowship and worked at the Netherlands Cancer Institute in Amsterdam between 1997 and 1999. Now, she is running her research group, "Mobile DNA", at the Max-Delbrück-Center in Berlin, Germany. In 2004, her research was evaluated by the European Science Foundation (ESF) as "strategically important" and was awarded by the European Young Investigator Award (EURYI) in 2004, and by ERC Advanced (European Research Council) in 2012. She is one of the inventor of the Sleeping Beauty transposon system, recently translated for clinical application to treat B-lineage malignancies. She is interested in Mobile or Transposable Elements (TEs). The evolutionary success of TEs is powerfully underscored by the finding that about 45% of the human genome is TE-derived. The vast majority of human TEs are not mobile, and have long been considered to constitute "junk DNA", seriously underestimating the impact of TE-associated activities. Dr. Izszak will present her work on how an endogenous retrovirus family has been co-opted to specify human naïve pluripotency.

S37

MODELING MYELODYSPLASTIC SYNDROMES IN MICE BY ALTERED HOXA1 SPLICEFORM EXPRESSION

PURTON, Louise
St. Vincent's Institute, Fitzroy, Australia

Myelodysplastic syndromes (MDS) are a heterogeneous group of incurable malignant blood cell diseases with largely unknown aetiology. Approximately 30% of MDS patients progress to acute myeloid leukaemia (AML). Mouse models that faithfully represent MDS are based on rare genetic abnormalities and currently represent only a very small subset of MDS patients. We have discovered that homeobox A1 (HOXA1) mRNA is upregulated 2-fold in 50% of MDS patients, irrespective of karyotype (n=183 MDS, 17 controls, $P<0.05$). Human HOXA1 and mouse Hoxa1 (WT-Hoxa1) are expressed as two different spliceforms generated by alternative splicing within exon 1: a full-length form (Hoxa1-FL) and a truncated form (Hoxa1-T), which lacks the homeobox domain. Mutations in splicing machinery have been recently identified in up to 85% of MDS patients. We therefore hypothesised that deregulated HOXA1 spliceforms may contribute to MDS. Retroviral overexpression of either WT-Hoxa1 (which generates both Hoxa1-FL and Hoxa1-T) or Hoxa1-T in murine bone marrow (BM) cells showed opposing effects, suggesting that Hoxa1-T may negatively regulate Hoxa1-FL. We therefore generated a mutant Hoxa1 (MUT-Hoxa1), which expresses normal Hoxa1-FL but not Hoxa1-T, by site-directed mutagenesis at the splice site of Hoxa1-T. We transplanted recipient mice with BM overexpressing MUT-Hoxa1, WT-Hoxa1 or empty vector control. All recipients of WT-Hoxa1 or MUT-Hoxa1 BM developed features resembling human MDS including peripheral blood macrocytic anaemia and thrombocytopenia, dysplastic features in BM erythroid and megakaryocyte lineages and increased apoptosis in BM erythroid cells. Strikingly, recipients of MUT-Hoxa1 BM developed a more severe MDS phenotype that spontaneously progressed to AML. In summary, altered HOXA1 spliceform expression likely contributes to MDS in up to 50% of patients. Our MUT-Hoxa1 and WT-Hoxa1 mouse models will therefore be highly valuable in identifying better therapies for a significant subset of MDS patients.

SPEAKER ABSTRACTS

Biography

Associate Professor Louise Purton received her PhD from The University of Melbourne and undertook post-doctoral studies at the Fred Hutchinson Cancer Research Center in Seattle. During this time she made novel discoveries that the vitamin A derivative all-trans retinoic acid (ATRA) has different effects in hematopoiesis and that ATRA enhances hematopoietic stem cell (HSC) self-renewal. Louise returned to Australia in 2000 to head her own research group at the Peter MacCallum Cancer Centre, focusing on the distinct effects of the different retinoic acid receptors in hematopoiesis. She was a visiting scientist in Professor David Scadden's laboratory at Massachusetts General Hospital in Boston, where she identified novel roles for cells of the bone marrow microenvironment in regulating myeloproliferative-like disorders, pioneering studies that were published in Cell in 2007. In 2008 Louise returned to Melbourne to establish the Stem Cell Regulation Unit at St. Vincent's Institute (SVI), where she investigates intrinsic and extrinsic regulation of hematopoiesis in normal and diseased states.

S38

EZH2 INHIBITORS AS POTENTIAL THERAPEUTICS IN GERMINAL CENTER B CELL LYMPHOMAS

SMITH, Jesse
Epizyme, Inc, Cambridge, MA, USA

Stem cell fate decisions, such as whether to self-renew or to differentiate, are regulated in large part by epigenetic mechanisms. Dysregulation of epigenetic machinery, such as histone methyltransferases (HMTs), in certain progenitor cell contexts can promote increased self-renewal, decreased differentiation and ultimately oncogenesis. For example, during B cell maturation, the HMT EZH2 is critical for formation of the germinal center (GC) reaction. EZH2-mediated repression of certain target sets allows GC B cells to undergo clonal expansion and somatic hypermutation. In order for GC B cells to exit GC reaction, EZH2 expression and activity must be attenuated -the failure to do so may contribute to oncogenic signaling in GC-derived lymphomas. Epizyme and others have previously reported on gain-of-function mutations in EZH2, lesions which result in increased trimethylation of H3K27 on polycomb target genes, including those required for B cell maturation and exit from the germinal center reaction. Importantly, lymphoma cell lines bearing these mutations demonstrate robust antiproliferative phenotypes in response to small molecule inhibitors of EZH2. Beyond these mutant EZH2 cell lines, there is evidence that small molecule inhibitors of EZH2 also have antiproliferative activity in GC lymphomas with wild type (wt) EZH2. These data are consistent with the idea that EZH2 is a "lineage factor" for GC B cells, and that GC B cell lymphomas may possibly become "addicted" to EZH2 through mechanisms other than gain-of-function mutations in said gene. The aggregate of these findings suggest EZH2 inhibitors warrant further investigation as therapeutics for GC B lymphomas. The focus of this presentation will be the discovery and development of EPZ-6438 (E7438), a small molecule inhibitor of EZH2, currently in a Phase I clinical trial for advanced malignancies. As described above, the seminal findings regarding the activity of EPZ-6438 in preclinical models of GC B cell lymphomas will be discussed. Additionally, preclinical investigations evaluating the combination of EPZ-6438 with multiple therapeutic modalities will be presented. Finally, a brief overview of the early clinical observations of EPZ-6438 will be discussed.

SPEAKER ABSTRACTS

Biography

Jesse Smith is the Executive Director of Biological Sciences at Epizyme (Cambridge, Massachusetts, U.S.A.), a clinical stage biopharmaceutical company creating innovative personalized therapeutics for patients with genetically defined cancers. Jesse earned his B.S. degree in Biochemistry and Molecular Biology from the University of Georgia, graduating with summa cum laude and Phi Beta Kappa honors. Subsequently, he earned his Ph.D from Duke University, in the laboratory of Dr. Sally Kornbulth. After a brief period as a post-doctoral fellow at the National Cancer Institute, Jesse began a career in the biopharma arena, including positions at Arqule, Sirtris (a GSK company), and Catabasis Pharmaceuticals. To date Jesse has contributed to seven IND submissions, one CTA filing, more than 20 scientific publications, and three patents.

S39

REGULATION OF NEURAL STEM/PROGENITOR CELL FATE IN THE EMBRYONIC AND ADULT MOUSE BRAINS

Furutachi, Shohei, Kuniya, Takaaki, Kishi, Yusuke, Hirabayashi, Yusuke, **GOTOH, Yukiko**
Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan

One of the fundamental questions in understanding tissue development is how multipotent progenitors/tissue stem cells give rise to various cell types in a defined order to achieve appropriate tissue organization. Neural stem/progenitor cells (NPCs) attract much attention since these cells give rise to neuronal and glial cell types in a developmental-stage dependent manner with striking precision. We have previously shown that polycomb group (PcG) complex and high mobility group A (HMGA) proteins play pivotal roles in driving fate switches of NSCs during neocortical development. I would like to talk about how these proteins are regulated and how they control the fate of NPCs in a developmental stage-dependent manner. In contrast to embryonic NPCs, adult neural stem cells (NSCs) maintain their differentiation potentials for a long time to continue to generate neurons for life. I would also like to talk about the mechanisms underlying their long-term maintenance and the differences between embryonic NPCs and adult NSCs.

Biography

Yukiko Gotoh received her Ph.D degree from the University of Tokyo and studied in Eisuke Nishida's laboratory where she biochemically identified and characterized vertebrate MAP kinase and its activator, MAPKK. After spending a few years in the USA as a visiting scientist in the labs of Jonathan A. Cooper at the Fred Hutchinson Cancer Research Center and Michael E. Greenberg at the Harvard Medical School, where she started working on signaling pathways in neurons, she returned to Japan and was appointed as an Associate Professor in 1999 and then as a Professor in 2005 at the University of Tokyo. Her group is interested in the mechanisms and logic underlying the regulation of neural stem/progenitor cell fate both during brain development and in the adult brain.

SPEAKER ABSTRACTS

S40

STEM CELLS FROM THE MAMMALIAN BLASTOCYST

ROSSANT, Janet

The Hospital for Sick Children Research Institute, Toronto, ON, Canada

The mammalian blastocyst contains about 100 cells and only three distinct cell types. One cell type, the epiblast, gives rise to all cell types of the body and to pluripotent embryonic stem cells, while the other two cell types give rise to placental and other support cells. Stem cell lines have been derived from all three blastocyst cell types in the mouse. These lines retain the cell lineage specificity of their embryonic origin. By studying both the embryo and its derived stem cells in the mouse, the key transcription factors and signaling pathways specifying cell fate in both situations have been identified. A detailed model of the development of the mouse blastocyst can now be developed. However much less is known about the development of the human blastocyst and the process of establishing pluripotency in this species. Delineating the comparative aspects of cell specification between mouse and human is key to understanding the complexities of the various pluripotent states of human and mouse stem cells.

Biography

Dr. Janet Rossant is a Senior Scientist in Developmental and Stem Cell Biology and the SickKids Chief of Research. Known for her studies of the pathways that control embryonic development in the mouse, Rossant has pioneered techniques for following cell fate and altering genes in embryos. Her current research focuses on stem cell development and cell differentiation in the developing embryo. Dr. Rossant is the immediate past President of the International Society for Stem Cell Research.

PAKAIR

THE TIME CRITICAL AIR CARGO SPECIALISTS

- Premium Life Science Courier Specialising in Stem Cell movements worldwide. Including:
  - Human embryonic stem cells
  - Mouse Embryonic Fibroblast cells
  - Other cell lines, cultures, media, enzymes and proteins
  - Cord Blood collections (at ambient or 2-8 deg C)
- Hire of PAKAIR Liquid Nitrogen dry shipper capable of carrying Cord Blood Bags.
- Smaller IATA Approved dry shippers for samples on canes.
- Assistance with Regulatory paperwork for quick border control clearance.
- Fastest possible transit door to door.
- Hand Carry (On-Board Courier) services available.

CONTACT: enquiries@pakair.com.au for further info.
WEB ADDRESS: www.pakairasia.com

POSTER HALL INFORMATION

POSTER SET-UP AND TAKEDOWN

The posters will be accessible throughout the duration of the Forum. Please place posters in their designated location on Level 4, Matrix Building by Wednesday, 5 November 2014 16.30. Velcro stickers for hanging posters will be provided.

Poster presenters are responsible for their own posters. Posters that have not been removed by Friday, 7 November at 18.00 will be discarded by the organizer.

POSTER PRESENTATION

Poster presenters are required to be present at their posters during scheduled poster presentations.

ODD-NUMBERED POSTERS

Wednesday, 5 November 2014 from 19.00 – 20.00

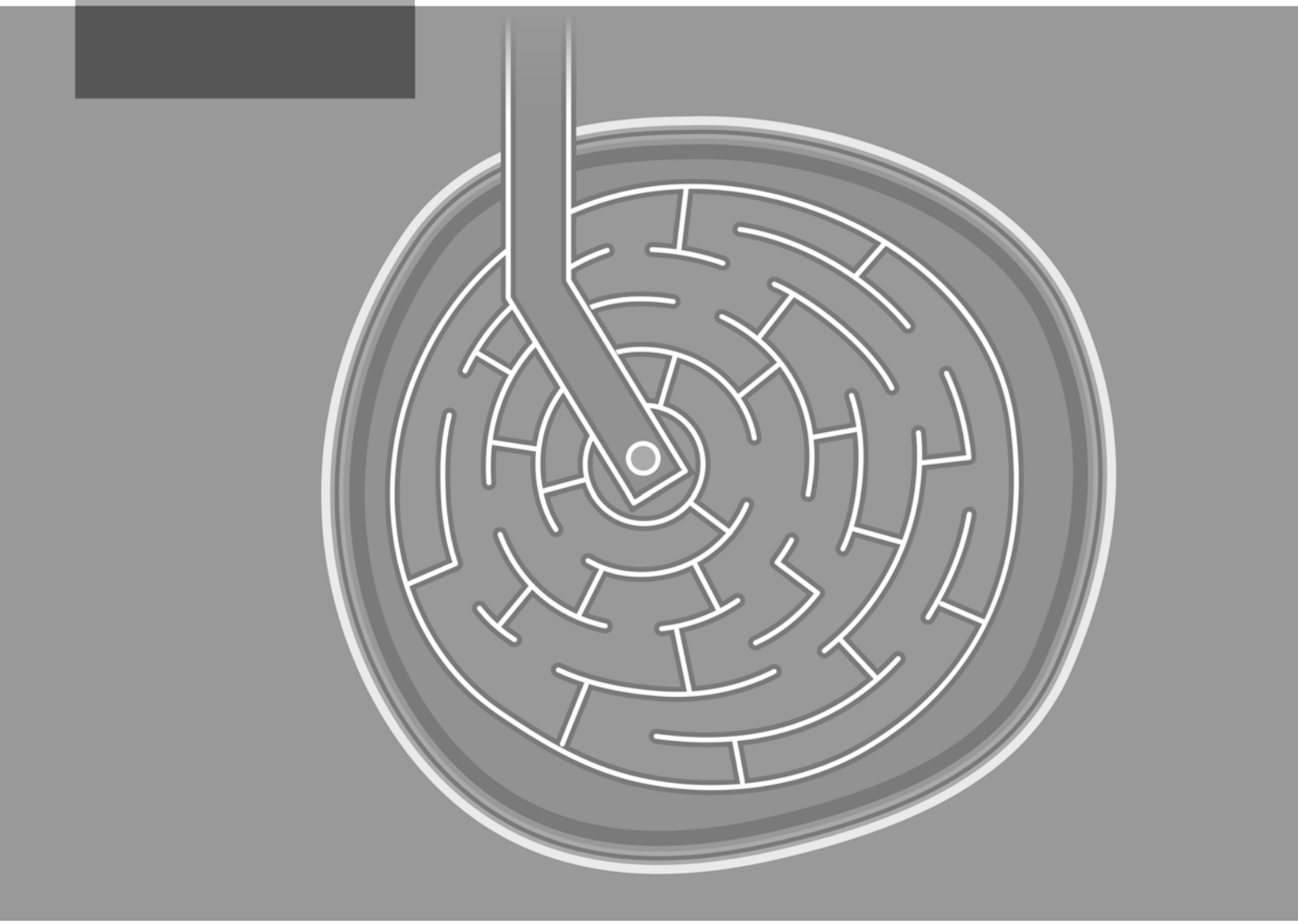
EVEN-NUMBERED POSTERS

Thursday, 6 November 2014 from 17.30 – 18.30

BioResearch

Lonza

It All Stems from Here



Simplify Your Reprogramming, Proliferation and Differentiation

Take the trial and error out of your stem cell workflow with optimized toolkits from Lonza.
Whether you work with iPSC or adult stem cells, your direct route to biologically relevant results starts here.

- NEW! L7™ Reprogramming and Culture System – robust system for generation and maintenance of hPSCs
- Nucleofector™ Technology – non-viral transfection for reliable reprogramming and genome editing
- Clonetics™ Primary Cells and Poietics™ Stem Cells – extensive range available

Streamline your stem cell workflow today.

© 2014 Lonza Walkersville, Inc

www.lonza.com/stem

POSTER ABSTRACTS

P1

ATTEMPT TO REPROGRAM SOMATIC CHICKEN CELLS INTO IPS CELLS

PAIN, Bertrand, Fuet, Aurélie, Maisin, Cécile, Aubel, Pauline, Voisin, Sophie
U846 INSERM, Bron, France

The Chicken Embryonic Stem (cES) cells, derived from chicken early blastoderm (stage IX-XII, EG & K), present typical features of ES pluripotent cells. Indeed, these cells are able to self renew for long term culture, to differentiate in vitro and in vivo into various cell types and to contribute to chimeras when injected into recipient embryos. The pluripotency of these unique cells is maintained through the cOCT4/cPOUV and cNANOG genes in the presence of other actors that were identified through a complete transcriptomic approach. In mammals, the introduction of the OSKM (Oct4, Sox2, Klf4 and c-Myc) genes into somatic cells has allowed to generate induced pluripotent stem (iPS) cells. So far, this process has been only clearly demonstrated in mammals. In an attempt to evaluate the reprogramming process in avian species, we have tried to reprogram primary chicken embryonic fibroblasts (cEFs) into IPS cells. Using first the mouse OSKM gene combination, we were unable to generate any substantial long term proliferating cells with ES-like features. We then cloned the different chicken cDNAs for OCT4 (POUV), NANOG, SOX2/SOX3, KLF4, c-MYC, LIN28 and inserted them into transposons and lentiviral backbones vectors. Several independent experiments provide drastic morphological changes of the transduced cEFs with various gene combinations. As previously observed the OSKM combination appears not to be efficient to induce a sustainable reprogramming process. The presence of additional genes is required to get long term proliferating alkaline phosphatase and SSEA1 positive cells. The ability of these cells to form embryoid body (EB), to express pluripotent associated genes and to contribute to chimeras when injected into recipient embryos will be presented and discussed. All these results indicate that those new reprogrammed cells present some of the specific traits of the cES cells.

P2

NOVEL SYSTEM TO ENHANCE THE PRODUCTION OF ERYTHROID CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS USING HIF -1 ALPHA

ALMURAIKHI, Nihal Ahmed, Mantalaris, A.
Imperial College London, London, United Kingdom

Blood shortage is one of the major global concerns as there is a significant imbalance in donations comparing to transfusion demands, which encouraged researchers to develop appropriate substitutes for donated blood using pluripotent stem cells. There has been a rising excitement lately that human induced pluripotent stem cells (iPS cells) could provide patient-specific cells for cellular therapy in addition to their differentiation capability into any cell type, which can be exploited in erythroid cell production. Erythropoiesis is the process of making erythrocytes that can be enhanced by hypoxia-inducible factors 1-alpha (HIF-1α), a transcription factor known to facilitates cellular adaptation to hypoxia by over-expressing specific genes and stimulating many metabolic processes, including erythropoiesis, and angiogenesis. In this study, we have established a novel protocol to generate erythroid cells from human iPS cells using HIF-1α as a key enhancer. Beside the use of the standard cytokine cocktail used for erythroid induction; Epo, SCF, FLT3, TPO, IL3, and IL6, other growth factors were used; BMP4, VEGF, FGF, in addition to 5% serum. Supplementing the cells in 2D culture system with our novel optimized concentrations of the said cocktail under hypoxic condition showed the highest yield erythroid markers, mainly CD235a. Further maturation of those cells is required in order to achieve fully mature and functional RBCs phenotype. These results must be supported by the detection of Rh type and ABO grouping to ensure the presence of RBCs antigens. Eventually, after optimizing an enucleation protocol, globin detection and O2 dissociation curve must be also

POSTER ABSTRACTS

made to ensure functionality of the hemoglobin (incomplete work). Thus, considering all the above, the ultimate aim of this study is the efficient production of mature and functional RBCs in vitro from patient-specific iPS cells using HIF-1α to facilitate erythroid progenitor maturation and proliferation

P3

PTEN AND TNC ARE NOVEL OCT4-REGULATING GENES REQUIRED FOR DRUG RESISTANCE AND METASTASIS IN LUNG CANCER

TANG, Yen-An[1], Chen, Chi-Hsin[1], Sun, H. Sunny[2], Cheng, Chun-Pei[3], Tseng, Vincent S.[3], Hsu, Han-Shui[4], Su, Wu-Chou[5], Lai, Wu-Wei[6], Wang, Yi-Ching[1]
[1]Department of Pharmacology, National Cheng Kung University, Tainan, Taiwan, [2]Institute of Molecular Medicine, National Cheng Kung University, Tainan, Taiwan, [3]Department of Computer Science and Information Engineering, National Cheng Kung University, Tainan, Taiwan, [4]Division of Thoracic Surgery, Taipei Veterans General Hospital, Taipei, Taiwan, [5]Department of Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan, [6]Department of Surgery, National Cheng Kung University Hospital, Tainan, Taiwan

Overexpression of Oct4, a stemness gene encoding a transcription factor, has been reported in several cancers. However, the mechanism by which Oct4 directs transcriptional program that leads to somatic cancer progression remains unclear. Here, using an integrative approach combining our Oct4 chromatin-immunoprecipitation sequencing and ENCODE datasets, we identify the genome-wide binding regions of Oct4 in lung cancer at promoter and enhancer of numerous genes involved in critical pathways which promote tumorigenesis. Notably, ***PTEN*** and ***TNC*** were previously undefined targets of Oct4. In addition, novel Oct4-binding motifs were found to overlap with DNA elements for Sp1 transcription factor, which facilitated chromatin recruitment of Oct4. Oct4 differentially interacted with Sp1 to suppress ***PTEN*** leading to drug resistance, or to transcriptionally activate ***TNC***, eliciting cancer invasion and metastasis. Clinically, lung cancer patients with Oct4 high, PTEN low, and TNC high expression profile significantly correlated with cancer progression and poor disease-free survival. Our study reveals a critical Oct4-driven transcriptional program that promotes stem-like features such as drug-resistance and metastasis through PTEN and TNC in lung cancer, respectively.

P4

NOTCH SIGNALING PLAYS IMPORTANT ROLES FOR THE MULTIPOTENCY OF MELANOBLASTS

WATANABE, Natsuki[1], Motohashi, Tsutomu[1], Hirobe, Tomohisa[2], Kunisada, Takahiro[1]
[1]Department of Tissue and Organ Development, Regeneration and Advanced Medical Sc, Gifu, Japan, [2]Active Radiation Protection Research Team, Radiation Risk Reduction Research Program, National Institute of Radiological Sciences, Chiba, Japan

Melanoblasts (MBs), precursors of melanocytes, are derived from neural crest cells and only differentiate into melanocytes in skin. We previously showed that MBs isolated from the back skin of mice were multipotent; they generated neurons and glial cells in addition to melanocytes. However, MBs only exhibited multipotency in a co-culture with ST2 stromal cells, not in a co-culture with keratinocytes, which surround MBs in skin. We herein investigated the multipotent mechanism of MBs using mouse ES cells designed to express green fluorescent protein under the control of the MB marker, Sox10 (Sox10-IRES-GFP ES cells). We isolated ES cell-derived MBs as Sox10+/Kit+ cells from the differentiation culture of Sox10-IRES-GFP ES cells. MBs derived from Sox10-IRES-GFP ES cells exhibited multipotency in a co-culture with ST2 stromal cells, but not in a co-culture with mouse embryonic fibroblasts (MEFs). MBs did not exhibit multipotency in a co-culture with MEFs, even in the presence of the culture supernatant of ST2 cells, suggesting that MBs may interact with ST2 cells, but not the factors secreted by these cells, to support their multipotency. Notch signaling is critical for the differentiation of MBs and

POSTER ABSTRACTS

we demonstrated that ST2 cells abundantly expressed the notch ligands, jagged1, jagged2, and dll1; therefore, we examined the effects of notch signaling on the multipotency of MBs. When the notch signal pathway was activated in ES cell-derived MBs by overexpressing the notch intracellular domain (NICD), MBs exhibited multipotency, even in a co-culture with MEFs. However, inhibiting notch signaling with DAPT prevented the multipotency of MBs, even in a co-culture with ST2 stromal cells. These results showed that notch signaling was important for the multipotency of MBs. We also isolated Sox10+/Kit+ MBs from the back skin of E14.5 mouse embryos generated from Sox10-IRES-GFP ES cells and examined the effects of notch signaling on their multipotency. MBs in which the notch signal pathway was activated by overexpressing NICD generated more multipotent colonies than MBs in which the pathway was not activated. MBs generated less multipotential colonies when notch signaling was inhibited than when it was not inhibited. These results strongly suggested that notch signaling may be important for maintaining the multipotency of MBs.

P5

NEURAL CREST CELLS SUSTAIN THEIR MULTIPOTENCY THROUGHOUT EMBRYOGENESIS

MOTOHASHI, Tsutomu, Watanabe, Natsuki, Kunisada, Takahiro
Regeneration and Advanced Medical Science, Gifu University Graduate School of Medicine, Gifu, Japan

Neural crest cells (NC cells) are migratory multipotent cells that give rise to a wide range of cell types including peripheral neurons, cranial mesenchymal cells, and melanocytes. The multipotency of NC cells is considered to be transient at the early stage of NC cell generation. NC cells then emerge from the neural tube, lose their multipotency, and become lineage-restricted precursors. Multipotent NC stem cells have recently been detected in various tissues, and some NC cell-derived cells were found to differentiate into other derivatives of NC cells, suggesting that the lineage of NC cells may be more flexible than previously considered. These findings prompted us to investigate whether NC cells sustained multipotency throughout embryogenesis. We examined the differentiation potency of NC cells soon after they emerged from the neural tube and also NC-derived cells after they entered into their target tissues. We employed mice that expressed green fluorescent protein under the control of the NC marker, Sox10 (Sox10-IRES-GFP mice) and another NC cell marker, Kit. Although Sox10+/Kit- and Sox10+/Kit+ NC cells were previously considered to be lineage-restricted precursors to neurons (N), glial cells (G), and melanocytes (M) respectively, after emerging from the neural tube, both exhibited potency to differentiate into N, G, and M in vitro. This result indicated that NC cells sustained multipotency even after delamination. After migrating to tissues, Sox10+/Kit+ cells in the skin and inner ear generated N, G, and M throughout embryogenesis, whereas those in the dorsal root ganglion only exhibited potency for a restricted period in the embryonic stages. This result demonstrated that NC-derived cells sustained their multipotency even after entering into the target tissues, whereas their differentiation potency appeared to have been strictly restricted by the tissue environment. The results of the present study revealed that although NC cells sustained their multipotency after delamination, potency changes depended on the environment in the target tissues.

P6

EARLY CARDIOMYOCYTE REPROGRAMMING - A DYNAMIC PROCESS

CHENG, Yuan Yuan, Lee, Sho Tone, Yan, Yu Ting, Hsieh, Patrick C.H.
Academia Sinica, Taipei, Taiwan

Although remnant cardiomyocytes (CMs) possess a certain degree of proliferative ability, the efficiency is too low for cardiac regeneration after injury. Therefore, induced pluripotent stem cells (iPSCs) may offer some hope for therapy. However, as terminally differentiated cells, CMs are considered difficult to successfully reprogram. In this study, we modified the conventional reprogramming protocol to accommodate

POSTER ABSTRACTS

the characteristics of CMs and decipher how they execute reprogramming. Post-natal CMs and non-CMs were isolated non-genetically by sorting populations based upon TMRM-staining. Troponin I was used to confirm that every CM contained well-structured sarcomeres. After that, a modified reprogramming method was employed to avoid damage to the CM, and these CM-derived iPSCs were characterized based on their pluripotency and differentiation abilities. During reprogramming of both CM and non-CM we observed sequential changes in cell proliferation, morphology, aggregation and colony formation. Microscopy and alkaline phosphatase assay revealed that CMs had a time-delayed sequence of events and fewer iPSC-like colonies formed compared to non-CMs. However, typical iPSC-like colonies were clearly observed after 7 days of both CM and non-CM reprogramming. Interestingly, we observed an increase in CM proliferation during days 1 to 3, though these proliferative cells did not always exhibit well-structured sarcomeres. A circular shape and aggregation of the cells began on reprogramming day 4, and colony formation started on day 6. In summary, we used a modified protocol to define the dynamics during CM reprogramming and demonstrated a sequential change in proliferation, morphology, cell aggregation, and colony formation. This study demonstrates that terminally differentiated CMs can be successfully reprogrammed and provides valuable information for inducing remnant CM proliferation for heart regeneration. Future studies are required to further clarify the epigenetic mechanism controlling CM reprogramming.

P7
ONLINE MONITORING OF STEM CELL FATE DECISIONS

Li, Bojun, Loebel, Claudia, Menzel, Ursula, Alini, Mauro, **STODDART, Martin J.**
AO Research Institute Davos, Davos Platz, Switzerland

Bone marrow derived mesenchymal stem cells (BMSCs) have been shown to have multipotentiality, with differentiation into bone, cartilage and fat being demonstrated. While this offers potential clinical therapies, the lack of methods to reproducibly induce stable differentiation severely limits their clinical use. In part this is due to the initial population used being heterogeneous, and the lack of methodologies to monitor cells at the individual, rather than the global level. Master transcription factors, such as Sox9 (cartilage) and Runx2 (bone) have been associated with cell differentiation pathways and can be used to investigate the cells phenotypic state. We have previously demonstrated that the differentiation of human MSCs into cartilage and bone can be assessed by quantifying the ratio of Runx2/Sox9 mRNA message within the first week of induction using real-time PCR. While offering advantages, this method is destructive and only generates data based on the population as a whole. Using SmartFlare fluorescence mRNA probes, we have developed an online dual fluorescence monitoring system for use in live cells. The cells can then be separated based on the relative intracellular fluorescence of Sox9 in relation to Runx2 using fluorescence activated cell sorting (FACS), and the purified cells further investigated at the functional and molecular level. This offers a more detailed analysis of the effectiveness of new therapeutics both at the individual cell level, e.g. number of responding cells, and the response of the population as a whole. By identifying and isolating differentiating cells at early time points, prospective analysis of differentiation is also possible, which will lead to a greater understanding of MSC differentiation.

P8
SGK196 CONTROLS STEM CELL FATES BY PROMOTING THE DEGRADATION OF TGF-BETA FAMILY RECEPTORS

TSANG, Stephanie, Wang, Fei
University of illilnois at Urbana-Champaign, Urbana, IL, USA

Much remains to be understood regarding the molecular mechanisms controlling stem cell fate. Through studies in human pluripotent stem cells (hPSCs), we have identified a novel regulator,

POSTER ABSTRACTS

SGK196, an atypical kinase of relatively unknown function. SGK196 depletion in human embryonic stem cells (hESCs) inhibits neural induction and enhances mesoderm induction, while SGK196 overexpression represses mesoderm and endoderm induction. SGK196 negatively regulates TGFβ family signaling by interacting with and promoting the degradation of TGFβ family receptors. Overall, we demonstrate that SGK196 is an essential regulator of stem cell fate determination. Through uncovering the regulatory mechanism of SGK196 we unveil valuable insight into receptor-level regulation of the TGFβ family pathway.

P9

TRANSPLANTATION OF HUMAN UMBILICAL CORD STEM CELLS WITH HIGHLY-EXPRESSED TGF-BETA RECEPTORS IN DEGENERATIVE INTERVERTEBRAL DISC OF RABBIT

AHN, Jongchan[1], Park, Eun-mi[1], Park, Seah[1], Han, Inbo[2], Lee, Soo-Hong[1]
[1]CHA University, Seongnam-si, Gyeonggi-do, South Korea, [2]Neurosurgery, CHA Hospital, Seongnam-si, Gyeonggi-do, South Korea

The goal of this study is to investigate the therapeutic effect of the mesenchymal stem cells derived from Wharton's jelly of the human umbilical cord (hUC-MSCs, Cordstem) for intervertebral disc degeneration. Recently, lumbar degeneration is increasing with age and other factors and causes discogenic low back pain. Therefore, the development of fundamental treatment is required to prevent or retard the degenerative process in early stages. In present study, we have used hUC-MSCs with highly-expressed transforming growth factor beta receptors (TGFβRs), presumably stimulating TGFβ signal through smad2/3 for cartilage regeneration. The 1 % hyaluronic acid(HA) hydrogel was also used as a scaffold to help adhesion of cells. For a disc degeneration model, we used New Zealand white rabbits and induced disc degeneration in each rabbit by a percutaneous technique at L3-4, L4-5, and L5-6 under C-arm procedure. Magnetic resonance images (MRI) were carried out after 2 weeks of injury to confirm disc degeneration and then hUC-MSCs were injected into each injured site. Histological observations were performed at 12 weeks after injection of hUC-MSCs. We found that the level of disc regeneration in the co-injection group of hUC-MSCs with highly-expressed TGFβRs and HA hydrogel was significantly higher than other groups. These results suggest that the treatment of hUC-MSCs with highly-expressed TGFβRs together with HA hydrogel could be an effective and potential technique for enhancement of cartilage regeneration in degenerative disc diseases.

P10

CELLS ARE NOT NECESSARY FOR MSC CONTRIBUTION TO SKIN WOUND HEALING IN DIABETIC MICE

ARANGO, Martha L., Conget, Paulette
Universidad del Desarrollo, Santiago, Chile

Pre-clinical and clinical studies have proved that a therapeutic effect results from mesenchymal stem cell (MSCs) transplant. Here we to evaluate whether the cells per se are necessary for MSC contribution to skin wound healing in diabetic mice. Vehicle (untreated, n=17), 1x106 allogeneic MSCs obtained from the bone marrow 8-33 and 50 weeks-old (allo MSCs, n=12-15) or 0.1 mL of an acellular derivate of these cells (acd-allo MSC-treated, n=8-10) were intra-dermally administered around 6 mm diameter wounds in the midline of 8 to 10 week-old female non-obese diabetic mice. Each two days after MSC transplant wound healing was macroscopically assessed (wound closure, retraction and granulation tissue formation) and microscopically (re-epithelialization, dermal-epidermal junction, leukocyte infiltration, collagen deposition pattern, fibrosis and skin appendage regeneration) at 16 day after MSC transplant.
ELISA was carried out to evaluate that paracrine factors were contents from acd-allo MSCs. While untreated or allo MSC-treated lesions healed at day 16, acd-allo MSCs lesions healed at day 12. Actually,

POSTER ABSTRACTS

at day 5, acd-allo MSC lesions were already half-closed. At day 16, allo MSC and acd-allo MSC lesions presented continuous epidermis; regular dermal-epidermal junction; normal vascularization, collagen deposition and skin appendages. Untreated lesions showed incomplete regeneration.
ELISA analyses showed paracrine factors such as: IGF-1, EGF, KGF, HGF, VEGF, ANG-1, ANG-2, MMP-1, MMP-3, CoL-1 and PGE2. Our data show that cells are not necessary for MSC contribution to wound healing in diabetic mice. Moreover, the administration of the cells delays the therapeutic effect. The later maybe explained by the fact that trophic factors secreted by MSCs and not the cells per se are critical for skin regeneration. Since, when MSCs are administered they required a time in order to secrete the trophic factors. Also, it maybe a negative effect due to allogeneic immune response.

P11

KRÜPPEL LIKE FACTORS REPROGRAM RABBIT INDUCED PLURIPOTENT STEM CELLS TO NAÏVE LIKE PLURIPOTENCY

AFANASSIEFF, Marielle[1], Tapponnier, Yann[1], Aubry, Maxime[1], Joly, Thierry[2], Savatier, Pierre[1]
[1]U846, INSERM, Bron, France, [2]ISARA, Lyon, France

Human and rabbit preimplantation embryos show unique characteristics in the timing of development and the shape of the conceptus, which are different from those described for rodent preimplantation embryos. In this respect, rabbit pluripotent stem cells harboring knockin or mutated genes involved in determining and regulating the fate of stem cells could be useful tools to investigate early mammalian development. To date, rabbit embryonic stem (ES) cells and induced pluripotent stem (iPS) cells have shown the cardinal features of primed pluripotency; therefore, these cells are unable to colonize rabbit preimplantation embryos and participate in the formation of the three germ layers. In an attempt to generate naïve-like pluripotent stem cells in rabbits, we engineered iPS cells expressing EOS lentiviral vector. The EOS vector expresses both green fluorescent protein (GFP) and puromycin-resistant genes under the transcriptional control of naïve state-specific distal element of *Oct4* enhancer. After selection in puromycin, iPS-EOS cells were further engineered to overexpress both KLF2 and KLF4 transcription factors. The resulting cells were called EK cells (for enhanced KLF activity). EK cells acquired some characteristic features of naïve pluripotency, including propagation in a medium supplemented with fetal calf serum, expression of naïve markers (*Rex1, Dazl, Fgf4, Esrrb, Dppa5,* and *Cdh1*), and permissiveness to single cell dissociation with trypsin. However, these cells remained dependent on FGF2 and activin receptors and not on LIF receptor and JAK kinase for self-renewal in the undifferentiated state. Interestingly, the gene expression profile of EK cells was closer to that of rabbit inner cell mass cells. To assess their capacity to colonize the epiblast, 5–10 EK cells were injected into 8-cell stage rabbit embryos. These embryos were further cultured to the blastocyst state ***in vitro*** or transferred to the oviducts of surrogates. Extensive colonization of the epiblast was observed in both mid- (E3) and expanded (E5) blastocyst stages in approximately 30% of the developing embryos. Colonization could also be observed in the epiblast of early gastrulation embryos (E6). However, whether the three germ layers were colonized by EK cells derivatives is currently under investigation.

P12

USING MICROFLUIDICS TECHNOLOGY TO PERFORM SINGLE-CELL DNA METHYLATION ANALYSIS

CHEOW, Lih Feng[1], Messerschmidt, Daniel[1], Lorthongpanich, Chanchao[2], Viswanathan, Ramya[1], Solter, Davor[2], Knowles, Barbara[2], Quake, Stephen[3], Burkholder, William[1]
[1]IMCB, Singapore, Singapore, [2]IMB, Singapore, Singapore, [3]Stanford University, Palo Alto, CA, USA

DNA methylation plays important roles in the epigenetic regulation of mammalian embryonic development. However, efforts to analyze its dynamic

POSTER ABSTRACTS

patterns in early embryos have been hampered by the scarcity of material. Conventional DNA methylation assays (e.g. bisulfite sequencing) typically require large amount of sample. These samples often include cells in different cell states, so that average bulk DNA methylation measurements would mask the epigenetic heterogeneity in subpopulations of cells. In order to measure DNA methylation with single cell resolution, we developed a method based on methylation-sensitive restriction digest that enables DNA methylation analysis of single genomes with locus-specific resolution. By using a microfluidics platform the assay can be multiplexed and many loci in a single cell can be addressed simultaneously. We applied this method to study the effect of maternal TRIM28 protein on genomic imprinting during early embryogenesis. By measuring imprinted genes methylation status of single blastomeres from 8-cell stage TRIM28 mutant mouse embryos, we observed mosaic imprinting defects in all the mutant embryos. This could account for the severe phenotypic variabilities observed in later stage embryos. This platform can be a useful tool for DNA methylation profiling in heterogeneous populations, as well as in vitro single cell epigenetic diagnostics.

P13

THE ROLE OF AMD1 AND THE POLYAMINE PATHWAY IN MOUSE ESC SELF-RENEWAL AND DIFFERENTIATION

JAMES, Christina[1], Tianyun, Zhao[1], Yang, Henry[2], Vardy, Leah A.[1]
*[1]Institute of Medical Biology, A*STAR, Singapore, Singapore, [2]Cancer Science Institute, Singapore, Singapore*

Polyamines are essential organic cations that play a role in a wide array of cellular processes including mRNA translation, transcription, mRNA stability, cell growth and proliferation. AMD1 and ODC1 are important polyamine pathway regulators that were previously reported to be involved in ESC self-renewal. AMD1 is required for ESC self -renewal and its over-expression can promote the ESC state in the absence of LIF in part by promoting high MYC levels. We identified a set of mRNAs that are regulated by AMD1 in ESCs by microarray analysis. We have been working to define how these genes are regulated by the polyamine pathway and to determine their roles in self-renewal. We show that one AMD1 regulated mRNA, mRAS, is essential for self-renewal and can promote self-renewal in the absence of LIF. A second AMD1 regulated gene is a highly conserved gene of unknown function. It is highly expressed in ESCs and rapidly down-regulated on differentiation. In addition, its mRNA levels are up-regulated in response to high levels of AMD1. We used gain- and loss-of-function strategies in mESCs in order to characterize the role of this gene in ESC self- renewal and differentiation. Our findings may provide new insights into our understanding of the interconnected regulatory network that maintains the self-renewal and pluripotency of mouse ESCs.

P14

EPIGENETIC REGULATION CONTRIBUTES TO UROCORTIN-ENHANCED MIDBRAIN DOPAMINERGIC NEURON DIFFERENTIATION

HUANG, Hsin Yi[1], Chiu, Tsung Lang[2], Chang, Hui Fen[1], Hsu, Hui Ru[1], Pang, Cheng Yoong[1], Liew, Hock Kean[1], Wang, Mei Jen[1]
[1]Medical Research, Buddhist Tzu Chi General Hospital, Hualien, Taiwan, [2]Neurosurgery, Neuro-Medical Scientific Center, Buddhist Tzu Chi General Hospital, Hualien, Taiwan

The production of midbrain dopaminergic (mDA) neurons requires precise extrinsic inductive signals and intrinsic transcriptional cascade at a specific time point in development. Urocortin (UCN) is a peptide of the corticotropin-releasing hormone (CRH) family that mediates various responses to stress. UCN was first cloned from adult rat midbrain. However, the contribution of UCN to the development of mDA neurons is poorly understood. Here, we show that UCN is endogenously expressed in the developing ventral midbrain (VM) and its receptors are exhibited in

POSTER ABSTRACTS

Nurr1+ postmitotic mDA precursors and TH+ neurons, suggesting possible roles in regulating their terminal differentiation. UCN treatment increased DA cell numbers in rat VM precursor cultures by promoting the conversion of Nurr1+ precursors into DA neurons. Furthermore, neutralization of secreted UCN with anti-UCN antibody resulted in a reduction in the number of DA neurons. UCN induced an abundance of acetylated histone H3 and enhanced late DA regulator Nurr1, Foxa2 and Pitx3 expressions. Using pharmacological and RNA interference approaches, we further demonstrated that histone deacetylase (HDAC) inhibition and late transcriptional factors upregulation contribute to UCN-mediated DA neuron differentiation. Chromatin immunoprecipitation analyses revealed that UCN promoted histone acetylation of chromatin surrounding the TH promoter by directly inhibiting HDAC and releasing of MeCP2-CoREST-HDAC1 repressor complex from the promoter, ultimately leading to an increase in Nurr1/co-activators-mediated transcription of TH gene. Moreover, UCN treatment in vivo also resulted in increased DA neuron differentiation. These findings suggest that UCN might contribute to regulate late mDA neuron differentiation during VM development.

P15

MODELING GERM CELL DEVELOPMENT FROM NON-OBSTRUCTIVE AZOOSPERMIA USING INDUCED PLURIPOTENT STEM CELLS

WANG, Yuan
East China Normal University, Shanghai, China

Maintenance of the germ cell lineage ensures the passage of genetic information through generations. So far, the etiology of male infertility has yet to be understood, partially due to the difficulties to obtain a large quantity of diseased human gametes. Although a number of studies demonstrated successful derivation of germ cells from human pluripotent stem cells, ***in vitro*** recapitulation of germ cell development remains to be one of the most fundamental challenges in biology. In this study, we developed a serum-free culture condition, and successfully induced spermatocyte-like cells and haploid spermatides from human embryonic stem cells. We found that this derivation process was regulated by several germ cell specific factors. In addition, several induced pluripotent stem cells from non obstructive azoospermic patients displayed various defects during ***in vitro*** differentiation toward germ cell lineages. Our study thus provides an useful tool to dissect molecular mechanism toward understanding of male infertility.

P16

MODULATING BIOCHEMICAL COMPOSITIONS OF POLYELECTROLYTE HYDROGELS TO REFINE THE CHONDROGENIC PHENOTYPES DERIVED FROM HUMAN MESENCHYMAL STEM CELLS

RAGHOTHAMAN, Deepak[1,3], Leong, Meng Fatt[2], Lim, Tze Chiun[2], Yang, Zheng[3], Wan, Andrew Chwee Aun[2], Lee, Eng Hin[3]
*[1]Bioprocessing Technology Institute, Agency for Science, Technology and Research (A*STAR), Singapore, [2]Institute of Bioengineering and Nanotechnology, Singapore, [3]Department of Orthopaedic Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*

Cartilage tissue engineering using mesenchymal stem cells (MSC) offers a huge potential, but the quality of clinical repair achieved remains suboptimal. One of the key challenges is the ability to regenerate cartilage with the typical zonal characteristics of native articular cartilage. In this study, the influence of different biochemical cues, namely Collagen type I (Col I), Chondroitin-6-sulfate (CS), and Hyaluronic acid (HA), in modulating the zonal chondrogenic outcomes of human bone-marrow derived MSCs was investigated. A chitin-alginate based fibrous hydrogel developed using interfacial polyelectrolyte complexation (IPC) was employed. MSC chondrogenic differentiation was assessed by the expression of cartilaginous markers and extra-cellular matrix (ECM) quantification, after 2-3 weeks of in vitro culture under chondrogenic differentiation conditions. Formation of zone specific cartilage was assessed by the analysis of phenotypic

POSTER ABSTRACTS

marker expression. Varying biochemical compositions of IPC-based hydrogels had distinct effects in deriving different zonal cartilage phenotypes. Unmodified, IPC-control hydrogels were found to favour a superficial zone-like phenotype with increased Proteoglycan-4 (PRG-4) expression, while incorporation of CS in IPC hydrogels was found to prime a middle zone-like cartilage phenotype, demonstrating higher Cartilage oligomeric matrix protein (COMP) expression, and enhanced ECM assembly. Significantly, Col I incorporated IPC hydrogels were found to facilitate superior chondrogenesis, with enhanced ECM deposition and Collagen type IX (Col IX) expression, favoring a relatively mature, mid-deep zonal phenotype. HA incorporated IPC hydrogels were found to derive a deep zone-like cartilage phenotype, with increased hypertrophic marker expression, namely Collagen type X (Col X). Collectively, these results demonstrated the significant potential of varying biochemical cues in IPC-based hydrogels to modulate the functional phenotype of chondrogenic differentiation. Such knowledge can augment current approaches for cartilage repair to improve functional tissue outcomes.

P17

NEURONAL DIFFERENTIATION DEMANDS PROGRANULIN EXPRESSION IN HUMAN NEURONAL STEM CELL CULTURES

HAO, Hsiao-Nan[1], Pei, Haitao[2], Peduzzi, Jean[3], Tang, Wei[4], Yao, Ruyong[5], Hao, Peter[3], Qu, Zhiqiang[5]
[1]Wayne State University, Detroit, MI, USA, [2]Neurology, Qingdao University Hospital, Qingdao, China, [3]Anatomy and Cell Biology, Wayne State University, Detroit, MI, USA, [4]Immunology, Shandong University School of Medicine, Jinan, China, [5]Medical Research Center, Qingdao University Hospital, Qingdao, China

Progranulin (PGRN) has been found to participate many physiological and pathological processes. Our hypothesis is that the PGRN expression might play an essential role in neuronal differentiation during brain development. Based on our previous reported results, it is most likely the neuronal differentiation (ND) occurs only among nestin+/CD133+ human fetal brain cells rather than that the cells with same markers isolated from adult brain tissue. We have noticed that the level of PGRN expression in human fetal neural stem cells (HFNSCs) is higher than that in adult HFNSCs. To explore the molecular mechanism of PGRN to regulate ND, both PGRN antisense deoxynucleotides (DON) and human recombinant PGRN protein were used for this study. Prior incubation of the nestin+/CD133+ HFNSC in the ND-inducible medium, cells were cultured with the PGRN antisense nucleotides contained culture medium. After PGRN antisense treatment, the level of PGRN expression was reduced in the HFNSCs. The decreased PGRN expression was confirmed using RT-PCR, Northern blotting and protein assays. Apoptotic signals were not detected after the cultures treated with PGRN antisense-DON. Interestingly, inhibition of PGRN expression declines ND among nestin+/CD133+ HFNSCs. In contrast, reducing of PGRN expression increases astrogliosis. Although the mechanism of ND may be not limited to the intracellular PGRN, effect of PGRN expression on DND deserves further investigation.

P18

MOUSE MESENCHYMAL STEM CELLS ISOLATED FROM DIFFERENT SOURCES AND STRAINS VARY IN GROWTH KINETICS AND IMMUNOPHENOTYPE

YAP, Fei Ling, Ha, Sieu Jeak, Ambu, Stephen, Tan, Seok Shin
International Medical University, Bukit Jalil, Malaysia

Mesenchymal stem cells (MSCs) have been extensively studied in regenerative medicine, cellular and gene therapies because of their unique characteristics such as self-renewal, relatively non-immunologic and multi-lineage differentiation abilities. Before clinical application of MSCs in human, pre-clinical studies in animal models are required to confirm their safety and efficacy. Isolation and characterisation of mouse MSCs (mMSCs) have been more difficult than human and rat MSCs. In this study, we aim to investigate mMSCs from 2 different sources (adipose tissue and lung) of 3 different commonly used mice strains

POSTER ABSTRACTS

(BALB/c, C57BL/6 and ICR) for the development of mMSCs in mouse model. mMSCs were isolated using the plastic adherent method and comparisons were done based on their morphology, growth kinetics and immunophenotypic profile. The growth kinetics of mMSCs was assessed by their growth rate and colony-forming-unit fibroblasts (CFU-F) formation. The morphologies of all newly isolated mMSCs were similar (fibroblastic-shaped) regardless of the mouse sources or strains. However, mMSCs from different sources and strains exhibited distinct growth patterns. Lung-derived mMSCs from C57BL/6 appeared to have a better growth rate where the cell number increased significantly up to day 12. Clonogenic potential of adipose tissue and lung-derived mMSCs from BALB/c were significantly lower than C57BL/6 and ICR. Generally, a higher number of initial cell density seeded increased the CFU-F formation. All mMSCs expressed CD29, CD44, CD90.2, and CD105 while lacking the expressions of CD11b, CD31, CD34, CD45, CD117 and I-Ak (MHC class-II) molecules. CD73 expression was found only in lung-derived mMSCs from BALB/c and C57BL/6. Based on the current data, we concluded lung-derived mMSCs from C57BL/6 might be the best mMSCs source as it has a better growth rate and CFU-F formation. Apparently, the choice of mMSCs from a particular source and strain maybe important factors in the experimental applications of mMSCs.

P19

REINFORCING STAT3 ACTIVITY REPROGRAMS HUMAN EMBRYONIC STEM CELLS TO NAIVE PLURIPOTENCY

CHEN, Hongwei[1], Aksoy, Irène[1], Gonnot, Fabrice[1], Voisin, Sophie[1], Osteil, Pierre[1], Vallot, Céline[2], Rougeulle, Claire[2], Stanton, Lawrence W.[3], Bourillot, Pierre-Yves[1], Savatier, Pierre[1]

[1]INSERM U846, Stem Cell and Brain Research Institute, Bron, France, [2]Université Paris Diderot, Paris, France, [3]Genome Institute of Singapore, Singapore

LIF/STAT3 signaling is hallmark of naïve pluripotency in rodents. However, FGF2 and activin/nodal signaling is required to sustain the self-renewal of human pluripotent stem cells in a state referred to as the primed state. The reason why LIF/STAT3 signaling alone fails to sustain pluripotency in humans remains unknown. Here we showed that forced expression of hormone-dependent STAT3-ER^{T2} in combination with LIF and tamoxifen allows human embryonic stem (ES) cells to escape from the primed state and enter a new state called TL, which is characterized by the activation of STAT3 target genes and long-term self-renewal in FGF2- and feeder-free conditions. Propagation of TL cells in the undifferentiated state is strictly dependent on both LIF and tamoxifen, indicating a synergistic effect of the two molecules for inhibiting differentiation. TL cells can be propagated in the presence of inhibitors such as GSK3 and MEK (the so-called 2i condition) along with LIF and tamoxifen. The resulting cells have been designated as TL2i. They remain pluripotent, as evidenced by their capacity to produce teratomas containing differentiated cells of the three germ layers. Moreover, they gradually acquire the characteristic features of naïve pluripotency, including the expression of naïve state-specific transcription factors (*ESSRB*, *TFCP2L1*, *KLF2*, *KLF4*, *NROB1*, *FGF4*, *GBX2*, *GDF3*), decreased levels of H3K27me3 repressive markers in their respective promoters, global genome hypomethylation, resistance to pharmacological inhibition of FGF receptor, elevated activity of the distal element of the *OCT4* enhancer, dependency on LIF and Jak2 kinase, and dramatically increased clonogenicity. Together, these results show that increasing STAT3 activity is sufficient (1) to switch human ES cells from FGF2 to LIF/Jak dependency and (2) to reprogram them to naïve-like pluripotency.

POSTER ABSTRACTS

P20

MOGRIFY: A DATA-DRIVEN APPROACH TO DISCOVERING THE FACTORS TO INDUCE DIRECT REPROGRAMMING BETWEEN ANY TWO CELL TYPES

RACKHAM, Owen John Llewellyn[1], Fang, Hai[2], Oates, Matt E.[2], Shin, Jay W.[3], Suzuki, Harukazu[3], Petretto, Enrico[1], Daub, Carsten O.[4], Forrest, Alistair R.R.[3], Polo, Jose[5], Gough, Julian[2]
[1]Medical Research Council, Clinical Sciences Centre, London, United Kingdom, [2]Department of Computer Science, University of Bristol, Bristol, United Kingdom, [3]Division of Genomic Technologies, RIKEN Center for Life Science Technologies, Yokohama, Japan, [4]Department of Biosciences and Nutrition, Karolinska Institutet, Stockholm, Sweden, [5]Department of Anatomy and Developmental Biology, Monash University, London, Australia

It is known that over-expression of sets of endogenous transcription factors in somatic cells can induce stem-cell-like pluripotency or trans-differentiation. These discoveries relied on exhaustive testing of large sets of transcription factors, an approach that is both inefficient and unscalable. Here we present a novel network-based method (Mogrify) that combines gene expression data with regulatory network information to identify targeted sets of transcription factors to induce cell conversion between any two cell types. We validate Mogrify by recovering known reprogramming factors for published cell conversions as well as providing further experimental evidence. Further to this, novel transcription factors for published conversions are identified, but critically transcription factor sets to induce novel cell conversions are presented, culminating in a trans-differentiation landscape of human cell types. Mogrify is available for every conversion between the 705 different human cell-types in the FANTOM5 dataset and made freely available to the community via a web interface. (Mogrify.net).

P21

EPIGENETIC REGULATORS INCREASE MULTILINEAGE DIFFERENTIATION CAPACITY OF MESENCHYMAL STROMAL CELLS

KIM, Hye Joung
Catholic University, Seoul, South Korea

This research designs to confirm enhancement of MSC multi lineage differentiation capacity when 5-aza, methylation inhibitor and TSA, HDAC inhibitor treat alone or together. Furthermore, this study addresses the expression of key regulator gene and change of epigenetic status which are involved in each lineage differentiation by epigenetic regulator. Neurogenic, osteogenic, and chondrogenic differentiation was induced following 7-21 day culture of cells in lineage specific differentiation medium and differentiation was assessed by immuno-histo/cytochemistry and immuno blotting. The changes of lineage specific transcription regulator gene expression and its relations to methylation status was investigated by real-time PCR and pyro-sequencing. In conclusion, our study demonstated that epigenetic regulator enhances lineage specific differentiation from BMMSCs. And also, suggested that gene activation and silencing controlled by epigenetic modulation, were crucial to MSC lineage differentiation. This study was supported by a grant (5-2013-A0154-00142) of the National Research Foundation of Korea

P22

ROLE OF PINX1 IN DETERMINING THE DIFFERENTIATION POTENTIAL OF MOUSE EMBRYONIC STEM CELLS

CHAN, Hing Chung, Lau, Yuen Ting, Li, Chun Kit, Waye, Mary Mui-Yee, Tsang, Suk Ying
The Chinese University of Hong Kong, Hong Kong

Telomerase is important to maintain the length of telomere. It is expressed in highly proliferating cells such as cancer cells and embryonic stem cells (ESCs), accounting for the unlimited proliferation

POSTER ABSTRACTS

capacity of these cells. Telomerase consists of two components: RNA component and reverse transcriptase component, known as telomerase reverse transcriptase (TERT). Recently, Pin2/TRF1-interacting protein (PinX1) has been found in many species including human and mouse. It was found to interact directly with TERT through its telomerase inhibitory domain at its C terminal. PinX1 overexpression led to shortening of telomere and many human cancers have been found to be deficit of PinX1. Apart from acting as a putative tumor suppressor, PinX1 has also been found to play a role in chromosome regulation as indicated by the formation of abnormal chromosome bridge in PinX1 knockdown cell lines. ESCs have two unique characteristics. ESCs can self-renew, meaning that they can proliferate indefinitely. In addition, ESCs are pluripotent, meaning that they can differentiate into all different cell types in our bodies. Since the expression of TERT was previously found to affect the differentiation of ESCs, and that PinX1 was found to be the regulator of telomerase, PinX1 may regulate the differentiation of ESCs. In addition, since PinX1 was found to regulate chromosome segregation, and that proper chromosome segregation is important for ESCs to self-renew and to differentiate, PinX1 may be important for maintaining ESC characteristics. The aim of this study was to investigate the role of mPinX1 in determining the differentiation potential of mouse ESCs (mESCs). Our group has found that mouse PinX1 (mPinX1) protein is expressed in mESCs. mPinX1 has been found to interact with TERT in HEK293 cells using pull-down assay. Interestingly, both the knockdown and overexpression of mPinX1 in mESCs led to a decrease in cardiac differentiation as revealed by the decrease in expression of multiple cardiac-specific markers. The results suggested that maintaining a critical expression level of mPinX1 is crucial for cardiac differentiation. Further experiments would be conducted to dissect the mechanism underlying the role of mPinX1 on cardiac differentiation.

P23

EFFECT OF ASPIRIN ON OSTEOGENIC POTENTIAL OF PERIODONTAL LIGAMENT STEM CELLS(PDLSCS)

ABD.RAHMAN, Fazliny[1], Vincent-Chong, Vui King[2], Md Ali, Johari[3], Rahman, Mohammad Tariqur[4], Abdullah, Mariam[1]

[1]Department Of Restorative Dentistry, Faculty Of Dentistry, University Malaya, Kuala Lumpur, Malaysia, [2]Oral Cancer Research Amd Coordinating (OCRCC), Faculty Of Dentistry, University Malaya, Kuala Lumpur, Malaysia, [3]Department Of Molecular Medicine, Faculty Of Medicine, University Malaya, Kuala Lumpur, Malaysia, [4]Department Of Biotechnology, Kulliyyah Of Science, International Islamic University Of Malaysia, Kunatan, Pahang, Malaysia

Aspirin is a non-steroidal anti- inflammatory drug (NSAIDs) mainly use as anti-inflammation and analgesic. Several studies have demonstrated that aspirin has adverse effects that could suppress formation and remodeling of bone. In patients who are candidates for bone disease, there is usually a history of taking aspirin or the need for the further use of aspirin. Mesenchymal stem cell (MSCs) has been used in orthopedics patients, as an emerging regenerative therapy to treat bone fractures and nonunion. Although the effect of aspirin on osteoblastic functions has been extensively studied, little information on whether aspirin has any adverse effect on the survival of MSCs to differentiate into osteoblast. Method: Periodontal ligament stem cells were identified and isolated from healthy periodontal ligament and undergo osteogenic differentiation. MTT assay was used to determine the effect of aspirin on periodontal ligament stem cell (PDLSCs) proliferation. Following differentiation, the presence of calcium deposition into the cellular matrix was analysed by Alizarin Red S staining and mRNA expressions. To better understand the effect of aspirin towards PDLSCs osteogenic potential, we analysed cDNA microarray analysis (10 μM, 200 μM, 500 μM, and 1,000 μM) and verified by qRT- PCR analysis. Results: MTT spectrophotometry results

POSTER ABSTRACTS

showed that lower concentration (1 to 5 μM) of aspirin doses have an inhibitory effect on growth. However significant differences were observed at increased concentrations. Following differentiation treated with aspirin, the mineralization nodule formation was most prominent in 500 and 1,000 μM. The microarray data analyses identified the numbers of up-regulated and down-regulated genes were almost similar at low and middle concentrations (10, 200, 500 μM). At 1,000 μM, small number of dysregulate genes with down-regulated expression was identified, indicating that PDLSCs cells had a more potential to differentiate to osteoblast at increased concentrations. Conclusions: The results suggest aspirin enhances the osteoblastic differentiation derived periodontal ligament stem cell (PDLSCs). The analysis of differential gene expressions on effect of aspirin towards osteoblast differentiation aids our understanding of functional differences between them at molecular level.

P24

THE ENDOGENOUS RETROVIRUS HERVH IS A PRIMATE-SPECIFIC LONG NON-CODING RNA REQUIRED TO MAINTAIN HUMAN EMBRYONIC STEM CELL IDENTITY

SACHS, Friedrich[1], Lu, Xinyi[1], Ramsay, LeeAnn[2], Jacques, Pierre-Etienne[3], Goeke, Jonathan[4], Bourque, Guillaume[2], Ng, Huck-Hui[1]
[1]Stem Cells & Developmental Biology, Genome Institute of Singapore, Singapore, [2]Department of Human Genetics, McGill University, Montreal, QC, Canada, [3]Cell & Molecular Biology, Sherbrooke University, Sherbrooke, QC, Canada, [4]Computational Biology, Genome Institute of Singapore, Singapore

The unique cellular state of embryonic stem cells is maintained by pluripotency-associated transcription factors such as OCT4. The transcriptional regulatory circuitries between human and mouse pluripotent stem cells exhibit notable differences. The identification and characterization of new regulators in this network will be key to deciphering the mechanisms behind human cellular pluripotency. Here, we report that the long-terminal repeat regions of the primate-specific HERVH endogenous retrovirus function as enhancers and that HERVH transcripts are preferentially expressed in human embryonic stem cells and are regulated by OCT4. Notably, we show that HERVH is a nuclear-localized long non-coding RNA that is required to maintain the undifferentiated state of human embryonic stem cells. Furthermore, we found association of HERVH with transcription factor OCT4 and co-activators such as p300, CBP and mediator subunits. These findings reveal that a primate-specific endogenous retrovirus has evolved a striking role in modulating pluripotency through a positive feedback loop involving the RNA itself.

P25

SERUM FREE MEDIA DEVELOPED FOR SUSPENSION CULTURES OF HUMAN MSC ON MICROCARRIERS

TAN, Kah Yong, Teo, Kim Leng, Reuveny, Shaul, Oh, Steve
Bioprocessing Technology Institute, Singapore

Mesenchymal stromal cells (MSCs) are being investigated as potential cell therapies for many different indications. Current methods of production rely on traditional monolayer culture on tissue-culture plastic, often relying on serum-supplemented growth media. However, serum-free media offers several advantages over serum-supplemented media, such as supply and contaminant issues. We demonstrate MSC growth in serum-free media in monolayer culture comparable to serum-supplemented media. Furthermore, we achieve MSC growth on Cytodex 1 microcarriers in spinner culture flasks under constant agitation with serum-free media. MSCs expanded this way express the ISCT markers, and demonstrate trilineage differentiation capability. Thus we have developed a protocol for MSC expansion which may prove useful for future industrial production of cells for therapy.

POSTER ABSTRACTS

P26

PERIPHERAL BLOOD MONONUCLEAR CELLS PROMOTE INCREASE MOUSE ENDOMETRIAL THICKNESS : A SAFE SOLUTION FOR INFERTILITY

LEE, EunJu
Seoul National University Hospital, Seoul, South Korea

To evaluate the effects of human peripheral blood mononuclear cells (hPBMCs) on endometrial thickness, human endometrial epithelial cell lines, SNU-685 or SNU-1077, were first co-cultured with hPBMCs. hPBMCs significantly increased the proliferation of endometrial epithelial cells in both SNU-685 ($p=0.0021$) and SNU-1077 ($p=0.00441$) cell lines. Next, an in vivo evaluation was performed in nonobese diabetic-severe combined immune-deficient (NOD-SCID) female mice. hPBMCs were isolated from the peripheral blood with Ficoll gradient and 1×10^5 hPBMCs were injected directly into the endometrium of the uterine horn of NOD-SCID female mice. The control was a counterpart mouse in whom the uterine horn was not treated. The 3-day specimens after hPBMC injection were significantly thicker ($p=0.001$) in the hPBMC treated group than in the non-treated control group. Histologically, the hPBMC injected group demonstrated a thick endometrium with no signs of an irregular mass. Finally, to evaluate the effects of hPBMCs on endometrial thickness during the events associated with the estrous cycle, the whole uterus was biopsied after one estrous cycle (in mice 4-6 days) from the day of the hPBMC injection. Both the non-treated uterine horn and the hPBMC injected uterine horn demonstrated normal cycling endometrium. Therefore, hPBMC treatment does not interrupt normal endometrial change according to the estrous cycle. Additionally, proinflammatory cytokine IL-6 levels decreased, and anti-inflammatory cytokine IL-10 levels dramatically increased in the supernatant from human endometrial epithelial cells co-cultured with hPBMCs (p value<0.05), as observed on ELISA. The results suggested that transplantation of PBMCs increase endometrial thickness with immunomodulating.

P27

INTRACELLULAR DOMAIN OF CD44 SUPPORTS TUMORIGENESIS BY ACTIVATION OF STEMNESS FACTORS

CHO, Yunhee, Chun, Kyung-Hee
Department of Biochemistry and Molecular Biology, Brain Korea 21 Plus Project for Medical Science, Yonsei University, Seoul, South Korea

The role of CD44 has been reported in the progression of tumors as well as for its expression on cancer stem cells. In this study, we demonstrated the effect of intracellular domain of CD44 (CD44ICD) on stemness factors, such as Nanog, Sox2 and Oct4 to promote breast tumorigenesis. We detected the sphere formation of breast cancer cells were correlated with the expression levels of CD44ICD rather than full-length CD44. Interestingly, CD44 depletion or GSI treatment decreased the expression levels and nuclear localization of stemness factors, whereas overexpression of CD44ICD without CD44 reversed these effects. The transcriptional activity of Sox-2 and Oct-4 was decreased by CD44 depletion or GSI treatment. Moreover, it was significantly increased by overexpression of CD44ICD, but not by overexpression of nuclear-localization sequence deleted CD44ICD. The interaction was detected between CD44ICD and these stemness factors. The deletion of C-terminal of CD44ICD did not interact with nor increase the transcriptional activation of them. Taken together, CD44 promotes tumorigenesis by its interaction and translocation of its intracellular-domain with stemness factors, and further we suggest that the prevention of CD44ICD formation is maybe a potent approach for breast cancer therapy.

POSTER ABSTRACTS

P28
LONG NONCODING RNAS OF THE DROSOPHILA GERMLINE

LIM, Robyn S. M.[1], Anand, Amit[1], Teo, Ryan Y. W.[1], Sharma, Vineet[1], Shirane, Kenjiro[2], Sasaki, Hiroyuki[2], Kai, Toshie[1]
[1]Temasek Life Sciences Laboratory, Singapore, [2]Department of Molecular Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan

Contrary to the position previously held by the central dogma of molecular biology, a substantial proportion of the genomes of diverse species are transcribed into functional transcripts without coding potential and cannot be dismissed as mere transcriptional noise. Apart from small non-coding RNAs, long non-coding RNAs (lncRNAs) have emerged as molecules that play significant roles in regulating many biological processes. Because many functional examinations of lncRNAs in animals have focused on vertebrate models or mammalian cell lines, there are fewer examples of lncRNAs that have been functionally analyzed in non-vertebrates or in developmental contexts. With research suggesting a role for lncRNAs in stem cells, we aim to uncover lncRNAs involved in germline stem cell (GSC) regulation and differentiation in Drosophila gonads, which are presently among the best-studied and genetically tractable animal systems to investigate stem cell–related phenomena in vivo. We employed fluorescence-activated cell sorting (FACS) to obtain purified populations of undifferentiated GSC-like cells from *Drosophila* ovaries. RNA sequencing was conducted on these FACS-sorted cells, as well as on mature stage 14 oocytes and cells from somatic *Drosophila* cell lines, which were selected to serve as references for differentiated female germ cell and somatic transcriptomes, respectively. The transcriptomes of mutant testes containing undifferentiated germline cells and wildtype testes were also sequenced. Differential gene expression analysis has identified several candidate annotated lncRNAs that are specifically expressed in undifferentiated GSCs. Furthermore, we have uncovered hundreds of novel, potentially non-coding intergenic, intronic, and antisense transcripts in the GSC transcriptome. Validation of GSC-enriched lncRNAs will be performed by qRT-PCR and in situ. By exploiting established genetic toolkits and mutagenesis approaches, we aim to explore the functional significance of these fascinating genes through the generation of knockdown lines and gene deletion mutants.

P29
STUDY OF HUMAN CESAREAN SCAR FIBROBLASTS AS A FEEDER CELLS FOR CULTURING THE HUMAN PLURIPOTENT STEM CELLS: EFFECT OF BASIC FIBROBLAST GROWTH FACTOR SUPPLEMENTATION

PAVARAJARN, Wipawee, Rungsiwiwut, Ruttachuk, Numchaisrika, Pranee, Virutamasen, Pramuan, Pruksananonda, Kamthorn
Obstetrics and Gynecology, Chulalongkorn University, Pathumwan, Bangkok, Thailand

Human pluripotent stem cells (hPSCs) are traditionally cultured on mouse embryonic fibroblast (MEFs). However, the components from MEFs could contaminate the hPSCs and that can limit the use of hPSCs for clinical application. On the other hand, several reports demonstrated that exogenous basic fibroblast growth factor (bFGF) could be depleted from the hPSCs culture media if the appropriated type of feeder cells was used. Our study were aimed to determine, i) the effect of exogenous bFGF supplementation in the hPSCs culture media on the morphology and gene expression of inactivated human cesarean scar fibroblasts (HSFs) and ii) the feasibility of using the inactivated HSFs as the feeder cells for culturing the hPSCs. The shrunk cells and increasing of the gap between the cells were observed in the inactivated HSFs that cultured in 4 and 8 ng/ml but not in 0 ng/ml bFGF. Expression of Activin A, bFGF, TGF-β and BMP4 were similar between inactivated HSFs cultured in 0, 4 and 8 ng/ml bFGF. After two hPSCs lines including hESCs line (Chula2.hES) and hiPSCs line (PFX12) were co-cultured

POSTER ABSTRACTS

with three conditions of inactivated HSFs for more than ten passages, the hPSCs lines were subjected to characterization. The results showed that the hPSCs cultured in 0, 4 and 8 ng/ml bFGF could maintain their undifferentiated state by positively immunostained for SSEA-3, Oct-4, Tra-1-60 and expressed transcriptional factor Oct-4 and Nanog. The hESC and hiPSC lines could differentiate in vitro into three embryonic germ layers and maintain their normal karyotype of 46, XY and 46, XX respectively. In conclusion, exogenous bFGF supplementation in the culture medium can be omitted when using HSFs as the feeder cells for culturing the hPSCs.

P30

THE DROSOPHILA ADP RIBOSYLATION FACTOR-LIKE 2 REGULATES ASYMMETRIC CELL DIVISION AND SUPPRESSES NEUROBLAST OVERGROWTH

CHEN, Keng, Koe, Chwee Tat, Wang, Cheng, Wang, Hongyan
Neuroscience & Behavioral Disorder Program, Duke-NUS Graduate Medical School Singapore, Singapore

The *Drosophila* larval brain neuroblast undergoes asymmetric cell division to generate one self-renewing neuroblast and one ganglion mother cell (GMC), which divides terminally to produce two neurons. During asymmetric cell division, the mitotic spindle is aligned along the apical-basal axis to ensure the correct segregation of asymmetric proteins into two daughter cells. Failure of asymmetric division could result in un-controlled proliferation of neuroblast, a phenotype resembling brain tumors. Here, we have identified ADP ribosylation factor-like 2 (Arl2) as a novel regulator of neuroblast asymmetric division. Disruption of Arl2 function, by either Arl2 knockdown or overexpression of a dominant-negative Arl2 (Arl2T30N), causes neuroblast overgrowth in the larval central brain. In arl2 null mutant clones, ectopic neuroblasts are generated in both type-I and type-II neuroblast lineages. Arl2 is required for the proper orientation of mitotic spindle during neuroblast asymmetric division. Interestingly, in arl2 null mutant clone neuroblasts, the centrosome function and microtubule organization are largely disrupted. Our results suggested that Arl2, which is important for the maintenance of microtubule dynamic, plays critical roles in regulating neuroblast asymmetric division and suppressing neuroblast overgrowth.

P31

DEPOT-SPECIFIC DIFFERENCES IN ROS PROFILE OF HUMAN ADIPOSE-DERIVED STEM CELLS FROM SUBCUTANEOUS AND VISCERAL FAT

SRIRAM, Sandhya[1], Ong, Wee Kiat[1], Takeda, Kosuke[1], Shabbir, Asim[2], Toh, Sue-Ann[3], Sugii, Shigeki[1]
[1]SBIC, Singapore, Singapore, [2]Department of Surgery, National University Hospital, Singapore, [3]Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Adipose-derived stem cell (ASC) proliferation, migration and regenerative potential are greatly dependent on intracellular Reactive oxygen species (ROS) levels. However, the ROS profiles and its effect on ASCs derived from the two anatomically distinct depots, subcutaneous (SC-ASC) and visceral(VS-ASC) white adipose tissue are unknown. In this study, human ASCs isolated from subcutaneous and visceral fat of omental region were used to determine ROS profiles and intrinsic differences in ROS properties between the two. Microarray analysis and further validation by qPCR of paired SC-ASC and VS-ASC from 6 human subjects revealed that SC-ASCs have higher expression of genes involved in anti-oxidant and anti-inflammatory pathways, while VS-ASCs have increased expression of genes involved in pro-oxidant and pro-inflammatory pathways. To further establish ROS profiles in SC-ASCs and VS-ASCs, Cell ROX Red and NO detection reagent were used to detect ROS and RNS levels, respectively. Quantitation using fluorescent multi-plate reader and image analysis using high content image screening and fluorescent microscope showed

POSTER ABSTRACTS

increased levels of ROS and RNS in VS-ASCs when compared to SC-ASCs. This finding establishes depot specific differences in ROS potential and oxidative capacity of adipose stem cells. These preliminary results could also explain the better adipogenic potential and antioxidant action of SC-ASCs. Further studies are being carried out to find the various pathways involved in the ROS production and its effects on proliferation, differentiation, migration and regenerative capacity of these ASCs.

P32

HUMAN MESENCHYMAL STEM CELLS BIOREACTOR CULTURE: OPTIMIZATION OF MICROCARRIER CONCENTRATION AND DESIGNING OF MEDIUM FEED

CHEN, Allen, Chew, Yi Kong, Tan, Hong Yu, Reuveny, Shaul, Oh, Steve K.
Bioprocessing Technology Institute, Singapore

Human Mesenchymal stem cells (hMSCs) emerge as the leading cell type of cell therapy targeting a variety of diseases. In order to supply the large amounts of hMSCs needed for these application, different types of microcarriers have been used for expansion of hMSCs isolated from various sources in scalable stirred bioreactors. We have previously described the expansion of hMSCs derived from human fetal tissues. However, our study as well as other publications was limited in exploring process optimization particularly in determination of the optimal correlation between microcarrier concentration and the design of an efficient nutrient feeding strategies needed to achieve higher cell yield and more efficient medium utilization. In this study, we firstly investigated the effect of basal medium selection on hMSCs expanded in stirred cultures on Cytodex 3 microcarriers in the presence of 10% FBS. We have found that αMEM supported faster MSC growth on microcarriers than DMEM (doubling time 31.6±1.4 vs 42±1.7h) and shortened the process time. At microcarrier concentration of 8mg/ml, a high cell concentration of 1.08×10^6 cells/ml with confluent cell concentration of 4.7×10^4cells/cm^2 was achieved. Instead of 50% medium exchange every 2 days, we have designed a full medium feed based on glucose consumption rate. The optimal medium feed that consisted of 1.5g/l glucose supported MSC growth to full confluency while achieving the low medium usage efficiency of 3.29ml/10^6cells. Finally, a controlled bioreactor with the optimized parameters achieved maximal confluent cell concentration with 16 fold expansion and a further improved medium usage efficiency of 1.68ml/10^6cells. In conclusion, we have optimized the microcarrier based platform for expansion of MSCs that generated high cell yields in a more efficient and cost effective manner. This study highlighted the critical parameters in the optimization of MSC production process.

P33

DIRECT REPROGRAMMING OF ADULT SOMATIC CELLS TOWARD RETINAL NEURONS

BANERJEE, Indranil[1], Bouwmeester, Tewis[2], Roska, Botond[1]
[1]*Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland,* [2]*Developmental and Molecular Pathways, Novartis Institutes for BioMedical Research, Basel, Switzerland*

Brain circuits are assembled from large numbers of morphologically, functionally, and gene-expression diverse cell types. The transcription factor repertoire that is needed to convert somatic or embryonic cells to specific neuronal cell types and the potential of converting one neuronal type to another within a connected brain circuit in vivo is not well known. The mammalian retina offers unique opportunities to address these questions. Many retinal cell types are well understood in terms of morphology, connectivity, and gene expression pattern. Retinal neurons can be manipulated in vitro and in vivo using genetic and viral approaches, and the function of the retina, the conversion of light to neural activity patterns, can be measured. Here, we address whether using adult retinal cell type-specific transcription factors we could create mouse cone and rod photoreceptors from adult somatic cells. A positive answer to this second question can have

POSTER ABSTRACTS

direct impact on the treatment of a set of blinding diseases. The insights gained from this work in mice could be used to understand and manipulate the retinal cell types in human within a circuitry.

P34

HEPATOCYTE-LIKE CELLS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELL WERE READILY PROLIFERATIVE WHILE MAINTAINING STABLE HEPATOCYTE

SA-NGIAMSUNTORN, Khanit[1], Wongkajornsilp, Adisak[2], Phanthong, Phetcharat[3], Borwornpinyo, Suparerk[4], Hongeng, Suradej[5]
[1]Department of Biochemistry, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand, [2]Department of Pharmacology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, [3]Department of Anatomy, Faculty of Science, Mahidol University, Bangkok, Thailand, [4]Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok, Thailand, [5]Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

The hepatocyte-like cells (HLC) derived from either human pluripotent stem cells or human embryonic stem cells have the potential to provide unlimited cell sources for in vitro assays for drug metabolism and toxicity. However, fully CYP-functional hepatocyte-like cell is still difficult to maintain and expand under conventional culture condition. Some HLC subpopulation remain in the immature stage that might not be suitable for drug metabolism assessment. In this study, the iPS cells were driven toward HLCs using the four stages protocol that mimics the liver development program in the embryonic stage. At the end of this protocol a homogenous population of HLC was developed. More than 95% of HLC population expressed albumin and HNF-4α at high level. The heightening hepatocyte-specific gene expression using real-time PCR and immunofluorescence confirmed that iPS cells were authentically differentiated along the hepatocyte lineage. The iPS-derived HLCs (iPS-HLCs) expressed all major CYP isozymes and transporter proteins comparable to those from tumorogenic HepaRG and HepG2 cell line. The CYP expressions in iPS-HLCs were first detected on day 22 after the initiation of the differentiation protocol and sustained or slightly increased until at least two weeks after the completion of the maturation. Moreover, a few iPS-HLC population could proliferate after being sub-cultured for additional 3 passages without losing their hepatocyte phenotypes. We proposed that the iPS-HLCs could serve as a superior model for hepatocyte substitution owing to their readily reproducible with stable phenotypes.

P35

REPLACEMENT OF FETAL BOVINE SERUM WITH HUMAN UMBILICAL CORD BLOOD SERUM FOR CULTURING THE SUPPORTIVE FEEDER CELLS OF HUMAN PLURIPOTENT STEM CELL LINES

RUNGSIWIWUT, Ruttachuk, Numchaisrika, Pranee, Virutamasen, Pramuan, Pruksananonda, Kamthorn
Obstetrics and Gynecology, Chulalongkorn University, Bangkok, Thailand

The self-renewal and differentiation ability of human pluripotent stem cells (hPSCs) provided the feasibility of using these cells in the field of regenerative medicine. In general, co-culture of hPSCs with feeder cells allowed the hPSCs enable to maintain their pluripotency and differentiation ability. Culture conditions that minimized the contact of animal products and/or pathogens to the feeder cells as well as the hPSCs, should be considered prior to clinical application. Here, we demonstrated that human umbilical cord blood-derived serum (hUCS) can be efficiently used for culturing human foreskin fibroblasts (HFFs) and subsequently co-cultured the inactivated HFFs with hPSCs. HFFs cultured in hUCS-containing medium displayed the fibroblastic feature, short population doubling time, normal cell cycle pattern, and maintain their normal karyotype after

POSTER ABSTRACTS

prolong culture. Moreover, HFFs cultured in hUCS-containing medium expressed the group of genes including, Activin A, FGF2, TGFβ1 that play an important role in maintenance of hPSC pluripotency. In addition, hPSC lines co-cultured with inactivated HFFs that previously cultured in hUCS-containing medium enable to maintain their undifferentiated stage as demonstrated by the expression of OCT-4, NANOG, SOX2 REX1 and hUTF. hPSC lines showed the positive results for SSEA-3, SSEA-4, OCT-4 and TRA-1-60 immunostaining . When hPSC lines were induced to differentiation in vitro, ectoderm (NESTIN), endoderm (AFP) and mesoderm (brachyury) were detected by immunocytochemistry and RT-PCR. Moreover, hPSC lines maintained their normal karyotype after prolong co-culture. The results of the present study promisingly demonstrated that hUCS could be applied for culturing the supportive feeder cells prior to co-culture with the hPSCs. These finding is beneficial for optimizing the xeno-free culture condition of hPSCs.

P36

EFFICIENT AND OPTIMIZED REPROGRAMMING CULTURE-EXPANDED HUMAN DERMAL FIBROBLASTS AND BLOOD-DERIVED CD34+ CELLS OR BLOOD-DERIVED ERYTHROID CELLS IN FEEDER-FREE AND XENO-FREE CULTURE CONDITIONS

Chang, Wing Y., **HUNTER, Arwen**, Chen, Annie, Ng, Alvin, De Jong, Susan, Peters, Carrie, Fairhurst, Maureen, Antonchuk, Jennifer, Thomas, Terry E., Eaves, Allen C., Louis, Sharon A.
STEMCELL Technologies Inc, Vancouver, BC, Canada

Skin and peripheral blood (PB) are widely used as the starting cell source for generating human induced pluripotent stem cells (hiPSCs). We have developed xeno-free media, TeSR™-E7™ and ReproTeSR™, and feeder-free culture protocols to efficiently reprogram and give rise to easily identifiable hiPSC colonies from skin fibroblasts and PB-derived cells, respectively. For working with PB, we have also incorporated upstream protocols to obtain high numbers of enriched populations of rare PB-derived $CD34^+$ cells and erythroid cells (ECs) from PB mononuclear cells (MNCs) by either immunomagnetic-based selection or culture expansion, prior to reprogramming. PB MNCs were fractionated using Ficoll™ in SepMate™-50 tubes and then enriched by EasySep™ $CD34^+$ positive selection for $CD34^+$ cells. The enriched population containing 35-78% $CD34^+$ cells was expanded for 7 days in StemSpan™ SFEM II Medium with $CD34^+$ Expansion Supplement, achieving 5 - 20 fold expansion (n=10). In separate experiments, ECs were expanded from PB-MNCs for 10 - 14 days in SFEM II with Erythroid Expansion Supplement yielding 64-95% $CD71^+GlyA^+$ cells. The culture-expanded fibroblasts, PB-derived $CD34^+$ or ECs were each transfected with episomal reprogramming vectors encoding OCT-4, SOX2, KLF4 and L-MYC. Post-transfection, fibroblasts were cultured on Matrigel™ in TeSR™-E7™ with daily media changes from day 3 - 28, whereas PB $CD34^+$ or erythroid cells were cultured on Matrigel in SFEM II Medium with supplements until day 3, followed by additions of 1 mL of ReproTeSR™ on days 3 and 5 and full daily media changes with ReproTeSR™ from days 7 - 28. Reprogramming efficiencies were 0.076 ± 0.004% (n=6) for adult fibroblasts, 0.026 ± 0.007% (n=6) for neonatal fibroblasts, 0.012 ± 0.003% (n=6) for $CD34^+$ cells and 0.013 ± 0.003% (n=3) for ECs. Resulting hiPSC lines were expanded using mTeSR™1 or TeSR™-E8™ maintenance media and were validated by cell and colony morphology, expression of pluripotency-associated markers and differentiation potential to all three germ layers. Together with our existing products, these new xeno-free reprogramming media provide a complete workflow for the isolation and expansion of cells prior to reprogramming, efficient feeder-free reprogramming of dermal fibroblasts and blood cells, and maintenance of hiPSCs in defined, conditions.

POSTER ABSTRACTS

P37

GALECTIN-1 AS POTENT REGULATORS OF STEMNESS PROPERTIES IN GASTRIC CANCER CELLS

KIM, Seok-Jun, Chun, Kyung-Hee
Department of Biochemistry and Molecular Biology, Brain Korea 21 PLUS Project for Medical Sciences, Yonsei University College of Medicine, Seoul, South Korea

Galectins are a family of carbohydrate-binding proteins with a high affinity for β-galactosides. Galectin-1 is differentially expressed by various normal and tumor tissues and appears to be functionally polyvalent, with a wide range of biological activity. Various articles were described function of galectin-1 in the intracellular and extracellular activity through the immune response and tumor progression and metastasis. In this study, we showed that down and over-expressed galectin-1 regulates mammosphere formation of gastric cancer cells using galectin-1 specific shRNA and expression vector. Therefore, to understand how galectin-1 can regulate cancer cell stemness, we knock-downed the expression of galectin-1 with small interfering RNA (siRNA) in gastric cancer cells, and monitored changes in gene expression, employing DNA microarray analysis. Those gene lists we found Sox-2 and Nanog like a stemness regulation transcriptin factor. As followed, we confirmed that galectin-1 regulate Sox-2 and Nanog expression levels using RT-PCR and Westernblot. Also, we found galectin-1 interact with Sox-2, Nanog and Oct-4. Take a results, Galectin-1 is potent regulater of gastric cancer cells stemness through binding with Sox-2, Nanog and Oct-4, thus regulation of galectin-1 was potential target in gastric cancer therapy.

P38

PDX1 REPORTER HUMAN ES CELL LINES

TSUNEYOSHI, Norihiro, Dunn, Ray
*Institute of Medical Biology, A*STAR, Singapore, Singapore*

Pancreatic and Duodenal Homeobox 1 (PDX1) encodes a homeobox transcription factor that is expressed at the earliest stages of human embryonic pancreatic development. Lineage tracing studies in the mouse have conclusively shown that Pdx1+ early pancreatic progenitor (ePP) cells are multipotent, giving rise to the exocrine, endocrine and ductal components of the adult organ. Consistent with this, loss of Pdx1 results in complete pancreatic agenesis both in mice and in man. This catastrophic phenotype clearly indicates that Pdx1 sits high atop the gene regulatory network that orchestrates the morphogenesis of this indispensible endocrine organ. To simulate human pancreatic development in vitro, we developed a human embryonic stem cell (hESC) differentiation protocol that tightly adheres to developmental logic and yields abundant PDX1+ ePP cells whose molecular signature closely approximates the incipient pancreatic primordium in vivo. To both better study the biology and developmental potency of human ePP cells, we developed a series of PDX1 reporter cell lines using TALEN gene editing technology. We introduced EmGFP by homologous recombination into intron 1 of PDX1, which results in a null mutation designated PDX1(1-136)-EmGFP, and constructed a second PDX1 reporter allele whereby the dosage of PDX1 is maintained with concomitant expression of EmGFP, which we designate as PDX1P2A-EmGFP. Homozygous PDX1(1-136)-EmGFP hESC clones were also obtained, and consistent with in vivo loss of function studies, PDX1 null cells fail to activate the pancreatic transcriptional program and divert to alternate fates in vitro. Overlapping microarray studies of wild-type and PDX1 null mutant hESC cells line with in-house PDX1 ChIP-Seq data has revealed a novel list of candidate PDX1 transcriptional targets. In addition, the PDX1P2A-

POSTER ABSTRACTS

EmGFP allele has afforded us the chance to isolate and to propagate for the first time PDX1+ ePP cells to homogeneity in chemically defined conditions. We anticipate that stable ePP culture will mitigate the need to differentiate ePP cells from pluripotent hESC and will provide a valuable platform for the screening of endocrine-inducing factors. Updates on these two projects will be presented.

P39

CHARACTERIZATION OF DOXORUBICIN TOXICITY IN HUMAN PLURIPOTENT STEM CELL DERIVED-CARDIOMYOCYTES

MAILLET, Agnes[1], Tan, Kim[1], Mehta, Ashish[2], Shim, Winston[2], Brunham, Liam[1]
[1]Translational Laboratory in Genetic Medicine, ASTAR, Singapore, Singapore, [2]Research and Development Unit, National Heart Centre, Singapore, Singapore

Anthracyclines, such as doxorubicin or daunorubicin, are widely used in the treatment of numerous cancers. However, their clinical utility is limited by a dose-dependent risk of cardiotoxicity and congestive heart failure. The precise mechanisms involved in doxorubicin-induced cardiotoxicity remain poorly understood. Recently, human Pluripotent Stem Cell (hPSC)-derived cardiomyocytes have emerged as a valuable tool for modeling drug toxicity on cardiac cells. The goal of this study was to investigate the response of hPSC-derived cardiomyocytes to doxorubicin treatment. hES cells were differentiated into cardiomyocytes using insulin free medium and Wnt inhibition. The differentiated cells were tested for their expression of cardiac Tropinin T and/or sarcomeric alpha actinin by immunofluorescence, FACS and qPCR. Cardiomyocytes were then treated with doxorubicin and, cell viability, apoptosis, reactive oxygen species (ROS) production, calcium handling and electrophysiological properties of the cells were assessed. Up to 90% of differentiated hES cells stained positive for cardiac specific markers indicating a high degree of efficiency. Furthermore, cardiomyocytes treated with increasing doses of doxorubicin (5nM to 50μM) showed a dose-dependent decrease in cell viability. Sensitivity to doxorubicin differed across different cell lines with IC50 between 500pM and 10μM. After doxorubicin treatment, the presence of apoptotic cells was detected as well as in increase in ROS production. We have characterized aspects of doxorubicin toxicity in hPSC-derived cardiomyocytes. Our results show marked difference in response to doxorubicin across different cell lines suggesting the importance of genetic background and highlighting that careful selection of appropriate controls will be essential to evaluate the influence of genetic variants on anthracycline toxicity.

P40

RAPID, UNBIASED GENE EXPRESSION PROFILING FROM LIMITED SAMPLES: ANALYSIS OF SINGLE EMBRYOID BODIES AND PLURIPOTENT STEM CELL BIOPSIES

Kibschull, Mark[1], Lye, Stephen J.[1], **OKINO, Steven T.**[2], Sarras, Haya[3]
[1]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada, [2]Gene Expression Division, Bio-Rad Laboratories, Hercules, CA, USA, [3]Gene Expression Division, Bio-Rad Laboratories, Mississauga, ON, Canada

Transcriptional profiling during stem cell differentiation and cellular reprogramming is a powerful tool for studying biological mechanisms of lineage determination and reprogramming progression. Current workflows require column-based RNA purification from high amounts of starting material; projecting in either limitation of analyzed targets or high running costs for conducting a thorough study. Here, we present a novel workflow using validated reagents that allow researchers the ability to assess up to 100 gene targets from as little as a single embryoid body (EB) or a small biopsy of human pluripotent stem cell (hPSC) colony. The 4-step workflow is initiated by the single step isolation of lysates containing genomic DNA-free RNA directly from the cultured cells. The lysate is then reverse transcribed to cDNA. The cDNA is then pre-amplified for a panel of 100 pre-validated gene targets previously shown to be involved in stem cell pluripotency and

POSTER ABSTRACTS

lineage determination. Lastly the amplified gene targets are quantified by SYBR Green qPCR. Using a single EB (~1000 cells) as starting sample, we demonstrate that our workflow enables the successful analyses of differentiation progression and lineage determination. We demonstrate excellent correlation and reproducibility in gene profiling between the pre-amplified cDNA samples compared to the non-amplified starting cDNA samples. Out of the 58 expressed targets 97% show a Cq difference below 0.75 cycles from predicted pre-amplified cDNA values, thus confirming that the workflow introduces no or minimal bias. Similarly, we successfully applied this workflow to gene expression profiling of small hPSC biopsies (<1000 cells) to monitor culture quality or reprogramming progression. In summary, we have developed a complete workflow enabling rapid, unbiased and reproducible gene expression profiling of a large panel of targets from very limited samples. The minimal amount of material required by this workflow highlights a more cost effective way for culturing and analyzing hPSC.

P41

THE BRM-HDAC3-ERM REPRESSOR COMPLEX SUPPRESSES DE-DIFFERENTIATION IN DROSOPHILA TYPE II NEUROBLAST LINEAGES

KOE, Chwee Tat[1], Li, Song[2], Rossi, Fabrizio[3], Wong, Jack Jing Lin[4], Wang, Yan[4], Wang, Yan[4], Zhang, Zhizhuo[5], Chen, Keng[1], Aw, Sherry Shiyang[6], Richardson, Helena[7], Robson, Paul[8], Sung, Wing-Kin[9], Yu, Fengwei[4], Gonzalez, Cayetano[10], Wang, Hongyan[1]

[1]Duke NUS / NGS, Singapore, Singapore, [2]Duke NUS, Singapore, Singapore, [3]Cell Division Group, Institute for Research in Biomedicine, Barcelona, Spain, [4]TLL, Singapore, Singapore, [5]Department of Computer Science, NUS, Singapore, Singapore, [6]IMCB, Singapore, Singapore, [7]University of Melbourne, Parkville, Australia, Australia, [8]GIS, Singapore, Singapore, [9]GIS/ NUS, Singapore, Singapore, [10]Cell Division Group, Institute for Research in Biomedicine, Barcelona, Spain

The control of self-renewal and differentiation of neural stem and progenitor cells is a crucial issue in stem cell and cancer biology. Drosophila type II neuroblast lineages are prone to developing impaired neuroblast homeostasis if the limited self-renewing potential of intermediate neural progenitors (INPs) is unrestrained. Here, we demonstrate that Drosophila SWI/SNF chromatin remodeling Brahma (Brm) complex functions cooperatively with another chromatin remodeling factor, Histone deacetylase 3 (HDAC3) to suppress the formation of ectopic type II neuroblasts. We show that multiple components of the Brm complex and HDAC3 physically associate with Earmuff (Erm), a type II-specific transcription factor that prevents dedifferentiation of INPs into neuroblasts. Consistently, the predicted Erm-binding motif is present in most of known binding loci of Brm. Furthermore, brm and hdac3 genetically interact with erm to prevent type II neuroblast overgrowth. Thus, the Brm-HDAC3-Erm repressor complex suppresses dedifferentiation of INPs back into type II neuroblasts.

P42

ALTERATION OF GLOBAL GENE EXPRESSION IN HUMAN SOMATIC CELLS USING DNA-BASED EPIGENETIC SWITCHES

NAMASIVAYAM, **Ganesh Pandian,** Taniguchi, Junichi, Le, Han, Sato, Shinsuke, Sugiyama, Hiroshi

iCeMS, Kyoto University, Kyoto, Japan

Cellular reprogramming involves profound alterations in global gene expression that is precisely controlled by a hypothetical epigenetic code. Since the epigenome is inherently flexible, it could be modulated through pharmacological interventions. Recently, cellular reprogramming of mouse embryonic fibroblasts (MEFs) into induced pluripotent stem cells got achieved with a cocktail of seven small molecules alone. However, requirement of several small molecules to modulate intricate multi-gene network and time taken to achieve completely reprogrammed cell line are the major concern. As an innovative chemical approach to control global gene expression via site-specific chromatin modifications, we developed a synthetic transcriptional activator called SAHA-

POSTER ABSTRACTS

PIPs by conjugating sequence-specific DNA binding pyrrole-imidazole polyamides (PIPs) to the histone deacetylase (HDAC) inhibitor SAHA. Screening studies suggested that SAHA-PIPs distinctively activated the pluripotency genes (Oct-3/4, Nanog, Sox2, Klf4 and c-Myc) in mouse fibroblasts by triggering the epigenetic marks associated with transcriptionally permissive chromatin. In human fibroblasts, a SAHA-PIP `1` induced the typically conserved germ cell genes and a SAHA-PIP `2` activated the core pluripotency gene network. Microarray studies and functional analysis revealed the remarkable ability of thirty-two distinct SAHA-PIPs to trigger the transcriptional activation of exclusive clusters of gene networks associated with certain cell fate. Since PIP conjugates can be tailored to bind predetermined DNA sequences, strategies to expand them could create an epoch-making approach in cellular reprogramming as they may precisely coax the somatic cells into pluripotent stem cells and/or a totally new type of cells.

P43

KLF2 IS A CRITICAL FACTOR DOWNSTREAM OF MEK/ERK SIGNALLING INHIBITION TO SUSTAIN GROUND STATE PLURIPOTENCY

YEO, Jia Chi[1], Jiang, Jianming[1], Tan, Zi Ying[1], Yim, Guo Rong[1], Ng, Jia Hui[1], Goke, Jonathan[1], Kraus, Petra[2], Chong, Han Chung[3], Tan, Nguan Soon[3], Lufkin, Thomas[2], Ng, Huck Hui[1]
[1]GIS, Singapore, Singapore, [2]Clarkson University, Potsdam, NY, USA, [3]Nanyang Technological University, Singapore, Singapore

The maintenance of undifferentiated mouse embryonic stem cells (mESCs) requires the presence of LIF and serum. Interestingly, by inhibiting both the pro-differentiative Fgf/Mek/Erk and Gsk3/Tcf3 pathways in mESCs (2i), a pluripotent “ground state” resembling the mouse pre-implantation epiblast can be established. While Gsk3-inhibition is known to alleviate Tcf3-mediated repression of Esrrb, the molecular mechanism downstream of Mek/Erk inhibition remains to be identified. Here, we uncover that Erk2 phosphorylates the Krüppel-like factor 2 (Klf2), leading to Klf2 proteasomal degradation. Mek/Erk inhibition during 2i thus serves to halt Klf2 phospho-degradation, leading to Klf2 protein stabilization and maintenance of ground state pluripotency. While Klf2-null mESCs are viable under LIF/Serum, they undergo apoptosis during 2i. In addition, we found that Klf2 overexpression is sufficient to replace Mek-inhibition, allowing for mESC self-renewal under Gsk3-inhibition alone. Taken together, our study highlights the importance of Klf2 during 2i, and defines the Mek/Erk/Klf2 pathway with the Gsk3/Tcf3/Esrrb axis to establish ground state pluripotency.

P44

GALECTIN-3 REGULATES THE CANCER STEMNESS BY MODULATING THE NOTCH1 INTRACELLULAR DOMAIN LEVEL IN OVARIAN CANCER.

KANG, Hyeok Gu, Chun, Kyung-Hee
Department of Biochemistry and Molecular Biology, Brain Korea 21 PLUS Project for Medical Science, Yonsei University. College of Medicine, Seoul, South Korea

Notch signaling is a central pathway in cells renewal and development. Recent studies havesuggested presence of cancer stem cell (CSC) subpopulationin various cancerous tissues including ovarian cancer. As in the case of normal stem cells, Notch signalingplays an important role in maintenance of CSCs. Activationof Notch signaling results in elevated self-renewal, as shown by increased mammosphere formation, whereas inhibition of Notch signaling had the opposite effect. Galectins are a family of β-galactosides binding proteins that recognize a variety of glycan-containing proteins atthe cell surface and are over-expressed in various tumors. Galectins over-expression as well as changes in their subcellular distribution has been associatedwith various cancer progression and poor prognosis.It is not well understood, however, how the interaction between galectins and transcription factors modulates CSC. In this study, our results

POSTER ABSTRACTS

demonstrated that galectin-3 modulate NOTCH1 intracellular domain (NOTCH1-IC)level. Using galectin-3 specific siRNA and expression vector, we showed that down-regulation or up-regulation of galectin-3 regulates the NOTCH1-IC level and Notch1 target genes such as HES1, HEY1, c-MYC. Through the IP experiments, we found galectin-3 binds to NOTCH1-IC. Also, we demonstrated that Galectin-3 is involved in the transcriptional regulation of NOTCH1-IC using reporter assay. Furthermore, to investigate the effect of galectin-3 on CSC population, we used mammosphere formation that used to reveal stem cell properties. Up-regulation of Galectin-3 increased mammosphere formation. The other way, Galectin-3 knockdown decreased mammosphere formation in the ovarian cancer cells. Take the results, we suggest that galectin-3 regulates ovarian cancer stemness through binding with NOTCH1-IC.

P45

ASSESSING FUNCTIONAL PLURIPOTENCY OF HPSCS USING THE TAQMAN® HPSC SCORECARD ASSAY

FERGUS, Jeffrey, Quintanilla, Rene, Lakshmipathy, Uma
Cell Biology, Thermo Fisher, Carlsbad, CA, USA

Efficient reprogramming technologies have led to the generation of patient-derived induced pluripotent stem cells from various sources and conditions, creating valuable tools in drug discovery and future cell therapies. Despite these advances, characterizing the resulting iPSCs is minimally addressed by current in vitro and in vivo cellular methods. However, molecular-based analysis methods offer an appealing solution for rapid, quantitative, and comprehensive characterization. We earlier reported the development of a TaqMan® hPSC ScorecardTM Panel comprised of a 94-gene panel and cloud-based analysis application which evaluates input sample gene signatures for self-renewal and lineage markers and evaluates the gene signature against a pluripotent reference standard. To further advance the TaqMan® hPSC ScorecardTM, the reference data has since been updated and the data visualization has been improved. In addition, over two hundred samples were analyzed using the TaqMan® hPSC ScorecardTM Panel to determine pluripotency along several stages of the iPSC workflow. Established clones, subjected to spontaneous differentiation via embryoid body formation to assess for trilineage differentiation potential and directed differentiation into specific lineages, were successfully characterized using the TaqMan® hPSC ScorecardTM Panel. Here we offer a much needed molecular characterization method that delivers standardization and effective qualification of hPSCs. We demonstrate the simplicity, ease and consistency of this method to predict hPSC functional pluripotency.

P46

HEMATOPOIETIC TRANSCRIPTION FACTORS PATTERN IS ASSOCIATED WITH FETAL HEMOGLOBIN EXPRESSION IN DEVELOPMENTAL STAGE-SPECIFIC

TANGPRASITTIPAP, Amornrat[1], Sripichai, Orapan[2], Suriyun, Thunwarat[2], Meaunpim, Patcharee[3], Satirapod, Chonthicha[4], Fucharoen, Suthat[2], Hongeng, Suradej[5]
[1]Research center, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, [2]Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Nakhon Pathom, Thailand, [3]Division of Instruments for Research, Office for Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, [4]Department of Obstetrics and Gynecology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Bangkok, Thailand, [5]Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Hematopoiesis is the maturation process of distinct lineages blood cells which controlled by the combination effects of growth factors and nuclear regulators (transcriptional factors). The human fetal and adult globin

POSTER ABSTRACTS

genes are sequentially expressed to achieve normal hemoglobin switching during human development. In an early infancy, γ-globin, rather than β-globin, is synthesized and combined with α-globin to produce fetal hemoglobin (HbF, α2γ2). However, γ-globin is normally developmentally switch-off around 6 to 8 months of age, while β-globin is switch-on to produce adult hemoglobin (HbA, α2β2). HbF production may be increased in β-thalassemia, one of the most common genetic disorders in the world, arises from a reduction of the β-globin synthesis. Clinical manifestations of β-thalassemia patients are related primarily to the extent of α-to non-α-globin chain imbalance and the precipitation of the unmatched α-globin. Hence, reactivation of fetal γ-globin gene in adults is the substantial interest for β-thalassemia management because persistent expression of HbF ameliorates its clinical symptoms. However, the mechanisms of regulation of HbF have not been fully elucidated. To address this issue, the relative expression level of selected hematopoietic transcription factors were investigated in CD34+-derived erythroblasts from adult normal, adult patients of β-thalassemia/HbE, and fetal liver from therapeutically aborted fetuses. The HbF levels in cultured erythrocytes were 4.4 + 0.2%, 38.4 + 31.1% and 95.4 + 1.5% in adult normal-, β-thalassemia/HbE and fetal liver-derived cells, respectively; n = 5. Quantitative RT-PCR was performed in sorting early stage erythroblasts. Fetal liver- and β-thalassemia/HbE-derived cells displayed lower expression of BCL11A, but increased levels of LSD1, MYB, NFE2 and TAL1. GATA2 expression was repressed in adult erythroblasts, while FOG1 and ID2 levels were correlated with HbF expression. The differences in ETO2, GATA1, GFI1B, KLF1, LDB1, LMO2, and SOX6 transcripts levels were not found. Additional experiments are underway to further explore the role of these factors in transcriptional regulation of the globin genes in both development stage-specific and β-thalassemia/HbE erythropoiesis.

P47

TRANSCRIPTIONAL AND EPIGENETIC MECHANISMS INVOLVED IN THE EFFICIENT DIFFERENTIATION OF HUMAN FETAL ENDOTHELIAL CELL-DERIVED INDUCED PLURIPOTENT STEM CELLS (IPSCS) TOWARD ENDOTHELIAL CELLS

TING, Chiao-Hsuan, Ho, Pai-Jiun, Yen, B. Linju
Natl Health Res Institutes, Zhunan Taiwan, Taipei City, Taiwan

Reprogramming of somatic cells using a few transcription factors has been shown to be an efficient method of producing pluripotent stem cells. Currently, a large number of studies on these induced pluripotent stem cells (iPSCs) are increasingly focused on clinical applications of these cells. As with human embryonic stem cells (hESCs)–the 'gold standard' for pluripotent stem cells–iPSCs have the ability to differentiate towards all three germ layers lineages and germ cells, making these cells valuable for generating specific, functional cells for therapeutic application. One of the most clinically relevant somatic lineages is endothelial cells (ECs), which can be used therapeutically to treat ischemic diseases of many organs. We have previously reported that human umbilical venous endothelial cells (HUVEC) can be used to generate iPSCs with the use of only two factors, OCT4 and SOX2. These 2 factor-HUVEC derived iPSCs (2F-iHUV) are able to undergo tri-germ layer differentiation both ***in vitro*** and ***in vivo*** in terms of teratoma formation. Since there is some recent data to suggest that iPSCs can retain epigenetic memory of the somatic cell from which it was generated from, we were curious to whether 2F-iHUV would be more efficient at differentiating into ECs compared to hESCs. We found that 2F-iHUV can acquire an EC phenotype without the need for embryoid body formation and collagen IV, in contrast to hESC derivatives, and within a very short duration (<1 week). Surface marker detection revealed that when cultured in EC medium, 2F-iHUV derivatives rapidly become positive for CD31/PECAM-1 and CD144/VE-cadherin, two EC markers. Furthermore, these differentiated 2F-iHUV EC-like cells can form

POSTER ABSTRACTS

tubal structures when plated on Matrigel and uptake acetylated-low density lipoproteins, both EC functional assays. To explore the specific mechanisms involved in the efficient EC differentiation of 2F-iHUV, we searched for the up-regulation of EC-transcription factors and found that FOXC1, FOXC2, and ER71 were expressed in these cells. We are currently investigating the role of epigenetic memory in the rapid activation of these endothelial transcription factors. Our findings suggest 2F-iHUV may be an efficient model for studying EC differentiation as well as a cell source suitable for generating EC for therapeutic application.

P48

ROLE OF THE VASCULAR NICHE IN HUMAN EMBRYONIC STEM CELLS DIFFERENTIATION INTO CARDIOMYOCYTES

GUPTA, Renuka Ravi[1], Pasquier, Jennifer[1], Hoarau-Vechot, Jessica[1], Abu-Kaoud, Nadine[1], Courjaret, Raphael[1], Al-Thawadi, Hamda[1], Elliot, David A.[2], Stanley, Edouard G.[2], Rafii, Arash[1]
[1]Genetic Medicine, Weill Cornell Medical College - Qatar, Doha, Qatar, [2]Monash Immunology and Stem Cell Laboratories,, Monash University, Clayton, Victoria, Australia

Cardiovascular diseases are becoming a leading cause of death in many countries. Therefore, understanding molecular mechanisms, cell-cell interactions and signaling pathways regulating cardiomyocytes (CM) development and expansion has become a major area of interest. Human Embryonic Stem cells (hESCs) have the ability to differentiate into different cell lineages. They provide an attractive platform for generating CM in cardiac disease modeling and regeneration. CM have been derived from hESCs through chemical agents, morphogens and growth factors. However these techniques have very low efficiency where contracting areas seen are minimal. The methods used were lengthy, inconsistent and the use of multiple growth factors makes them expensive. There thus emerges an urgent need to develop a more efficient and less costly method to differentiate hESCs into CM. The vascular endothelium is highly versatile and multifunctional having many synthetic and metabolic properties. Many growth factors present within the endothelial cells have been shown to play a major role at different stages of cardiac repair and development. We thus hypothesize that the cocktail of factors could help to improve the efficiency of cardiogenesis. Using endothelial cells as a platform, differentiation of hESCs into CM was carried out, and output efficiency was compared to general method of differentiation. We used hESCs cells where eGFP sequences targets the NKX2-5 locus, which is a marker for cardiac cells, and a model of autonomous Akt-activated endothelial cells as a layer. In combination with cytokines treatment, we were able to show an increase of GFP positive cells denoting the activation of NKX2-5 in the hESC population, after only 8 days of differentiation. Important beating areas with a high frequency of beating rate were also seen during this time frame. Our study demonstrated that the use of endothelial cells as a feeder layer during the differentiation improved cardiac efficiency. This confirmed the important role of endothelial paracrine factor in the hESC differentiation into CM. Future studies should focus on identifying the essential factors of the endothelial niche responsible for the maintenance and expansion of CM and enabling widespread use in clinical applications.

P49

MESENCHYMAL STEM CELLS SUPPRESS T CELL IMMUNE RESPONSE BY TRANSCRIPTOME MODULATION

RAMASAMY, Rajesh[1], Vellasamy, Shalini[2], Tong, Chih Kong[1], Azhar, Atiqah[3], Soon Choy, Chan[3], Veerakumarasivam, Abhimanyu[3]
[1]Department of Pathology, Universiti Putra Malaysia, Serdang, Malaysia, [2]Department of Biomedical Science, Faculty of Medicine, Universiti Malaya, Petaling Jaya, Malaysia, [3]Perdana University Graduate School of Medicine, Serdang, Malaysia

Transplantation across individuals with genetic variability becomes more feasible with immunosuppressant regiment such as radio and chemotherapy. Although such generalised

POSTER ABSTRACTS

immunosuppression is crucial and lifesaving at times; however prolonged immunosuppression often leads to severe adverse effects. Hence, stem cell therapy emerged as partial substitutes for immunosuppression therapy whereby their therapeutic effect is govern by specific mechanisms towards tissues regeneration and balanced immune response. The most commonly used stem cell in allogeneic transplantation setting is mesenchymal stem cells (MSC). Mesenchymal stem cells have been demonstrated to possess immunomodulatory properties such as suppressing lymphocytes proliferation, preventing dendritic cells (DC) maturation and ameliorating graft versus host disease (GVHD). Most of these studies have addressed the immunosuppression mechanisms at cellular and protein levels yet failed to decipher such effect at gene and transcriptional levels. By using global gene expression profiling, this study aimed to decipher the molecular pathways associated with umbilical cord-derived MSC (UC-MSC) mediated immunosuppression of activated T cells. UC-MSCs exerted a dose-dependent inhibitory effect on T cell proliferation. This inhibition was mainly mediated through direct cell-cell contact rather than soluble factors. The treated T cells did not appear to undergo apoptosis but was significantly arrested at the G0/G1 phase. The expression of many genes in the activated T cells were found to be dysregulated by UC-MSCs. For example, IFNG, CXCL9, IL2, IL2RA and CCND3 were downregulated while IL11, VSIG4, GJA1, TIMP3 and BBC3 were upregulated. Dysregulated gene clusters that were associated with lymphocyte proliferation/activation, apoptosis, and cell cycle and immune response ontologies were selected for further analysis. Using the Ingenuity Pathway Analysis, 13 canonical pathways were identified as enriched with these dysregulated genes. These pathways include T helper cell differentiation, cyclins and cell cycle regulation as well as gap/tight junction signalling. In conclusion, this study indicates that MSC mediated immunosuppression is multifactorial that involve modulation of many genes via specific transcriptomic changes.

P50

BIOREACTOR PROCESSES FOR BRAIN, BONE, HEART AND BLOOD DEVELOPMENT

OH, Steve[1], Chen, Allen[1], Lam, Alan[1], Jian, Li[2], Birch, William[2], Shekaran, Asha[1], Lin, Melissa[1], Ting, Sherwin[1], Sivalingam, Jaichandran[1], Laco, Filip[1], Reuveny, Shaul[1]

[1]Bioprocessing Technology Institute, Singapore, [2]Institute of Materials Research and Engineering, Singapore

As the varieties of tissues in development from stem cells are being mastered in the lab, many of these differentiated cells can and must eventually be manufactured and supplied in industry scale bioreactors. In the past decade at the Bioprocessing Technology Institute, A*STAR, we have created and adapted a microcarrier bioreactor platform which is capable of manufacturing human embryonic stem cells, induced pluripotent stem cells, human mesenchymal stem cells (hMSC) from fetal, cord blood, adipose and bone marrow material. Integrated processes to differentiate these cells to neuroprogenitors, cardiomyocytes have been published, while bone/cartilage and blood production in bioreactors are in progress. We have demonstrated that human pluripotent stem cell (hPSC) aggregates can be produced on polystyrene and commercial microcarriers with defined coatings of polylysine combined with either vitronectin or laminin. Alternatively, in the absence of coatings, application of the ROCK inhibitor also allows microcarrier cell expansion in suspension cultures. We have then differentiated these hPSC to neuroprogenitors achieving 10e7 cells/ml and demonstrated tri-lineage neural differentiation and function in animal models. hMSC have been expanded on microcarriers and differentiated to bone and cartilage forming cells in an integrated bioprocess that yields at least 50% more calcium and collagen than monolayer cultures. More recently we have demonstrated the reproducible and facile production of 3 million cardiomyocytes/ml with the use of Wnt activation and Wnt inhibition small molecules in 2 types of agitated bioreactor systems.

POSTER ABSTRACTS

As a final example, we are advancing protocols for expanding and differentiating hPSC into enucleated red blood cells (RBC) through a 3 stage process via the formation of embryoid bodies, hemangioblasts and erythroblasts that is capable of producing at least a logarithmic improvement (over monolayer cultures) in RBC yield to achieve 10e12 cells all within a 1 litre bioreactor. These 4 examples of differentiation to brain, bone, heart and blood tissues demonstrate the feasibility to manufacture cells for research and therapy in controlled and integrated microcarrier bioprocesses.

P51

CLEC-2 SIGNALING REGULATES THE PRODUCTION OF THROMBOPOIETIN FOR QUIESCENT HEMATOPOIETIC STEM CELLS.

NAKAMURA-ISHIZU, Ayako, Suda, Toshio
Cancer Science Institute, National University of Singapore, Singapore

Bone marrow (BM) hematopoietic stem cells (HSCs) are maintained in a specialized microenvironment ("niche") compromised of various cells and secretory factors. Megakaryocytes (Mks) reportedly are independent niche cells for HSCs. We have recently shown that Mks maintain cell cycle quiescence of HSCs through the production of the niche cytokine, thrombopoietin (Thpo). Mk production of Thpo implicates a paracrine manner by which the cytokine functions for the quiescence of HSCs. Yet, Thpo is also produced systemically in hepatocytes and serum levels of Thpo largely depend upon circulating platelet number, which complicates the regulations on Thpo production in the BM. Analysis of a mice specifically deleted of membrane protein C-type lectin like receptor-2 in Mks (Clec2MkΔ/Δ) revealed decline in both BM and serum Thpo levels despite thrombocytopenia. Mks obtained from Clec2MkΔ/Δ mice exhibited lower production of Thpo confirmed at both gene and protein levels. Mks from Clec2MkΔ/Δ mice also lost potential to sustain HSC numbers in a co-culture system. Due to the low production of Thpo, HSCs from Clec2MkΔ/Δ exhibited loss of cell cycle quiescence and lower repopulation potentials. Furthermore, Clec2MkΔ/Δ mice exhibited massive splenomegaly due to extramedullary hematopoiesis. The loss of stem cell potential in HSCs from Clec2MkΔ/Δ mice was rescued through administration of recombinant Thpo. Finally, knockdown of CLEC-2 downstream signaling diminished the Thpo production in cultured Mks. These findings indicate that Mk specific molecule, CLEC-2, can modulate the HSC niche functions of Mks via production of Thpo. Our data also identifies a novel signaling pathway for systemic Thpo production along with the Mk niche.

P52

EFFECT OF EX VIVO CULTURE CONDITIONS ON IMMUNOSUPPRESSIVE ACTIVITY OF HUMAN BONE MARROW MESENCHYMAL STROMAL/STEM CELLS: INVOLVEMENT OF PROSTAGLANDIN E2 SYNTHASE

KO, Young Jong, Lee, Myoung Woo, Kim, Dae Seong, Park, Yoojin, Park, Hyun Jin, Lee, Soo Hyun, Sung, Ki Woong, Koo, Hong Hoe, Yoo, Keon Hee
Samsung Medical Center, Seoul, South Korea

Mesenchymal stromal/stem cells (MSCs) have been of particular interest due to their immunosuppressive capacities; however, few clinical trials revealed satisfactory results despite a large number of successfully demonstrated pre-clinical data. In this study, a microarray analysis was performed to investigate whether ex vivo culture conditions affect the valuable characteristics of MSCs for therapy. Gene expression profiles were mainly influenced by the level of cell confluence rather than initial seeding density. The analysis showed that 276 genes were up-regulated and 230 genes down-regulated in MSCs harvested at ~90% versus ~50% confluence ($P < 0.05$, $FC > 2$). The genes that were highly expressed in MSCs largely corresponded to chemotaxis, inflammation, and immune responses, indicating direct or indirect involvement in immunomodulatory functions. Especially, chemokine receptor type 7 (CXCR7), mucin 1 (MUC1), prostaglandin E (PGE) synthase (PTGES), and UL16 binding protein 1 (ULBP1), known to be

POSTER ABSTRACTS

potentially involved in various immune responses, were found to be significantly up-regulated in MSCs harvested at a higher cell density. PTGES and ULBP1 were further confirmed for their immunosuppressive activities so that MSCs with PTGES or ULBP1 siRNA were found to restore the T-cell proliferation. Moreover, mouse injected hPBMC with naive MSCs have higher survival rate than mouse injected hPBMC with modified MSCs that reduced PTGES expression by siRNA targeting PTGES, indicating that PTGES and PGE2 are involved in immunosuppressive activity of MSCs for therapeutic efficacy in GVHD. Therefore, the results of this study may provide useful guidelines for the harvest of MSCs that have high expression of the immune response-related genes including PTGES for the enhanced cell therapy in GVHD, thereby controlling culture condition.

P53

A COMPARISON OF GENE EXPRESSION PROFILES OF HUMAN ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS BY

KIM, Dae Seong, Lee, Myoung Woo, Ko, Young Jong, Park, Hyun Jin, Park, Yoojin, Lee, Soo Hyun, Sung, Ki Woong, Koo, Hong Hoe, Yoo, Keon Hee
Sungkyunkwan University, Seoul, South Korea

Because of the heterogeneity of human mesenchymal stem cells (MSCs), methods for cell expansion in culture and the effects on gene expression are critical factors that need to be standardized for preparing MSCs. In this study, differences in gene expression profiles of adipose tissue (AT)-derived MSCs were examined after harvesting cells cultured at different densities. AT-MSCs from three different donors were plated at a density of 200 or 5,000 cells/cm^2. After 7 days in culture, detailed gene expression profiles were investigated using a DNA chip microarray, and subsequently validated using a reverse transcription polymerase chain reaction (RT-PCR) analysis. Gene expression profiles were influenced primarily by the level of cell confluence at harvest. In MSCs harvested at ~90% confluence, 177 genes were up-regulated and 102 genes down-regulated relative to cells harvested at ~50% confluence ($P < 0.05$, FC > 2). proliferation-related genes were highly expressed in MSCs harvested at low density, while genes that were highly expressed in MSCs harvested at high density (~90% confluent) were linked to immunity and defense, cell communication, signal transduction and cell motility. Several cytokine, chemokine and growth factor genes involved in immunosuppression, migration, and reconstitution of damaged tissues were up-regulated in MSCs harvested at high density compared with MSCs harvested at low density. Stemness genes, including Oct4, Nanog, Sox2, Klf4, c-Myc, and Lin28, were up-regulated in early passage (P4) MSCs harvested at low density that exhibits high proliferation rate relative to MSCs harvested at high density. On the other hand, long-term cultured MSCs (P15 MSCs) exhibited low stemness gene expression and proliferation rate, positive βgal staining, high p53 and p21 expression relative to early-passage MSCs, though cells were cultured at low density. There was no significant difference in MSCs from three donors, which means that long-term cultured cells lost their stemness gene expression and proliferation potential. The cell density at harvest modulates the gene expression profile of AT-MSCs, which is consistent with the result in BM-MSCs; therefore, the results of this study may provide useful guidelines for the harvest of MSCs for use in cell therapies.

P54

RAPID IDENTIFICATION OF HOMOZYGOUS MUTANT MOUSE EMBRYONIC STEM CELL CLONES SHOWING DIFFERENTIATION RESISTANT OR DIFFERENTIATION PRONE PHENOTYPE

YOSHIDA, Junko[1], Horie, Kyoji[2]
[1]Osaka University Graduate Sch of Medicine, Suita, Japan, [2]Nara Medical University, Kashihara, Japan

Forward genetics is a powerful method for the discovery of novel genes involved in the regulation of various biological processes in an unbiased manner. However, recessive genetic screening is difficult in mammalian cells due to the diploidy of the genome. Generating homozygous mutant cells through two rounds of gene targeting is labor-intensive. Therefore, a more streamlined high-throughput approach is

POSTER ABSTRACTS

anticipated in order to achieve forward genetics in mammalian cells. Embryonic stem cells (ESCs) are attractive for forward genetics because various biological processes can be studied using in vitro differentiation protocols. We recently reported a method to rapidly generate homozygous mutant mouse ESCs from heterozygous mutant ESCs by conditional regulation of the Bloom's syndrome gene (Blm). This method takes advantage of the high rate of loss of heterozygosity associated with Blm-deficient condition. We have so far generated ~200 homozygous mutant ESC clones. To accelerate phenotype analyses of the homozygous ESC clones in various culture conditions, we devised a system to conduct phenotype screening by pooling homozygous ESC lines. To keep track of the behavior of each homozygous mutant ESC clone, we introduced synthetic short nucleotide barcode sequences into homozygous ESC clones. Barcode-labeled homozygous ESC pool was cultured in various conditions and populations of undifferentiated and differentiated ESCs were quantified by counting barcode reads using the Illumina GA2 sequencer. This method was highly quantitative, allowing us to rank a large number of mutant clones according to the strength of the phenotype. Now we are conducting further analyses of representative clones showing strong differentiation-resistant or differentiation-prone phenotype.

P55

REPROGRAMMING A NON-GERMLINE INCORPORATING EMBRYONIC STEM CELL INTO A FULLY FUNCTIONAL STATE WITH ESRRB AND ATRX

ROSSELLO, Ricardo A.[1], Pfenning, Andreas Pfennig[2]
[1]*Biochemistry, Metropolitan University and University of Puerto Rico, Guaynabo, PR, USA,* [2]*Computational Biology, Massachusetts Institute of Technology, Cambridge, MA, USA*

Derivation of mouse embryonic stem cells (ESCs) is a lengthy process that sometimes produces cell lines that fail to incorporate into the germline. Identifying this limitation may take up to several months, while the cell line is established and appropriate . This presents a significant limitation, as time invested may not yield the desired results. Identifying these cells early in the process, in order to disregard them or employ them for other non-ESC use, could enhance the efficiency in which research is conducted. Furthermore, overcoming these differences may lead to cell lines that can be used in ESC studies. We have cultured 10 pairs of ESC lines from different mouse strains that either were germline transmitting cells or not. A slate of assays, including proliferation, teratoma formation, gene expression assays were performed to detect differences. Microarray analysis was utilized to assess cross strain differential expression, determine gene ontology categories and networks. A priority list of genes was validated using RTPCR. Two efforts were then made with the data and analysis. First, the determination of a diagnostic test that could discriminate in early stages of the ESC derivation process which cells are likely to be incorporating, and which ones would not. 23 lines of mouse ESCs were derived and analyzed using the screen. The system was able to predict all of the cells that could produce germline transmission (n=8), while it only failed to identify one non-incorporating cell (n=14). Secondly, non-incorporating ESCs were transduced with some of the differentially expressed genes, to assess if they could be reprogrammed to incorporate into the germline. Several genes were used, but at least two (ESRRB and ATRX) were able to reprogram the cells to incorporate and produce germline transmission. Taken together, our results show that there is a cross-strain set of differentially expressed genes between ESCs that incorporate into the germline and those that do not, that can be successfully utilized as a early screen and that the non-germline transmitting state can be reprogrammed by overexpressing some of these genes. The latter result could be a platform to study the differences in human primed (Epi Stem Cells) vs. ground state (naïve) cells, providing insights into the nature of these two cell types.

POSTER ABSTRACTS

P56

ADDRESSING THE PHYSIOLOGICAL CUES OF THE LIMBAL MICROENVIRONMENT INFLUENCES THE PROLIFERATION AND TRANSDIFFERENTIATION OF DENTAL PULP STEM CELLS INTO LIMBAL STEM CELLS

HENNESSY, Ciara Ellen[1], Shawcross, Sue[1], Kushnerev, Evgeny[1], Brahma, Arun[2], Carley, Fiona[2], Yates, Julian[3], Hillarby, Chantal[1]
[1]Manchester Medical School, University of Manchester, Manchester, United Kingdom, [2]Royal Manchester Eye Hospital, Manchester, United Kingdom, [3]Manchester Dental Hospital, Manchester, United Kingdom

Background: Limbal stem cells play a major role in providing a never-ending supply of corneal epithelial cells. When this is compromised alternative sources need to be found. Dental pulp stem cells DPSC) have been shown to differentiate into corneal epithelial cells; however, their life cycle in vitro is markedly reduced. In this study, we examined certain factors unique to the microenvironment of the limbus, which are believed imperative for the stem cycle life cycle, were investigated. The attraction of isolated DPSC to the corneoscleral junction (the site of the limbus) and the effects of conditioned medium on DPSC transdifferentiation were explored. Method: Using a QuasiVivo™ (Kirkstall Ltd, Yorkshire) flow circuit, we subjected DPSC to shear forces and diffusible factors released by corneoscleral rims within the circuit. And assessing its role in the proliferation rate and viability of these cells by carrying out viability assays and microscopy. GFP transfected DPSC were placed in the flow circuit and the corneal rims were analyzed with fluorescent microscopy and immunocytochemistry to further evaluate the differentiation of DPSC for Keratin 3. Results: Light microscopy revealed greater confluence and proliferation rates from those cells in the circuit as compared to the static wells by 32% after 48 hours. The fluorescent DPSC appeared to settle on the corneoscleral junction of the rims and expressed cytokeratin 3 on Immunocytochemistry. Conclusion: Addressing the physiological cues of the limbal microenvironment in vitro appears to promote greater proliferation and transdifferentiation of DPSC than DPSC in static wells.

P57

GENE EXPRESSION PROFILING OF LIF/JAK/STAT3-DEPENDENT HUMAN EMBRYONIC STEM CELLS

BOURILLOT, Pierre-Yves[1], Chen, Hongwei[1], Gonnot, Fabrice[1], Voisin, Sophie[1], Genthon, Clémence[2], Donadieu, Cécile[2], Acloque, Hervé[2], Savatier, Pierre[1]
[1]Stem-Cell and Brain Research Institute Inserm U846, Lyon, France, [2]UMR1388 Génétique, Physiologie et Systèmes d'Elevage, INRA, Toulouse, France

We generated human embryonic stem cell lines harboring genetic and epigenetic features of naïve pluripotency. In brief, we used ligand-dependent STAT3-ERT2 to activate STAT3 target genes, which resulted in the bypassing of FGF2 dependency and installing of LIF dependency. Reprogramming involved two steps: transition from F (FGF2) to TL (tamoxifen + LIF) and then from the TL to TL2i state (tamoxifen + 2i/LIF). The experimental procedure and the characteristic features of the resulting cells at both genetic and epigenetic levels are described in the accompanying poster ("Reinforcing STAT3 activity reprograms human embryonic stem cells to naïve pluripotency"). Here we explored the transcriptome of TL and TL2i cells at both multi- and single-cell levels using RT-qPCR and Affymetrix DNA arrays. We observed that (1) LIF and tamoxifen act synergistically to activate the transcription of STAT3 target genes and sustain self-renewal of TL cells; (2) TL2i cells show uniform expression of the three naïve markers ESRRB, PECAM1, and TDGF1 and heterogeneous expression of KLF4 and TFCP2L1. Next, we compared the transcriptome of F, TL, and TL2i cells with that of human morula and blastocysts, and well as with that of some recently published human naïve ES cells [NHSM cells: propagated with FGF2, TGFβ1, LIF, and inhibitors of MEK, GSK3β, JNK, and p38MAPK; 6i/L/A cells: propagated N2B27 basal medium with LIF and activin,

POSTER ABSTRACTS

and inhibitors of MEK, GSK3β, ROCK, BRAF, and SRC],. We observed that TL, NHSM and 6i/L/A cells exhibit the highest similarities in their global gene expression profiles. They cluster close to morula and blastocyst samples. We also observed that the TL2i cells have a unique gene expression profile, which was not observed in any other cell types analyzed. Notably, one cluster of genes upregulated in TL2i cells, human morula, and blastocysts versus all other categories was identified. Gene ontology analysis of this cluster pointed to 17 cellular functions, including (1) response to reactive oxygen species, (2) regulation of exit from mitosis, and (3) positive regulation of apoptotic process. These data strongly suggested that TL2i cells acquired some characteristic features of human preimplantation embryo that were not found in other naïve-like human ES cells.

P58

LONG-TERM CONDITIONAL DELETION OF PTC IN GLAST-EXPRESSING CELLS LEADS TO AN ACCUMULATION OF QUIESCENT NEURAL STEM CELLS IN THE ADULT RODENT BRAIN.

DAYNAC, Mathieu[1], Faure, Hélène[1], Traiffort, Elisabeth[2], Hahn, Heidi[3], Gauthier, Laurent[4], Mouthon, Marc-André[4], Boussin, François[4], Ruat, Martial[1]

[1]Neurobiology&Development, CNRS UPR-3294, Gif-Yvette, France, [2]Laboratory of Neurobiology, CNRS, Gif-Yvette, France, [3]Tumor Genetics Group, Institute of Human Genetics, Göttingen, Germany, [4]Laboratory of Radiopathology, CEA, Fontenay-aux-Roses, France

The Sonic Hedgehog (Shh) signaling pathway is active in the adult rodent subventricular zone (SVZ) of the lateral ventricle where it has been proposed to regulate the maintenance of neural stem and precursor cells. To explore the role of the Shh receptor Patched (Ptc) in adult SVZ neurogenesis, we used a tamoxifen-inducible Cre transgene under the control of the astrocyte-specific glutamate transporter (GLAST) expressed in astrocyte-like neural stem cells (NSCs) and took advantage of a conditional Ptc knockout mouse line. We recently proved with this model that Ptc inactivation in adult NSCs leads to a dramatic decrease of neurogenesis, and to a shift in NSCs division mode from asymmetric to symmetric. However, the role of Ptc deletion on the distinct NSCs (quiescent vs activated) and SVZ populations has not been explored. Using an immunofluorescence and FACS technique to distinguish all SVZ neurogenic populations, including quiescent vs activated NSCs, we dug out the wide role of Ptc deletion on the different steps of neurogenesis. Ptc deletion (6 months) in Glast expressing NSCs leads to a progressive and large accumulation of quiescent NSCs without increasing the pool of mature non neurogenic astrocytes. Unexpectedly, we found that the neurosphere forming potential as well as neurosphere size in the presence of active Shh (10 nM) was increased specifically for activated NSCs from WT animals (by almost 2 fold) and not altered for transit amplifying cells nor immature neuroblasts. Experiments are underway to further analyze the distinct role of Shh signaling in quiescent and activated NSCs as well as in the other SVZ populations.

P59

THE DOSAGE OF PATZ1 MODULATES REPROGRAMMING PROCESS

WU, Qiang

Biochemistry, National University of Singapore, Singapore

The acquisition of pluripotent cells can be achieved by combined overexpression of transcription factors Oct4, Klf4, Sox2 and c-Myc in somatic cells. This cellular reprogramming process overcomes various barriers to re-activate pluripotency genes and re-acquire the highly dynamic pluripotent chromatin status. Recent studies have revealed that many genetic and epigenetic factors are essentially involved in the reprogramming process. We recently reported that Patz1 (POZ/AT hook containing zinc finger 1) is required for ES cell identity. Surprisingly, we found that Patz1 plays an inhibitory role in OKSM-induced reprogramming process. We found that there were more iPS colonies inducted from Patz1+/- MEFs than wild type MEFs; while the addition of Patz1 significantly repressed reprogramming efficiency. Patz1-/- MEFs gave the lowest reprogramming efficiency which may result

POSTER ABSTRACTS

from cell senescence trigged by up-regulated *Ink4a/Arf* locus. However, Patz1+/- MEFs appeared to surpass the senescence barrier of *Ikn4a/Arf* locus, thus enhancing iPS colonies formation. Interestingly, microarray analysis revealed altered expressions of many epigenetic factors in Patz1+/- MEFs. ChIP assay showed that Patz1 could bind to and potentially regulate many epigenetic regulators. Moreover, Patz1+/- MEFs displayed higher levels of acetylated histone H3, H3K4me3, H3K36me3 and lower levels of histone H3K9me3 and H3K27me3, indicating that heterozygous knockout of Patz1 results in a globally open chromatin which is more accessible for transcriptional activation. Together, we have found that Patz1 can interplay with epigenetic factors and modulate reprogramming process.

P60

GENERATION AND CHARACTERIZATION OF NON-INTEGRATING HUMAN INDUCED PLURIPOTENT STEM CELLS FROM TRISOMY 21 HUMAN FETAL FIBROBLASTS

KITIYANANT, Narisorn[1], Phanthong, Phetcharat[2], Promthep, Kornkanok[1], Hongeng, Suradej[3], Kitiyanant, Yindee[2]
[1]*Stem Cell Research Group, Institute of Molecular Biosciences, Mahidol University, Nakhon Pathom, Thailand,* [2]*Department of Anatomy, Faculty of Science, Mahidol University, Bangkok, Thailand,* [3]*Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand*

Trisomy 21 is a common chromosome abnormality found in humans and is associated with hematopoietic abnormalities in the fetal liver and cognitive impairment which the roles of extra chromosome 21 materials are unclear. Induced pluripotent stem (iPS) cells hold a great potential for many applications such as drug discovery, disease modeling, and as a cell source in regenerative medicine. Therefore, trisomy 21-iPS cells might be a valuable tool for understanding the syndrome molecular mechanisms. In this study, we reprogrammed Trisomy 21 fibroblasts from aborted fetus by introducing episomal human reprogramming factors with electroporation. The cells were established in feeder-free and serum free condition. After electroporation, the iPS-liked colonies were manually picked, individually expanded, and characterized for their pluripotency properties. Our results showed that generated Trisomy 21-iPS cells expressed pluripotency markers. Moreover, these cells still had an extra chromosome 21 as demonstrated by FISH. Trisomy 21-iPS cells would be a disease model and could be further used for studying molecular mechanism underlying neurogenesis in Down syndrome.

P61

EFFICIENT IN VITRO DIFFERENTIATION OF RAT AMNIOTIC FLUID STEM CELLS INTO HEPATOCYTES-LIKE CELLS VIA DEFINITIVE ENDODERM

YEO, Pei Ling, Ramasamy, Thamil Selvee
Department of Molecular Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Adult hepatocytes is an imperative cellular resource in developing cell based hepatotoxicity assay for pharmaceutical application and disease modeling. However, the scarcity in primary hepatocytes availability and the inability to maintain the functionality of these cells in vitro demand an alternative cell sources such as stem cell derived-hepatocytes. As such, C-kit positive (C-kit+) amniotic fluid stem (AFS) cells represent a promising tool in which these cells are consider to be broadly multipotent stem cells with intermediate characteristics between embryonic and adult stem cells. These cells are easier to maintained and manipulated in culture compare to embryonic stem cells. Hence, this study aims to develop an efficient two step hepatic differentiation protocol from C-kit+ rat AFS cells through definitive endoderm (DE). C-kit+ rat AFS cells were treated with Activin-A and Sodium Butyrate for DE specification and followed by their differentiation into hepatoblasts and hepatocytes-like cells using cocktail of cytokines: Hepatocytes Growth Factor and Oncostatin M in commercial available

POSTER ABSTRACTS

Hepatocytes Culture Media (HCM). Preliminary data of this two-step hepatic differentiation protocol showed sequential morphological changes of AFS from elongated morphology to a more triangular morphology suggesting the formation of DE. During the later stages of differentiation, cluster of round densely packed cells together with more cuboidal morphology resembled hepatocytes-like cells were observed. Efficiency of hepatic differentiation protocol will be assess by gene expression analysis using PCR, Immunocytochemistry and Western Bloting techniques. The hepatocytes-like cells generate from rat AFS cells will be potential sources for in vitro cell-based assay replacing the use of animal model in hepatotoxicity study and disease modelling.

P62

GENETIC CORRECTION OF ABERRANT SPLICING OF BETA THALASSEMIC ERYTHROID CELLS WITH IVS-2 654/BETA^{E} DERIVED FROM PATIENT-SPECIFIC IPS EXPRESSING ANTISENSE U7 SNRNA

BORWORNPINYO, Suparerk[1], Phanthong, Phetcharat[2], Kitiyanant, Narisorn[3], Jearawiriyapaisarn, Natee[4], Nuntakarn, Lalana[5], Kitiyanant, Yindee[2], Hongeng, Suradej[6]
[1]Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok, Thailand, [2]Department of Anatomy, Faculty of Science, Mahidol University, Bangkok, Thailand, [3]Stem Cell Research Group, Institute of Molecular Biosciences, Mahidol University, Nakhon Pathom, Thailand, [4]Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Bangkok, Thailand, [5]Department of Obstetrics and Gynecology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, [6]Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Induced pluripotent stem (iPS) cells are promising tools in medical research exclusively with human disease modeling, drug screening, gene and cell replacement therapy. One such genetic disorder is β-thalassemia, one of the most common genetic diseases among the people living in Southeast Asia. Here, we report successful generation of iPS cells derived from a β-thalassemic patient carrying IVS-2 654/β^{E} mutations fully characterized by pluripotent marker expressions and teratoma formation. The C→T substitution at position IVS-2 nt 654 causes to alternative aberrant spliced β-globin mRNA, consequently leading to the reduction in the production of β-globin chain. In this present study, we successfully created the iPS cells to express antisense U7 snRNA targeting the aberrant splice site of the thalassemic pre-mRNA. Subsequently, the established thalassemic iPS cells were found to differentiate to erythroid cells and stably express the antisense snRNA. To demonstrate the restoration of correct splicing pattern, the β-globin gene expression and hemoglobin synthesis will be further analyzed. Once proven, various mutations with abnormal splicing β-globin pre-transcripts could be corrected in thalassemic iPS cells and differentiated to hematopoietic stem cells (HSCs) before transplantation to the patients using this gene therapy approach.

P63

IDENTIFICATION OF EPIGENETIC MODULATORS GOVERNING EMBRYONIC STEM CELLS MAINTENANCE AND DIFFERENTIATION BY INSERTIONAL MUTAGENESIS APPROACH

LENKA, Nibedita[1], Krishnan, Shruthi[1], Sinha, Sangeeta[1], Rangasamy, Danny[2]
[1]National Centre for Cell Science, Pune, India, [2]John Curtin School of Medical Research, Australian National University, Canberra, Australia

The selective activation and repression of many transcriptional and epigenetic factors or networks of regulatory genes is associated with the precision and complexities with which the hierarchy in developmental events commences and progresses. Our current understanding of the epigenetic factors that regulate self-renewal and differentiation of embryonic stem cells (ESCs) is

POSTER ABSTRACTS

far from being complete. Since loss of function studies by gene disruption reckons the function of specific genes and their downstream relevance, we have followed an insertional mutagenesis approach using Line1 retrotransposon-mediated loss-of-function genetic screening in murine ESCs and tried to identify and elucidate the chromatin and epigenetic regulatory complexes that underlie commitment and specification of undifferentiated ESCs to specific lineages. The mapping of the trapped genes has yielded a couple of known and hither-to unknown candidates. Amongst the identified genes with no known functions ascribed during ESCs maintenance and differentiation, Tollip is known to act in the immune response to invading pathogens by controlling IRAK phosphorylation in the TLR and IL-1R signaling pathways, while USP, a family of proteins that are known to function as a histone ubiquitin hydrolase. The functional relevance of these genes was studied by knocking down the same in ESCs. While Tollip knock down led to impairment in differentiation of ESCs indicating its crucial role during differentiation, USP knockdown did not yield any difference with respect to ESCs morphology and pluripotent gene signature when maintained under both maintenance and spontaneous differentiation conditions when compared to control. The USP knock down cells did generate teratoma upon injection into the SCID mice reflecting to their pluripotent characteristic in vivo. Interestingly however, the in vitro differentiation into cardiomyocytes was promoted in these clones thereby suggesting negative modulatory effect of USP on cardiomyogenesis with no appreciable difference with respect to neural differentiation. Collectively, our investigation has unleashed a crucial and context dependent influence of epigenetic factors during cell fate specification in ESCs and paved way for further investigation on their mechanistic basis underlying the same.

P64

A CONTACT LENS BASED TECHNIQUE FOR HDPSC CULTURING AND A POTENTIAL NOVEL CARRIER FOR TRANSPLANTATION ONTO THE HUMAN CORNEA

SOTHIRACHAGAN, Shankari[1], Kushnerev, Evgeny[1], Shawcross, Sue[1], Brahma, Arun[2], Carley, Fiona[2], Yates, Julian[3], Hillarby, Chantal[1]
[1]University of Manchester, Manchester, United Kingdom, [2]The Royal Manchester Eye Hospital, Manchester, United Kingdom, [3]Manchester Dental Hospital, Manchester, United Kingdom

Background: Corneal epithelial homeostasis relies on a healthy limbus. Patients with limbal stem cell deficiency (LSCD) require stem cell transplantation for ocular surface reconstruction. Current treatments include limbal stem cell transplantation with amniotic membrane, which involves a surgical procedure, and autologous epithelial progenitor transplantation requires the existence of a viable limbus. In patients with bilateral LSCD, an alternative source of stem cells is required. Studies thus far have demonstrated successful of human dental pulp stem cells (hDPSC) application in animal models with LSCD. Aims: The aim was to demonstrate adherence and proliferation of hDPSCs on contact lenses and migration of cells from the lens surface to a human cornea with denuded epithelium. Methods: Extended wear contact lenses (Purevision and Purevision 2; Bausch and Lomb) and daily wear lenses (Focus dailies, Alcon) were incubated in fibronectin and fetal bovine serum. Green Fluorescent Protein (GFP)-labelled and non-GFP labelled hDPSCs were seeded on these lenses supported in growth medium. CellTiter 96® AQueous One Solution Cell viability assays were conducted on each non-GFP seeded lens. Lenses seeded with GFP-labelled hDPSCs were positioned on denuded human corneas obtained from the Manchester Hospital eye bank and observed for migration onto corneal surfaces. Results: No cells survived on the daily wear lenses. However, extended wear lenses incubated in fibronectin and

POSTER ABSTRACTS

fetal bovine serum supported cellular adherence and proliferation. Conclusion: This study provides evidence that hDPSCs can survive and proliferate on extended wear contact lenses. In terms of clinical use, this approach has the potential to provide both an alternative source of stem cells for bilateral LSCD treatment and a non-surgical robust physical carrier for their transplantation.

P65

SOX17-MEDIATED CONVERSION OF MOUSE EMBRYONIC STEM CELLS (ESCS) INTO FUNCTIONAL EXTRAEMBRYONIC ENDODERM STEM (XEN) CELLS IDENTIFIES DYNAMIC NETWORKS CONTROLLING CELL FATE DECISIONS

MCDONALD, Angela C.H.[1], Biechele, Steffen[2], Rossant, Janet[1], Stanford, William L.[3]
[1]The Hospital for Sick Children Research Institute, Toronto, ON, Canada, [2]Institute for Regenerative Medicine, University of California at San Francisco, San Francisco, CA, Canada, [3]Sprott Centre for Stem Cell Research, Ottawa Hospital Research Institute, Ottawa, ON, Canada

The extraembryonic endoderm (ExEn) of the mammalian conceptus is important for patterning of the embryo proper, gives rise to support tissues such as the primary yolk sac, and can be maintained in vitro as self-renewing XEN cells. Little is known about the regulatory networks distinguishing XEN cell lines from the extensively characterized ESC. An intriguing regulatory network candidate is the transcription factor Sox17, which is essential for XEN cell derivation and self-renewal. Previous research has shown that forced expression of Sox17 in ESCs can induce ExEn gene expression. However, the ability of Sox17 to convert ESCs to functional XEN cells has not been explored. To address this, we overexpressed Sox17 in ESCs using a doxycycline-inducible system (Sox17-ESCs), and generated cells with cell morphology indistinguishable from embryo-derived XEN cells. Sox17-ESCs rapidly induced ExEn gene expression and subsequently repressed pluripotency genes. In contrast, fluorescent activated cell sorting reveals a stepwise loss of pluripotency cell surface proteins and a subsequent induction of XEN cell surface proteins. Single-cell Sox17-mediated conversion is highly efficient with over 90% of cells converting by day 30. Stable Sox17-XEN cells can be maintained independent of transgene expression for greater than 30 passages. In vivo, Sox17-XEN cells integrate and proliferate in the parietal endoderm of E8.5 mouse embryos. To identify dynamic regulatory networks driving Sox17-mediated XEN conversion, we performed times series RNA-sequencing and used these data to create a dynamic regulatory map of gene expression bifurcation points throughout conversion. We overlaid transcription factor binding data on our dynamic regulatory map, and identified three classes of novel putative ExEn cell fate regulators including ExEn cell fate repressors, ExEn cell fate activators and transcription factors active in both ESCs and XEN cells, acting to either repress or activate ExEn genes, respectively. By perturbing the expression of putative ExEn regulators, we identified a role for Nr5a2 in repressing ExEn differentiation in ESCs. Taken together, our findings show that Sox17-mediated XEN conversion is a robust system that can be used to identify novel regulatory network modules that regulate cell fate decisions.

P67

ANALYSIS OF THE SPLICING-REGULATORY NETWORK IN HUMAN EMBRYONIC STEM CELLS

GOEKE, Jonathan, Lu, Xinyi, Ng, Huck-Hui
Genome Institute of Singapore, Singapore

Human embryonic stem cells (hESCs) harbour the ability to undergo lineage-specific differentiation into clinically relevant cell types. Transcription factors and epigenetic modifiers are known to play important roles in the maintenance of pluripotency of hESCs. However, little is known about regulation of pluripotency through splicing. In this study, we analyse genome-wide RNA profiling after depletion of the RNA binding protein SON. We find that depletion of SON induces

POSTER ABSTRACTS

intron retention and alternative splicing. We confirm that SON regulates the proper splicing of transcripts encoding for pluripotency regulators such as OCT4, E4F1, MED24 and PRDM14. For PRDM14 we further show that loss of SON induces an alternative isoform that appears to be non-functional in the context of pluripotency. Finally we compare the SON regulated splicing network in hESCs to the splicing network of early human embryos.

P68

SELECTIVE INHIBITION OF TCF-DEPENDENT BETA-CATENIN TARGET GENE EXPRESSION IMPROVES SELF-RENEWAL AND INHIBITS DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS

Chatterjee, Sujash S.[1], **SAJ, Abil**[2], Gocha, Tenzin[1], Gonsalves, Foster C.[1], Madar, Aviv[1], Dasgupta, Ramanuj[2]
[1]New York University Langone Medical Center, New York, NY, USA, [2]Genome Institute of Singapore, Singapore

Regulation of the core Pluripotency Transcriptional Network (PTN) comprising Nanog,Oct4 and Sox2 is critical in the maintenance of mouse embryonic stem cell (mESC)self-renewal. While the canonical Wnt/β-catenin pathway has been implicated inmodulating PTN function, the specific molecular mechanisms remain controversial. Afocused RNAi screen to assess the effect of knocking down individual Wnt-pathwaycomponents on the expression of Nanog-GFP revealed a surprising inverse correlation between mESC pluripotency and β-catenin/TCF4- driven target gene activation.Surprisingly, β-catenin/TCF4-dependent transcription coincided with the activation ofdifferentiation and loss of self-renewal. Meanwhile, abrogation of β-catenin/TCF4interaction by a selective small molecule inhibitor (iCRT3) blocked mESC differentiationand significantly improved self-renewal characteristics in both long-term cultures anddifferentiation-promoting conditions. Notably, biochemical investigation of themolecular mechanisms revealed that iCRT3-induced release of β-catenin from theactivating TCF complexes resulted in its enhanced association with Oct4 at the E-cadherin-mediated adherens junctions (AJs). Taken together, these results suggest aTCF-independent function of β-catenin in promoting mESC pluripotency by modulatingthe sub-cellular localization of Oct4 in order to limit the expression of Oct4-drivenlineage priming factors.

P69

ABLATION OF CDK1 IN PLURIPOTENT CELLS INDUCES RAPID LETHALITY: A POTENTIAL THERAPEUTIC APPROACH FOR TARGETING CANCER STEM CELLS

SYMONDS, Catherine E., Santamaria, David, Barbacid, Mariano
Molecular Oncology, CNIO - Spanish National Cancer Research Center, Madrid, Spain

Cyclin-dependent kinase 1 (Cdk1) plays an essential role in the progression of eukaryotic cells through the mitotic cell cycle by coordinating the phosphorylation of multiple substrates. To further study the role of Cdk1 during the cell cycle, we have generated germ-line and conditional *Cdk1* knock out strains of mice. As expected, mice lacking *Cdk1* are early embryonic lethal. Likewise, ablation of the conditional *Cdk1* alleles in adult animals leads to rapid tissue atrophy and lethality. We have used these conditional alleles to interrogate whether Cdk1 plays differential roles in stem vs. lineage-committed cell cycles. To this end we isolated embryonic (ES) and hematopoietic stem cells (HSCs), embryonic fibroblasts (MEFs) and keratinocytes (KTs), and generated induced pluripotent stem cells (iPS). We observed that ablation of *Cdk1* in differentiated cell types (MEFs and KTs) induced a permanent arrest at the G2-M transition (with a 4N DNA content) followed by several cycles of endoreplication up to a 32N DNA content. However, when ES, HSCs, or iPS cells were depleted of Cdk1, we observed a transient arrest at the G2-M transition, but then cells entered apoptosis instead of undergoing endoreplication. This phenotype was strictly dependent on the

POSTER ABSTRACTS

undifferentiated state of the cells, since when ES and iPS cells were grown under culture conditions permissive to differentiation, they also became permanently arrested at G2-M and underwent endoreplication. Elimination of *Cdk1* in ES cells as well as early embryos resulted in a significant amount of DNA damage, which is likely to be the cause of the observed apoptosis and early embryonic lethality. We are also using the conditional *Cdk1* strain to interrogate the possible role of Cdk1 inhibition on the proliferation of cancer stem cells (CSCs). To this end, we inserted the *Cdk1* alleles within the Tg.*MMTV-PyMT* mammary mouse tumor model and generated primary cell lines. We are now analyzing the therapeutic potential of targeting *Cdk1* in various tumor cell populations, including putative CSCs, using a panel of selective markers including Lin, CD24, CD29, CD44, CD49f and Sca-1. These studies may unveil a novel and therapeutically relevant synthetic lethality between the stemness state of tumor cells and Cdk1 inhibition.

P70

COMPARISON OF GENE EXPRESSION OF MESENCHYMAL STROMAL CELLS FROM ONTOGENICALLY DIFFERENT SOURCES SHOWS GOOD CORRELATION WITH THEIR FUNCTIONAL DIFFERENCES

VAZ, Candida, Tan Bee Tee, Betty, Ann Yong Cheen May, Delicia, Yi Lee, Qian, Tanavde, Vivek
*BII A*STAR, Singapore, Singapore*

Mesenchymal stromal cells (MSCs) are multipotent cells, known to differentiate into a variety of cell types mainly adipocytes, chondrocytes and osteocytes. MSCs can be obtained from several sources and the significant differences in their properties, makes it crucial to determine their optimal source. In this study, we compared the gene expression of MSCs derived from three ontogenically different sources: Embryonic stem cells (hES-MSCs), Fetal limb (Flb-MSCs) and Bone Marrow (BM-MSCs). Each microarray dataset comprised of an undifferentiated control and three differentiated cell types : Adipocytes, Chondrocytes and Osteocytes, taken at an early stage (Day7) and a terminal stage (Day14/21). The differentially expressed genes between the differentiated cells and undifferentiated controls were compared across the three MSC sources. We found that the differentially expressed genes were highly dissimilar with very less extent of overlap (2-4%). Pathway and functional analysis of the differentially expressed genes revealed a large variation in the top functions and canonical pathways. The similar canonical pathways among the three sources were lineage specific. The similar pathways for adipocytes, chondrocytes and osteocytes were: 'LXR/RXR Activation' and 'Acute Phase Response Signaling'; 'Inhibition of Matrix Metalloproteases'; 'Agranulocyte and Granulocyte Adhesion and Diapedesis' respectively. Analysis of the enriched biological functions showed that the top common functions of all the three sources were 'Cellular Growth and Proliferation' and 'Cellular Development'. However, certain functions such as 'DNA Replication, Recombination and Repair'; 'Cardiovascular System Development'; 'Skeletal System Development' were highly enriched in hES-MSCs, Flb-MSCs and BM-MSCs respectively. These source specific functions correlates well with the nature of the source and proves that MSCs from the three ontogenically different sources utilize different biological functions to obtain similar differentiation products. A deeper understanding of various MSC sources will enable the application of MSCs in tissue specific disorders. Thus our study advocates the utilisation of biological pathways and networks to ascertain optimal sources of MSCs for specific clinical applications.

P71

DISSECTING THE ROLE OF THE CELL CYCLE IN REPROGRAMMING THROUGH NUCLEAR TRANSFER

CHIA, Gloryn, Egli, Dieter
Columbia University, New York, NY, USA

Reprogramming, either through ectopic expression of a defined set of transcription factors or through the transfer of a somatic nucleus into unfertilized

POSTER ABSTRACTS

oocytes, enables the conversion of one cell type to another. Most work has focused on understanding the role of transcription factors in reprogramming of cell- type specific gene expression. Addressing the role of the cell cycle in reprogramming has proven more challenging. In many cases, it is impossible to separate the two. Cell cycle progression is often required for cell survival; prolonged experimental arrest of the cell cycle is often lethal. The literature provides different and sometimes conflicting accounts of the role of the cell cycle in reprogramming. While we have previously reported that the transition through mitosis facilitates transcriptional reprogramming, others have demonstrated somatic cell reprogramming without progression through the ell cycle. An advantage of somatic cell nuclear transfer is that reprogramming occurs within a short time frame, as short as a single cell cycle. This allowed us to carefully dissect of the role of different cell cycle phases in reprogramming.

P72

UNCOVERING REGULATORY PATHWAYS FOR THE EXIT FROM PLURIPOTENCY

GONZALES, Kevin Andrew, Liang, Hongqing, Lim, Yee Siang, Ng, Huck Hui
Genome Institute of Singapore, Singapore

The potential of human embryonic stem cells (hESCs) lies on their ability to differentiate into any specific somatic lineage, making it imperative to understand the genetic network governing the exit from pluripotency. Here, we report the first high-throughput RNA interference (RNAi) screen for factors crucial for the exit from pluripotency of hESCs in multiple differentiation conditions. Our study systematically identified a multitude of novel regulators of the exit from pluripotency, and notably found specific chromatin modifying complexes to be centrally important for the exit from pluripotency.

P73

ANTIOXIDANTS CAUSE RAPID EXPANSION OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS VIA CDK AND CDK INHIBITOR REGULATION

SUN, Li-Yi[1], Pang, Chen-Yoong[1], Li, Dian-Kun[2], Chang, Yao-Jen[3], Cheng, Ching-Feng[1]
[1]Department of Medical Research, Buddhist Tzu Chi General Hospital, Hualien, Taiwan, [2]Department of Hematology and Oncology, Taichung Tzu Chi General Hospital, Taichung, Taiwan, [3]Department of Surgery, Taipei Tzu Chi General Hospital, Taipei, Taiwan

Background: Antioxidants have been shown to enhance proliferation of adipose-derived mesenchymal stem cells (ADMSCs) in vitro, but the detailed mechanism(s) and potential side effects have not been fully understood. Methods: In this study, human ADMSCs cultured in ImF-A medium supplemented with antioxidants (N-acetyl-L-cysteine and ascorbic acid-2-phosphate) and fibroblast growth factor 2 (FGF-2) were compared with ADMSCs cultured with FGF-2 alone (ImF) or with FGF-2 under 5% pO2 conditions (ImF-H). Results: Exposure to ImF-A resulted in a higher percentage of ADMSCs in S phase of the cell cycle and a smaller percentage of cells in G0/G1 during log-phase growth. This resulted in a significantly decreased cell doubling time and increased number of cells in the antioxidant-supplemented cultures compared to FGF-2 alone, with ~225% higher cell density after 7 days. Western blotting showed that levels of the CDK inhibitors p21 and p27 and CDK4 inhibitor B (p15) decreased after ImF-A treatment, but CDK2, CDK4, and CDC2 levels clearly increased. In addition, ImF-A led to significant reductions in the expression of CD29, CD90, and CD105, but relative telomere length, osteogenesis, adipogenesis, and chondrogenesis were enhanced. However, results were similar between ADMSCs treated with antioxidants and those under hypoxic conditions. Conclusion: Treatment with antioxidants promotes ADMSC entry into S phase by suppressing cyclin-dependent kinase inhibitors, resulting in rapid cell proliferation similar to that observed under hypoxic conditions.

POSTER ABSTRACTS

P74

MESENCHYMAL STROMAL CELLS IMPROVE CARDIAC FUNCTION AND LEFT VENTRICULAR REMODELING IN A HEART TRANSPLANTATION MODEL

MONTANARI, Sonia[1], Dayan, Victor[1], Yannarelli, Gustavo[1], Billia, Filio[2], Viswanathan, Sowmya[1], Connelly, Kim A.[3], Keating, Armand[1]
[1]Cell Therapy Program, Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada, [2]MaRS Centre Toronto Medical Discovery Tower 3rd Floor 3-706, Toronto, ON, Canada, [3]Keenan Research Centre, St. Michael's Hospital, Toronto, ON, Canada

Introduction: Ischemia/reperfusion (I/R) injury is an inevitable consequence of organ transplantation and a major determinant of patient and graft survival in heart transplantation. Bone marrow-mesenchymal stromal cells (BM-MSCs) are a potentially effective cell therapy for cardiac disease. We investigated the effects of intravenous delivery of BM-MSCs in the acute phase post-transplant on cardiac performance, degree of tissue fibrosis, left ventricular reverse remodeling, neovascularization density and frequency of apoptotic cells in a heterotopic heart transplantation (HHT) model associated with (I/R) injury. Methods: BM-MSCs were isolated from wild-type (WT) Lewis (LEW) rats and cultured to early passage. Hearts of WT LEW rats were harvested and transplanted heterotopically into the necks of recipient WT LEW rats, using the Cuff technique. 48 hours after HHT, 3x106 BM-MSCs/ml were injected intravenously into animals in the experimental group while controls received an equivalent volume of saline. Echocardiographic evaluation was assessed at baseline, at 8 and 28 days after MSC injection. Recipient heart function was also evaluated. Histochemical analysis of host and transplanted hearts was performed 28 days after BM-MSC infusion. Results: 8 days after BM-MSC injection, fractional shortening of transplanted hearts was significantly higher and left ventricular systolic diameter was lower in the BM-MSC group versus controls. No differences were found between groups 28 days after infusion. Cardiac fibrosis was lower in the BM-MSC group. A reduction in ventricular remodeling was observed by histochemical analysis and confirmed by cardiac Magnetic Resonance Imaging. The perivascular stromal cells density and the number of capillaries were increased while apoptotic cells significantly decreased in transplanted hearts receiving BM-MSCs compared to the control group. Conclusion: We showed early improvement in cardiac function and subsequent enhanced ventricular remodeling, reduced cardiac fibrosis, augmented neovascularization and decreased cardiomyocyte apoptosis of the transplanted heart in a HHT model after intravenous infusion of BM-MSCs. Our data suggest that clinical studies with BM-MSCs are warranted to investigate effects on cardiac graft and transplant recipient survival.

P75

A NEW SOFT AGAR COLONY FORMATION ASSAY BASED ON HIGH-CONTENT IMAGING FOR SENSITIVE DETECTION OF TUMORIGENIC CELLULAR IMPURITIES IN HUMAN CELL-PROCESSED THERAPEUTIC PRODUCTS

KUSAKAWA, Shinji[1], Yasuda, Satoshi[2], Kuroda, Takuya[2], Kawamata, Shin[1], Sato, Yoji[2]
[1]FBRI, Kobe, Japan, [2]National Institute of Health Science, Tokyo, Japan

Contamination with tumorigenic cells is one of the most concerns for human cell-processed therapeutic products (hCTPs). The soft agar colony formation assay is a well-known in vitro assay for detection of malignant transformed cells showing anchorage-independent cell growth. However, when the soft agar colony formation assay is applied to the process control in manufacturing of hCTPs, the sensitivity of the assay for transformed cells needs to meet the criteria of quality assessment of hCTPs. In the present study, we modified the conventional protocol of soft agar colony formation assay and established a new assay system with high sensitivity and precision for the detection of transformed cells as tumorigenic impurities in hCTPs. If we equally divide a cell preparation into wells of cell culture plates, so

POSTER ABSTRACTS

that each well contains 0 or 1 transformed cell, the wells are supposed to be "positive" or "negative" for the colony derived from a single transformed cell. Theoretically, based on the number of "positive" wells, this assay system can exactly determine the number of transformed cells in the cell preparation. To test our idea, primary human mesenchymal stem cells (hMSCs) containing a specific number of HeLa cells were seeded into 96-well plates with soft agar culture medium. After staining of the colonies and dissolving of the soft agar media, we counted the number of the colonies using an automated high-content imaging system. The errors by oversight were found to be decreased in our assay system, compared with the conventional method. The new assay system detected a colony generated from a single HeLa cell spiked into 1.3 x 10^4 hMSCs in a well of the 96-well plate. When a cell suspension containing one HeLa cell spiked into 1.0 x 10^6 hMSCs was aliquoted into 80 wells and cultured in the soft agar media, our assay system detected only one "positive" well, indicating its ability to detect as low as 0.0001% HeLa cells in hMSCs. These results suggest that our new assay system is highly sensitive, precise, and quantitative to detect a trace amount of tumorigenic cellular impurities in a cell preparation, which can be useful in the manufacturing and quality assessment of hCTPs.

P76

IDENTIFICATION OF A NATIVE EPIBLAST-LIKE HUMAN PLURIPOTENT STEM CELL STATE

CHAN, Yun Shen Winston, Ng, Huck Hui
Genome Institute of Singapore, Singapore

Embryonic stem cells (ESCs) are prized for their potential in regenerative medicine and as a primary model for understanding cell fate and development. These cells are derived from early pre-implantation embryos and are often referred to as the in-vitro counterpart of the inner cell mass (ICM). However, multiple studies have suggested that significant differences exist between the ***in vivo*** and ***in vitro*** pluripotent cell state. Moreover, an intermediate progenitor cell state could be isolated when human ESC is derived from the blastocyst, further supporting a transition of cell fate during the derivation process. These differences potentially arise from the switch of cell fate program where the native pluripotent stem cells are directed to self-renew continuously in culture whereas the ICM progresses to form the embryo. These differences will likely pose challenges for the use of hESC in modeling ***in vivo*** developmental processes. Here in, we describe a novel culture condition that allows the induction of a human ESC state that more closely resemble pluripotent cells of the native blastocyst.

P77

SME-MIR-124 C IS ESSENTIAL FOR CEPHALIC GANGLION REGENERATION IN PLANARIAN SCHMIDTEA MEDITERRANEA

SASIDHARAN, Vidyanand, Marepally, Srujan, Vemula, Praveen, Palakodeti, Dasaradhi
inStem, Bangalore, India

In recent years, the planarian Schmedtea mediterranea has emerged as a tractable model system to study stem cell biology and regeneration. Planarians are bilaterally symmetrical fresh water animals capable of regenerating the whole body or lost tissues and organs. They have specialized cells called neoblasts, which are functionally equivalent to embryonic stem cells. MicroRNAs are small RNA species that control gene expression by modulating translation repression, mRNA stability and have been implicated in the regulation of various cellular processes. Though recent studies have identified several miRNAs in Schmedtea mediterranea, their expression in neoblast sub population and during regeneration has not been examined. we also identified several planarian miRNAs whose expression is enriched in different neoblast subpopulations and in regenerating tissues at different time points during regeneration. Some of these miRNAs where enriched within 3 hrs post amputation and might therefore play a role in wound healing and/ or neoblast migration. Our results also revealed miRNAs, such as sme-mir-2d-3p and sme mir 124 family, whose expression is enriched in the cephalic ganglia, are also expressed in the brain primodia during CNS regeneration. This study aims

POSTER ABSTRACTS

in understanding the functional activities of miRNAs. We were able to knockdown sme-miR 124c-3p using LNA anti-miRs which is highly expressed in cephalic ganglia. Previous studies on miR 124 inhibition showed many differentiation defects, but here we are interested to study the brain regeneration upon sme-miR 124c-3p knockdown. Knockdown of miR 124C using LNA anti-miRs showed varied phenotypes like under developed or no development of photo receptor, abrupt neuronal connections between cephalic ganglia and ventral nerve cord and lesions. These phenotypes explain the extensive requirements of miR 124C-3p in the brain regeneration. Future studies aims in the identification of potential targets of miR 124C-3p. Thus this study will help us to elucidate the potential roles of microRNAs as a key player in post transcriptional modification during the course of head regeneration.

P78

STEM CELL-MEDIATED EXON SKIPPING OF THE DYSTROPHIN GENE BY THE BYSTANDER EFFECT

TORRENTE, Yvan[1], Meregalli, Mirella Angela[1], Beley, Cyriaque[2], Farini, Andrea[3], Sitzia, Clementina[3], Razini, Paola[3], Cassinelli, Letizia[4], Colleoni, Federica[3], Tavelli, Alessandro[3], Belicchi, Marzia Laura[1], Garcia, Luis[2]

[1]Department of Pathophysiology and Transplantation, Università degli Studi di Milano-Fondazione IRCCS Ca' Granda OMP, Centro Dino Ferrari, Ystem Srl, Milan, Italy, [2]UFR des sciences de la santé Simone Veil, Université Versailles Saint-Quentin, Montigny-le-Bretonneux, France, [3]Department of Pathophysiology and Transplantation, Università degli Studi di Milano-Fondazione IRCCS Ca' Granda OMP, Centro Dino Ferrari, Milan, Italy, [4]Ystem Srl, Milan, Italy

An attractive, therapeutic strategy for Duchenne muscular dystrophy (DMD) is to induce exon skipping in affected muscle cells, in order to restore the open reading frame of the dystrophin gene. Such an approach has been made in preclinical and clinical studies, using antisense oligonucleotides (AONs), however many hurdles remain due to the narrow therapeutic range of AONs. AON delivery is hampered by its short period of transient correction in dystrophin expression, and the chronically high dose it requires to achieve a sustained benefit in DMD patients. We used engineered stem cells as vectors for a permanent and efficient delivery of AONs. We engineered human DMD stem cells with a lentivirus, which permanently expresses the cloned AONs, and transplanted them into scid/mdx mice, the animal model of DMD. These engineered stem cells were fused with myoblasts and skeletal muscles of dystrophic scid/mdx mice, rescuing their dystrophin expression. We assessed that functional AONs were released in exosomes by these engineered, human DMD stem cells. These results demonstrated a bystander effect, mediated by the use of engineered stem cells for the delivery of AONs, and provided a possible new treatment method for DMD.

P79

AUTOLOGOUS SKIN DERIVED STEM CELLS AND PLATELET RICH PLASMA AS TREATMENT FOR TRAUMATIC SPINAL CORD INJURIES

TORRENTE, Yvan[1], Grimoldi, Nadia[2], Belicchi, Marzia Laura[1], Erratico, Silvia Alice[3], Pluderi, Mauro[2], Giordano, Rosaria[4], Tiberio, Francesca[2], Marconi, Maurizio[4], Rampini, Paolo[2], Bresolin, Nereo[5]

[1]Department of Pathophysiology and Transplantation, Università degli Studi di Milano-Fondazione IRCCS Ca' Granda OMP, Centro Dino Ferrari, Ystem Srl, Milan, Italy, [2]Neurosurgery, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Università degli Studi di Milano, Milan, Italy, [3]Ystem srl, Milan, Italy, [4]Center of Transfusion Medicine, Cellular Therapy and Cryobiology, Department of Regenerative Medicine, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [5]Department of Pathophysiology and Transplantation, Università degli Studi di Milano-Fondazione IRCCS Ca' Granda OMP, Centro Dino Ferrari, Milan, Italy

Traumatic spinal cord injury (SCI) results in a devastating loss of neurological function and in psychologically shattering condition that affects young healthy people that are in their most productive years. Currently, there are over

POSTER ABSTRACTS

2 million SCI patients worldwide and at present, there are no universally accepted treatments for this neurological disorder. Recently, particular attention is paid to the potential of stem cells in treating SCI, but there are only few clinical studies and insufficient data. This clinical study explored the feasibility and efficacy of autologous skin derived stem cells (SDSCs) transplantation in two patients with complete and chronic spinal cord injury. We hypothesized that the combination of autologous SDSCs as accessible sources of stem cells combining with platelet-rich plasma (PRP), rich of growth factors, was a possible treatment of SCI. PRP behave as natural scaffold and is able to improve stem cells survival, proliferation and axon regeneration and remyelination. Preoperative and postoperative neurological functions were evaluated with neurological clinical examination, MRI, and electrophysiological studies every two months after the treatment for one years. Results showed that in the treated patients had a clinical improvement in terms of pin prick sensory and sphincter control. No signs of adverse events such as wound infection, and no sign of tumor were evident until 6 months postoperatively. Based on the results of this clinical study, we consider that SDSCs and PRP transplantation have a possible role in promoting neurological rehabilitation for the complete and chronic spinal cord injury, increasing patient quality of life.

P80

MIR-125B REGULATES CELL PROLIFERATION AND SURVIVAL IN NEONATAL MEGAKARYOCYTES

KANDI, Ravinder, Undi, Ram Babu, Gutti, Ravi K., Gutti, Ravi K.
Department of Biochemistry, University of Hyderabad, Hyderabad, India

Developmental differences between neonatal and adult megakaryocyte (MKs) contribute to the vulnerability of neonates to develop severe thrombocytopenia (platelet count <150 x 109/L) and 20-35% of infant admitted to the NICU develop thrombocytopenia. Neonates are also affected by disorders of megakaryocytopoiesis that are predominantly present during this developmental stage. The regulatory mechanisms underlying these developmental differences are unknown. However, there are no reports demonstrating the role of miRNA in developmental megakaryocytopoiesis. We hypothesized that miRNAs would be differentially expressed in neonatal and adult MKs, and that these differences would contribute to their biological differences. To test this, we cultured human cord blood (CB) and peripheral blood (PB) CD34+ cells in serum free media with thrombopoietin. Our studies found that out of 88 miRNAs involved in the stem cell development and differentiation. All samples (n=3) expressed detectable amounts of all 88 screened miRNAs. We observed that miR-125b levels in CB were significantly higher (~4.5-fold) compared to PB-derived MKs, and these differences were consistent at all stages of MK development (0, 7, 11 and 14 days, $p < 0.05$). We checked miR-125b levels in two MK cell lines of different developmental origin, CMK (child) and MEG-01 (adult). Consistent with their developmental origin, we found that miR-125b levels in CMK cells were significantly higher than in MEG-01 cells (~7.5-fold). To explain the potential roles of miR-125b in megakaryocyte development, we predicted the targets of miR-125b via the algorithms: TargetScan, PicTar, miRwalk and miRanda and P53, BAK1 and CDK6 were found to be a predicted target by bioinformatic analysis. Here, we show that miR-125b downregulates p53, BAK1 and CDK6 in CB-MKs. The high levels of miR-125b expression in CB-MKs might contribute in their rapid proliferation, resulting in increased megakaryocyte numbers. Thus, it could be a potential therapeutic target in blood disorders.

POSTER ABSTRACTS

P81

GENE EXPRESSION VARIABILITY IS A UNIFYING ELEMENT OF THE PLURIPOTENCY NETWORK AND STEM CELL SUB-POPULATIONS

MASON, Elizabeth A.[1], Hough, Shelley[1], Mar, Jessica C.[2], Laslett, Andrew L.[3], Quackenbush, John[4], Wolvetang, Ernst J.[5], Wells, Christine A.[5], Pera, Martin[6]
[1]University of Melbourne, Melbourne, Australia, [2]The Albert Einstein College of Medicine, Bronx, NY, USA, [3]Materials Science and Engineering, CSIRO, Melbourne, Australia, [4]Dana Farber Cancer Institute, Harvard University, Boston, MA, USA, [5]AIBN, University of Queensland, Brisbane, Australia, [6]Florey Neuroscience and Mental Health Institute, and Walter and Eliza Hall Institute of Medical Research, University of Melbourne, Melbourne, Australia

Pluripotent stem cells display significant heterogeneity in gene expression, but whether this diversity is an inherent feature of cells poised between self-renewal and differentiation remains unknown. We have previously used surface antigens to define stem cell population subsets with different capacity for self-renewal and pluripotency, and different average expression of canonical pluripotency genes (Pou5f1, Sox2 and Nanog). Here we have further characterized these subsets at single cell resolution, demonstrating that gene expression variability is a metric which describes phenotypic and molecular heterogeneity within the larger stem cell population. Gene expression variability was associated with network structure in a generalizable manner, such that low-variance genes were the most highly connected. Known drivers of pluripotency (Pou5f1, Sox2, Nanog) were amongst these, suggesting these are the most stable elements of the gene regulatory network. Our data suggest that gene expression variability predicts those genes in the network under the highest degree of regulatory constraint, supporting a model of inherently metastable self-renewing population subset that gives rise to a continuum of intermediate pluripotent phenotypes.

P82

A COMPARISON OF THE HEPATIC DIFFERENTIATION PROPENSITY BETWEEN TESR AND 3IL CULTURED HESCS

TNG, John, Hu, Yuan-Yu, Chan, Yun-Shen
Genome Institute of Singapore, Singapore

Wei-Quan Tng[1,2,6], Yun-Shen Chan[1,6], Yuan-Yu Hu[1], Jonathan Go¨ke[1], Jia-Hui Ng[1], Xinyi Lu[1], Kevin Andrew Uy Gonzales[1,2], Cheng-Peow Tan[1], Zhong-Zhi Hong, Yee-Siang Lim[1] and Huck-Hui Ng[1,2,3,4,5]
[1]Gene Regulation Laboratory, Genome Institute of Singapore, Singapore, [2]Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore, [3]Department of Biological Sciences, National University of Singapore, Singapore, [4]School of Biological Sciences, Nanyang Technological University, Singapore, [5]Department of Biochemistry, National University of Singapore, Singapore
[6]These authors contributed equally to this work

Pluripotent stem cells are defined by two key characteristics namely self-renewal and the capacity to differentiate into any cell type in the body, making them ideal tools for investigating development and disease. In addition, they generate hope for regenerative medicine in the future such as replacing diseased cells. However, not all pluripotent cell lines are equal in their capacity to differentiate into desired cell types in vitro. A landmark study by Osafune and colleagues demonstrated that different human embryonic stem cell (hESCs) lines possess different in-vitro differentiation propensities. Using a well-established endodermal differentiation protocol that specifies for the hepatic lineage, we showed a difference in differentiation propensity between H1 hESCs cultured under TeSR and 3iL conditions for hepatocytes (characterized by key hepatic markers such as HNF4α and Albumin). These crucial differences in developmental potential among hESCs lines is most likely due to molecular variations that arise due to different culture conditions and our results highlight the importance of screening and deriving lines for lineage-specific differentiation.

POSTER ABSTRACTS

P83

ISOHD: AN ISOGENIC CAG ALLELIC EMBRYONIC STEM CELL PANEL TO STUDY HUNTINGTON DISEASE

Ooi, Jolene, Sim, Bernice, Rahadi, Nurul Dinie, Hayden, Michael R., **POULADI, Mahmoud A.**
TLGM, ASTAR and National University of Singapore, Singapore

Huntington disease (HD) is an autosomal-dominant, progressive neurodegenerative disease. It results in motor, cognitive and psychiatric dysfunction in affected patients. There is no known cure and current treatments merely alleviate the symptoms. The causative gene, HTT, was identified in 1993. Mutant forms of the gene display a trinucleotide CAG repeat expansion in its first exon. A number of pathogenic mechanisms thought to contribute to HD have been identified, although their relative importance remains unclear. The length of the CAG tract in the mutant allele has been demonstrated to correlate with a number of clinical observations, including age of onset, body weight and severity of neuropathology. As the study of CAG-dependent effects may shed light on the molecular mechanisms behind the etiology of HD, this study aims to generate a CAG allelic HD human embryonic stem (hES) cell panel. The panel was designed to be isogenic, so as to allow fair comparisons across CAG lengths. For ease of nomenclature, this isogenic panel has been termed isoHD. The isoHD panel consists of hES cells carrying 18, 30, 45, 65 and 81 CAG repeats in the first exon of HTT and was generated using transcription activator-like effector nuclease (TALEN)-mediated homologous recombination. In brief, a pair of TALENs that cleaves a sequence adjacent to the CAG repeat tract was constructed. The TALENs, which are tagged to Blasticidin and Zeocin resistance cassettes, were co-introduced into H9 hES cells with donor DNA carrying selected CAG lengths and a puromycin resistance selection cassette. We enriched for ES cells that incorporated both TALENs and the donor DNA using triple antibiotic resistance and subsequently identified targeted clones using PCR, sequencing, and western blotting techniques. These cells retain their pluripotent identity and will be differentiated into neurons for functional analyses. In summary, we have generated an isoHD hES cell panel that can be manipulated to serve as a platform to illuminate CAG-dependent pathways that contribute to the pathogenesis of HD.

P84

GENERATION OF INDUCED PLURIPOTENT STEM CELL (IPS) CELL LINES FROM NORMAL AND PRE-CANCEROUS HUMAN BREAST STROMAL AND EPITHELIAL TISSUES AND RE-DIFFERENTIATION TO BREAST EPITHELIAL CELL FATE

CAPALDO, Bianca D.[1], Wang, Bei[2], Vaillant, Francois[1], Lee, Lily[1], Chung, Tung-Liang[2], O'Brien, Carmel M.[2], Lindeman, Geoffrey J.[1], Laslett, Andrew L.[2], Visvader, Jane E.[1]
[1]*ACRF Stem Cells and Cancer Division, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia,* [2]*Stem Cells, CSIRO Manufacturing Flagship, Clayton, Victoria, Australia*

Breast cancer is the most common cancer to affect women worldwide. To understand breast cancer heterogeneity, it is essential to study normal epithelial subtypes and tissue development. Germline mutations in the BRCA1 and BRCA2 genes predispose women to developing breast cancer, with a lifetime risk approximating 65% and 45%, respectively. Within breast epithelium, an aberrant luminal progenitor population is expanded in BRCA1 mutation carriers. This cell type is the likely cell of origin for basal-like breast tumors arising in BRCA1 mutation carriers. To further understand the molecular defects occurring in precancerous breast tissue from women carrying BRCA1 and BRCA2 mutations, we have generated human induced pluripotent stem cell (hiPS) lines from breast epithelial and stromal cells obtained from BRCA1 and BRCA2 mutation carriers and from non-carriers. These new hiPS lines display a normal karyotype while retaining their relevant mutations post re-programming and have been validated for pluripotency using teratoma and in vitro differentiation assays. Currently, we are establishing functional differentiation assays

POSTER ABSTRACTS

using transcription factors and growth factors to re-direct human pluripotent cells to a mammary epithelial fate. The establishment of these in vitro models should provide a valuable resource to define the master regulators of mammary epithelial fate and to study the mechanisms that contribute to breast cancer in BRCA1 and BRCA2 mutation carriers.

P85

MICROFLUIDIC FABRICATION OF POLY-ε-CAPROLACTONE MICROSPHERES

LI, Jian[1], Toh, Jessica Pei Wen[1], Lam, Alan[2], Liu, Jolene Mei Jun[1], Reuveny, Shaul[2], Oh, Steve K.W.[2], Birch, William R.[1]
*[1]Institute of Materials Research and Engineering, A*STAR, Singapore, Singapore, [2]Bioprocessing Technology Institute, A*STAR, Singapore, Singapore*

Human embryonic stem cells are self-renewing, pluripotent cells, which have the ability to differentiate into a wide variety of cell types, offering application potential in tissue regeneration and drug discovery. Therapeutic applications require large numbers of cells, thus strengthening the need to develop scalable culture systems. To realize this goal, there is a need for efficient bioprocesses that are suitable for the large-scale expansion and/or differentiation of clinical-grade cells. With this in mind, a defined, scalable, three-dimensional culture system is being developed using polymer spherical microcarriers that serve as a support for cell culture. Poly-ε-caprolactone (PCL) is an excellent candidate as microcarrier materials because of its biocompatibility and mechanical properties. Here we prepared the PCL microspheres using a simple microfluidic device, which forms microspheres with relatively uniform size. We probed effects of PCL molecular weight on the size and mechanical properties of the microspheres. The diameter and size distribution of the PCL microspheres can be tuned by varying PCL molecular weight, concentration of the PCL solution, and the fluid flow rates. These PCL materials may be used as a matrix for expanding human embryonic stem cells.

P86

A HUMAN PLURIPOTENT STATE WITH DISTINCT TRANSCRIPTIONAL CIRCUITRY

NG, Jia Hui, Chan, Yun Shen, Goke, Jonathan, Ng, Huck Hui
Genome Institute of Singapore, Singapore

Embryonic stem cells serve as a cell model to study pluripotency and other early embryonic processes such as differentiation. Recent studies have highlighted significant differences between the cultured hESCs and the pluripotent cells of the in vivo embryos. Using a combination of small molecules and LIF, we induced a 3iL hESC state that expresses elevated levels of NANOG and other epiblast-enriched genes. Transcriptome profiling reveals that the 3iL hESCs more closely resemble the native preimplantation epiblast cells, and these changes are accompanied by concomitant changes in histone modifications. Lastly, based on the genome-wide binding data, we show that there is substantial re-localisation of the key transcription factors OCT4 and NANOG in the 3iL hESC state.

P87

FABRICATION OF PLGA HYBRID NANOPARTICLES COATED WITH RECONSTRUCTED MEMBRANE OF HUMAN-ADIPOSE DERIVED MESENCHYMAL STEM CELL

RAJENDRAN, Jagathesh Chandra Bose[1], Arai, Yoshie[2], Ahn, Jongchan[3], Park, Hansoo[4], Lee, Soo Hong[5]
[1]Chung Ang University, Seoul, South Korea, [2]CHA University, Seoul, South Korea, [3]CHA Biocomplex, CHA University, Seoul, South Korea, [4]Chung Ang University, Seoul, South Korea, [5]Department of Life Science, CHA Biocomplex, CHA University, Seoul, South Korea

Polymeric nanoparticles with various forms have been broadly studied and developed as a delivery platform of drugs, genes, and growth factors in the area of stem cell engineering.

POSTER ABSTRACTS

They have many appreciable advantages such as high encapsulation efficiency, tuneable and sustained release profile, with excellent serum stability, long circulation time, and potential for differential targeting of cells or tissues depending on their compositions and structures. Recently, cell membrane-camouflaged hybrid nanoparticles as delivery vehicles of vaccines showed promising results in preclinical trials. Herein, we developed polymeric hybrid nanoparticles coated with adipose-derived stem cells (ASC) membrane as long-circulating therapeutic carriers that would be functionally localized to the disease sites and. The morphological structure, size, surface charge and surface markers of reconstructed ASC membrane alone (Nanoghost) and poly(L-lactic acid-co-glycolic acid) (PLGA) hybrid nanoparticles coated with ASC membrane have been confirmed by transmission electron microscopy, dynamic light scattering, agarose gel electrophoresis, flow cytometry, respectively. We also studied the interfacial interactions between lipid vesicles of reconstructed ASC cell membrane and polymeric nanoparticle by changing polymer surface charge. By using stem cell membrane, this hybrid nanoparticle system would provide a useful tool with high delivery efficiency and cellular compatibility

P88

IDENTIFYING THE NICHE SIGNALS THAT PROMOTE STEM CELL SELF-RENEWAL

WADA, Aya, Yeo, Jia Chi, Goke, Jonathan, Ng, Huck Hui
Stem Cell and Regenerative Biology, Genome Institute of Singapore, Singpaore

Microenvironment surrounding stem cells greatly influence balance between self-renewal and differentiation. The microenvironment, niche, has been described as cellular structure such as vascular network or growth factors expressed by surrounding mesenchyme. When stem cells are cultured *in vitro*, local niche signal should be coming from feeder cells. Recent study showed that organ matched mesenchymal cells support different stages of stem/progenitor cells suggesting that mesenchyme/feeder cells do produce specific signal, niche factors, in vitro culture system. To investigate in vitro niche signals, we turned mouse liver as a model system. Liver is a vital organ and possess amazing ability to regenerate. Regeneration mechanism of liver, identity and the location of stem/progenitor cells involved and their niche are still in debate, yet, studies have shown bipotential adult stem/progenitors cell give rise to regenerated tissues. Isolated liver cells in vitro, also showed capacity of self-renewal and generation of liver organoids suggesting adult liver contains self-renewing multipotential cells. While several growth factors expressed by mesenchyme support hepatocyte growth during regeneration, more insight into niche factors can lead to identifying liver stem/progenitor cells and their growth and differentiation mechanism. Here, our goal is to define adult liver stem/progenitor cell types and niche factors drive those cells to grow and generate robust adult functional hepatocytes. First, we established hepatic cell and feeder co-culture system and maintaining hepatic cells derived from primary mouse liver cells for long-term. To determine whether the feeder cells play important role in supporting stem cells and drive the growth of hepatocytes, we have compared and analyzed transcriptome of supportive and non-supportive feeder cells. Currently, we are characterizing supportive feeder and hepatic cell co-culture system and generating supportive cell specific gene expressing feeder lines to test candidate factors ability to support stem cells.

POSTER ABSTRACTS

P89

GENE-DOSAGE DEPENDENT NEURITE DEFECTS IN A HUMAN INDUCED PLURIPOTENT STEM CELL MODEL OF SPG4 RELATED HEREDITARY SPASTIC PARAPLEGIA

HAVLICEK, Steven[1], Kohl, Zacharias[2], Mishra, Himanshu[1], Prots, Iryna[1], Eberhardt, Esther[3], Boyer, Leah[4], Marchetto, Maria Carol[4], Lampert, Angelika[5], Schloetzer-Schrehardt, Ursula[6], Winkler, Juergen[2], Gage, Fred[4], Winner, Beate[1]
[1]IZKF Junior Research Group and BMBF Research Group Neuroscience, IZKF, Friedrich-Alexander University Erlangen-Nuernberg, Erlangen, Germany, [2]Molecular Neurology, Friedrich-Alexander University Erlangen-Nuernberg, Erlangen, Germany, [3]Institute for Physiology and Pathophysiology, Friedrich-Alexander University Erlangen-Nuernberg, Erlangen, Germany, [4]Laboratory of Genetics, The Salk Institute for Biological Studies, La Jolla, CA, USA, [5]Institute of Physiology, RWTH Aachen, Aachen, Germany, [6]Department of Opthalmology, Friedrich-Alexander University Erlangen-Nuernberg, Erlangen, Germany

The hereditary spastic paraplegias (HSPs) are a heterogeneous group of motorneuron diseases characterized by progressive spasticity and paresis of the lower limbs. Mutations in Spastic Gait 4 (SPG4), encoding Spastin, are the most frequent cause of HSP. To understand how mutations in SPG4 affect human neurons, we generated human induced pluripotent stem cells (hiPSCs) from fibroblasts of two patients carrying a c.1684C>T nonsense mutation, and from two controls. These SPG4 and control hiPSCs were able to differentiate into neurons and glia at comparable efficiency. All 4 known Spastin isoforms were reduced in patient cells. Strikingly, the complexity of SPG4 neurites was decreased, which was paralleled by an imbalance of axonal transport of mitochondria. In addition, prominent neurite swellings with disrupted microtubules were present at an ultrastructural level in SPG4 neurons. Overexpression of either the M1 or M87 Spastin isoforms restored neurite length, branching, numbers of primary neurites and reduced swellings in SPG4 neurons. We conclude that neurite complexity and maintenance in HSP patient-derived neurons are critically sensitive to Spastin gene dosage. Our data show that elevation of single Spastin isoform levels is sufficient to restore neurite complexity and reduce neurite swellings in patient cells. Furthermore, our human model offers an ideal platform for pharmacological screenings with the goal to restore physiological spastin levels in SPG4 patients.

P90

M6A RNA METHYLATION FACILITATES ADEQUATE RESOLUTION OF NAIVE PLURIPOTENCY AND COMPETENCE FOR LINEAGE PRIMING

Geula, Shay[1], Moshitch-Moshkovitz, Sharon[2], Dominissini, Dan[2], Mansour, Abed AlFatah[1], Kol, Nitzan[2], Pinto, Yishay[3], Levanon, Erez[3], Stern-Ginossar, Noam[1], **NOVERSHTERN, Noa**[1], Rechavi, Gideon[2], Hanna, Jacob[1]
[1]Molecular Genetics, Weizmann Institute, Rehovot, Israel, [2]Cancer Research Center, Chaim Sheba Medical Center, Ramat Gan, Israel, [3]Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel

The naïve pluripotent epiblast in the inner cell mass (ICM) is an important ground state configuration where the epigenetic landscape is reset and prepared for subsequent lineage priming that incurs in the pluripotent post-implantation epiblast, after which overt lineage differentiation occurs. The in vivo naïve and primed pluripotent states can be modeled in vitro by capturing naïve embryonic stem cells (ESC) and primed Epiblast stem cells (EpiSC), respectively. While global profiling has indicated transcriptional and epigenetic differences between naïve and primed pluripotent states, limited knowledge is available on how their circuitry interprets defined epigenetic perturbations and whether cardinal differences exist in their lineage decision making. In this study we identify Mettl3, an m6A RNA

POSTER ABSTRACTS

modification writer, as a critical regulator for terminating naïve pluripotency and a positive maintainer of primed pluripotency in vitro and in vivo. Remarkably, Mettl3 knockout pre-implantation epiblasts and naïve ES cells, entirely lack m6A on mRNAs and are viable. Yet, they fail to adequately terminate the naïve pluripotent state, and subsequently undergo aberrant and restricted early lineage priming at the post-implantation stage. A comprehensive functional and genomic analysis involving profiling of m6A, RNA transcription and translation in Mettl3 wild-type and knockout pluripotent and differentiated cells, identified m6A as a critical determinant that destabilizes secondary naïve specific pluripotency genes Nanog, Klf2, Klf4 and Esrrb, but not Oct4 (that is expressed in both pluripotent states), and restrains their transcript stability and translation efficiency, subsequently driving rapid exit from naïve pluripotency. In established EpiSCs, m6A depletion perturbs the primed pluripotent state towards differentiation or towards naïve pluripotency when ground state conditions are applied. In summary, we identify a mechanism that functionally regulates mouse naïve and primed pluripotency in a divergent manner and demonstrate for the first time a critical role for an mRNA epigenetic modification in early mammalian development in vivo.

P91

THE ROLE OF T-CADHERIN IN MELANOMA GROWTH, VASCULARIZATION AND STROMAL CELL RECRUITMENT

RUBINA, Kseniya A., Sysoeva, Veronika Yu, Semina, Ekaterina V., Yurlova, Ekaterina
Lomonosov Moscow State University, Moscow, Russian Federation

T-cadherin is a GPI-anchored member of cadherin superfamily. T-cadherin mediates guidance of migrating cells rather than strong cell-cell adhesion. Since tumor growth and metastasis are dependent on cell movement and invasion, T-cadherin was suggested to be involved in cancer progression. T-cadherin is expressed in human melanocytes and its expression is lost in human melanoma cells. We examined T-cadherin expression in normal human skin melanocytes, melanoma cells, blood vessels of the primary melanomas, and melanoma metastasis. We found that T-cadherin was expressed in normal epidermis, blood vessels, and melanocytes in the human skin but its expression was decreased during malignant transformation in primary melanomas and metastasis. We also studied the in vivo role of T-cadherin in an animal model using murine melanoma cell line B16F10 with lung metastasis. This murine melanoma has been proved to be an adequate model for development of new anticancer drugs and their preclinical evaluation. T-cadherin overexpression in B16F10 cells led to the enlarged growth of primary tumor site. While T-cadherin inhibited tumor neoangiogenesis, it increased the metastatic and invasive potential of these cells, which was associated with the recruitment of mesenchymal stromal cells (MSC) into the primary tumor. The quantitative PCR and PCR Array Assay showed that the overexpression of T-cadherin in melanoma cells induced 6-fold increase in the expression of HGF receptor c-Met, suggesting the role of HGF-c-Met signaling in stimulation of melanoma cell proliferation and increased growth of the primary tumor. Gene expression analysis showed that T-cadherin-positive B16F10 cells had elevated levels of mRNAs encoding integrins α5, αV, αE, β3, matrix protein laminin α3, metalloproteinase MMP14 and chemokines CXCL 10, CCL5, CXCL 11 and CCL7. The increased expression of these molecules was suggested to be an additional factor, contributing to the increased invasion of melanoma and MSC recruitment into the tumor. In this work, we provided a clinical and experimental evidence for multiple roles of T-cadherin in tumor progression: the stimulation of growth and invasion of melanoma cells, MSC recruitment to the primary tumor site, and inhibition of tumor neoangiogenesis.

POSTER ABSTRACTS

P92

TOPOGRAPHICAL INFLUENCE ON DIFFERENTIATION OF PLURIPOTENT STEM CELLS INTO NEURONS OF DOPAMINERGIC SUBTYPE

TAN, Kenneth K B[1], Chai, Chou[2], Yim, Evelyn K F[1]
[1]Mechanobiology Institute, National University of Singapore, Singapore, [2]National University of Singapore, Singapore

Parkinson's disease is a neurodegenerative disease attributed to the loss of midbrain dopaminergic (DA) neurons. Pluripotent stem cells hold great promise in the study and clinical treatment for this neurodegenerative disease but progress has been hampered by the acquirement of robust cells. Appropriate biophysical cues have been known to direct stem cell fate but the role of topography in differentiating stem cells into subtype specific cells has hitherto not been well understood. Here, we aim to develop an in vitro substrate that will accelerate the derivation of midbrain dopaminergic (DA) neurons from human pluripotent stem cells (hPSCs). By using human embryonic stem cells as well as patient-derived human induced pluripotent stem cells (iPSCs), we have examined the ability of topographical patterns to influence the differentiation of hPSCs into subtype-specific and regionalized dopaminergic (DA) neurons. We have made minor modifications to the protocol based on dual SMAD inhibition method and optimized on patterned substrates to further improve the efficiency for DA neuron derivation. The size of embryoid bodies was standardized on fabricated PDMS chambers consisting of microwells. Results indicate that hPSCs are able to form neurospheres with ~80-90% efficiency on the substrates as early as Day 5, suggesting optimal guidance into the neuronal lineage and subsequent DA neuronal derivation. We have also optimized the use of region specific markers to study regionalized specification of DA neurons on patterned substrates. Our current progress in using patterned substrates for DA differentiation will be presented here. It is hoped that this will also provide novel insights into mechanisms underlying DA neuronal development and ultimately discover new therapeutic approaches for this neurodegenerative disease.

P93

THE POTENTIAL ROLES OF PIRNA REGULATORY NETWORK IN CHICKEN SPERMATOGONIA STEM CELL FORMATION

Tseng, Yen-Tzu, **CHANG, Kai-Wei**, Liao, Hung-Fu, Mo, Chu-Fan, Lin, Shau-Ping
Institute of Biotechnology, National Taiwan University, Taipei City, Taiwan

Genome-wide epigenetic reprogramming during the formation of germ cells is accompanied with the transcription of transposable elements (TEs) which threaten the genetic integrity. P-element-induced wimpy testis (PIWI) and piwi-interacting RNA (piRNA) are responsible for suppressing TEs during the epigenetic transition. PIWI-deficient male germ cell will lead to infertility. While our preliminary results show most of piRNAs in mouse and chicken target at TEs, a portion of piRNAs target at exons, introns, and some regulatory regions. Hence, how piRNA regulatory network is involved in other regulatory pathways that lead to proper germ cell development is becoming of interest. We use chicken as the animal model, to characterize the mechanisms of PIWI and piRNA mediated gene regulation in addition to TE suppression. We emphasize on piRNA composition before (E11) and after (E14) spermatogonial stem cell (SSC) formation. Taking the advantage of the 3'end-2'O-methylation signature of piRNA, we performed 3'end-2'O-methylated enrichment prior small RNA sequencing. We found only 40% piRNAs from E11 and E14 gonad targeting at TEs, compare to that of 70% piRNAs from stage X embryos. Interestingly, another 40% of the piRNAs from E11 and E14 gonad are mapped to intergenic regions. In order to identify whether those piRNAs associated intergenic regions are transcribed and can be regulated by piRNA machinery, we performed strand-specific RNA sequencing for

POSTER ABSTRACTS

Stage X embryos and the testes of E11 and E14 embryos, followed by de novo assembly for expanding the chicken transcriptome database. Utilizing the strand specificity information from the custom constructed transcriptome, we will be able to gain additional insights of the uncharacterized piRNAs and experiment on their correlation with SSC formation.

P94

SUCCESSFUL GENERATION OF DISEASE-SPECIFIC INDUCED PLURIPOTENT STEM CELLS FROM WAGR SYNDROME PATIENT WITH GERMLINE MUTATIONS HARBORING 11P13 DELETION

CHOMPOONUCH, Supachat[1], Borwornpinyo, Suparerk[2], Phanthong, Phetcharat[3], Kitiyanant, Narisorn[4], Sommani, Piyanart[5], Nuntakarn, Lalana[6], Saengsawang, Witchuda[7], Trachoo, Objoon[8], Hongeng, Suradej[9]

[1]Molecular Medicine Program, Multidisciplinary Unit, Faculty of Science, Mahidol University, Bangkok, Thailand, [2]Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok, Thailand, [3]Department of Anatomy, Faculty of Science, Mahidol University, Bangkok, Thailand, [4]Institute of Molecular Bioscience, Mahidol University, Bangkok, Thailand, [5]Department of Electrical Engineering, Faculty of Engineering, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand, [6]Department of Obstetrics and Gynecology, Mahidol University, Bangkok, Thailand, [7]Department of Physiology, Faculty of Science, Mahidol University, Bangkok, Thailand, [8]Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, [9]Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Wilms tumor, aniridia, genitourinary anomalies, and mental retardation (WAGR) syndrome is a rare genetic disease with hemizygous deletion of chromosome 11p13. The lack of suitable human disease models has haltered the progress in studies of disease mechanism and development of therapeutics for this genetically complex mutation. Generation of WAGR patient-specific induced pluripotent stem cells and differentiation into affected phenotypes could provide in vitro models potentially for the understanding of disease and platform for drug screening. Here we report the successful reprogramming of patient fibroblasts with germline mutations of WAGR syndrome to pluripotency using transduction of Oct4, Sox2, Klf4, c-Myc polycistronic lentiviral vector. iPS cells derived from WAGR syndrome patient fibroblast exhibited common pluripotent stem cell genes and surface markers such as Oct4, Sox2, Nanog, Tra-1-60, Tra-1-81 and SSEA4 along with teratoma formations showing tissues characteristic of all three germ layers. Karyotyping of iPS cells confirmed 11p13 deletion without other chromosomal abnormality. Further studies to demonstrate the differentiation potential of 11p13 deleted iPS cells to renal and neural lineages would provide disease-specific in vitro model for therapeutic drug screening to treat WAGR syndrome and Wilms tumor carcinogenesis.

P95

MODELLING SPINAL BULBAR MUSCULAR ATROPHY (SBMA) WITH THE USE OF IPS CELLS

SHEILA, Marianne
Genome Institute of Singapore, Singapore

Spinal bulbar muscular atrophy (SBMA) or Kennedy's Disease is an X-linked recessive neuromuscular disorder characterized by the selective degeneration of lower motor neurons within the spinal cord and brainstem.SBMA results from the abnormal expansion of the CAG repeat in exon 1 of the androgen receptor (AR) gene and thus is categorized as one of a group of nine trinucleotide repeat disorders. The selective loss of motor neurons in SBMA results in limb and bulbar muscle atrophy and weakness, mobility problems, swallowing and speech difficulties,

POSTER ABSTRACTS

gynaecomastia and reduced fertility. Although there is widespread expression of the AR in various tissues and organs, the exact mechanism of selective motor neuron loss within the spinal cord and brain stem remains elusive. Several lines of evidence point to the possible perturbation in the miRNA network which underlies the pathology in SBMA. To further understand the pathology of SBMA and the dysregulation in the miRNA network, our research in SBMA is focused on two main areas: (1) modelling the selective death of motor neurons in SBMA using patient-derived iPS cells which are induced to differentiate into motor neurons (2) adopt a transcriptomic approach in identifying candidate miRNAs that may be involved in motor neuron degeneration via wide-scale genomic screen platforms such as RNA-seq and microRNA microarrays. This approach aims to shed light on the molecular basis and pathways dysregulated in SBMA resulting in motor neuron degeneration and the eventual goal of establishing novel therapeutic strategies for SBMA treatment.

P96

ULTRASHORT PEPTIDES AS BUILDING BLOCKS FOR THREE-DIMENSIONAL SCAFFOLDS TO PROMOTE PLURIPOTENCY AND PROLIFERATIVE POTENTIAL OF EMBRYONIC STEM CELLS

Jaspreet, J, Jadhav, Sandip V., Loo, Yihua,
HAUSER, Charlotte A. E.
Institute of Bioengineering and Nanotechnology, Singapore

Pluripotent stem cells (PSC) including embryonic and induced pluripotent stem cells are highly promising candidates for regenerative medicine, attributable to their totipotency and high proliferative potential. However, current limitations in their large-scale culture dampen developmental progress within the clinical landscape. Flat and rigid two dimensional substrates are commonly used to culture stem cells, which exposes them to a very different microenvironment relative to conditions experienced in the body. Extracellular matrix (ECM) creates a three-dimensional and relatively soft microenvironment ***in vivo***. Therefore, a 3D niche provides a closer biomimetic avenue as a stem cell scaffold relative to 2D systems. While conceptually appealing, construction of an artificial 3D microenvironment poses challenges whereby cell viability remains a major problem. In our study, biophysical factors such as mechanical property and architecture of scaffold materials are accounted for as they play an important role in the regulation of stem cell fate. Self-assembly of small building block molecules such as peptides has been shown to encapsulate and support the viability of PSCs. We report the use of a novel class of gel-forming peptides derived from natural amino acids as a scaffold for PSC expansion. These amphiphilic ultrashort peptides are 3-7 amino acids long and self-assemble in water to form networks of interconnected fibre with a striking morphological resemblance to collagen 1. The 3D scaffolds demonstrate a large degree of interconnected porosity, favoring cell migration and nutrient exchange through macromolecular diffusion. Their high water retention capacity of up to 99.9% results in the formation of clear and transparent hydrogels, enabling the use of optical techniques for high-throughput screening. In our setup, embryonic stem cells (ESCs) were exposed to a defined 3D hydrogel environment by embedding them ***in situ***. ESCs remained viable following multiple passaging (n≥7) and the expression of relevant pluripotency markers were confirmed with quantitative PCR. These findings render our peptide-based 3D hydrogel a potential novel solution toward maintenance and robust expansion of PSCs, possibly supporting their relevance in clinical applications and in the healthcare industry.

POSTER ABSTRACTS

P98

DYNAMIC EXPRESSION OF SYSTEM A AMINO ACID TRANSPORTERS IN MOUSE PLURIPOTENT CELLS IN VIVO AND IN VITRO

TAN, Boon Siang Nicholas[1], Rathjen, Peter D.[2], Gardner, David K.[1], Rathjen, Joy[2]
[1]*Deparment of Zoology, University of Melbourne, Parkville, Victoria, Australia,* [2]*Menzies Research Institute Tasmania and University of Tasmania, Hobart, Tasmania, Australia*

Many strands of evidence suggest that nutrients, and specifically amino acids, play regulatory roles in early mammalian embryogenesis and pluripotence. Despite this, little is known of the regulatory roles of amino acids in pluripotent lineage progression. In culture, we have shown that L-proline induces differentiation of mouse ES cells into a second pluripotent cell population, early primitive ectoderm-like (EPL) cells. These data suggest active roles for amino acids in pluripotent cell regulation. Radioactive amino acid uptake assays, gene expression analysis and immuno-fluorescent techniques identified a system A amino acid transporter, SLC38a2 as the major transporter required for L-proline transport into mouse ES cells. The use of pharmacological inhibitors of cell signalling pathways determined requirements of Src tyrosine kinases, p38 MAPK and Erk in the formation and maintenance of EPL cells. Expression of SLC38a2, and another system A transporter, SLC38a1, was determined in early mouse embryos (from 2-cell to early post-implantation stages). Both transporters showed dynamic expression patterns. SLC38a1 was preferentially expressed on outer cells of the compacted morula that are fated to form trophectoderm (TE). After this point, SLC38a1 was detected in all cells of the embryo. The preferential expression of SLC38a1 in the outer cells of the morula may indicate a requirement for amino acids in TE formation. SLC38a2 was detected in the TE, and specifically in the nucleus of these cells. SLC38a2 expression was also seen in pluripotent cells, with an up regulation of expression in the epiblast prior to primitive ectoderm formation. ES cells cultured in naïve conditions showed a reduction in SLC38a2 mRNA, suggesting that SLC38a2 is regulated at this point of pluripotent cell development. Understanding the role of amino acids in the development of embryonic cell populations will contribute to optimisation of media and protocols used in regenerative medicine.

P99

DIRECTED DIFFERENTIATION OF MOTOR NEURONS AND ASTROCYTES FROM HUMAN INDUCED PLURIPOTENT STEM CELLS FOR THE STUDY OF SPINAL MUSCULAR ATROPHY

LOH, Sharon, Stanton, Lawrence W.
Genome Institute of Singapore, Singapore

Spinal Muscular Atrophy (SMA) is a neurodegenerative disorder affecting spinal cord Motor Neurons (MNs), leading to muscle weakness. It has been reported that low expression level of SMN protein and other neuronal cell types cause MNs to degenerate in SMA. However, key downstream regulatory genes of SMN protein responsible for inducing MNs death and the roles of astrocytes in SMA pathology remain elusive. To study we successfully established methodologies that efficiently differentiate WT and SMA human induced pluripotent stem cells (hiPSCs) to MNs and astrocytes. Our differentiation methods generated MNs that expressed MNs specific markers (FOXP1, ISL-1, ChAT) and astrocytes that expressed astrocytic markers (GFAP, S100b, CD44). These MNs and astrocytes after being patterned by morphogens also achieved caudal ventral identity and expressed caudal ventral marker HOXB4. It's no surprise that dead cells started appearing only in the SMA MN culture during the second week of MN differentiation, suggesting that SMA iPSCs-derived MNs were degenerating. Interestingly, it was also observed that SMA-iPSCs derived astrocytes expressed glutamate transporter less abundantly than WT-iPSCs derived astrocytes, implying that SMA astrocytes may have impaired glutamate

POSTER ABSTRACTS

uptake function that cause excitotoxicity to MNs. These preliminary findings suggest that SMA iPSCs-derived MNs and astrocytes exhibited abnormalities and these affected cell types obtained through directed differentiation from hiPSCs, as we have demonstrated, will serve as a valuable resource to study dysregulated molecular mechanisms in SMA.

P100

DIFFERENTIATION OF POLYCYSTIC OVARY SYNDROME DERIVED INDUCED PLURIPOTENT STEM CELLS INTO OVARIAN GRANULOSA-LIKE CELLS

LAN, Chen-wei
National Taiwan University, Taipei, Taiwan

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women at the reproductive age. Although its etiology and pathogenesis remain unclear, recent studies suggest that ovarian granulosa cell dysfunction may partly be responsible. Human pluripotent stem cells (hPSCs) have an unlimited capacity for self-renewal and can differentiate into all the specialized cells. These features make hPSCs potentially valuable for the study of early embryo development, establishment of disease models, and applications in regenerative medicine. This study aimed to establish an effective culture system in order to direct the differentiation of induced pluripotent stem cells (iPS) from patients with PCOS into granulosa cells. Through multi-step approaches comprising in vitro treatments with cocktails of growth factors, gene expression analyse the progress of PCOS-iPSCs to granulosa-like cells that expressed the granulosa cell-specific forkhead transcription factor FOXL2, estrogen synthetase CYP19A1, anti-Müllerian hormone (AMH), the type 2 AMH receptor (AMHR2), and the follicle stimulating hormone receptor (FSHR), but not luteinizing hormone receptor (LHR). Next, we have identified cell-surface markers AMHR2 and FSHR for the enrichment of granulosa-like cell types derived from PCOS-iPSCs. These cells will aid investigations that use granulosa-like cells generated from PCOS-derived pluripotent stem cells to study granulosa cell development, folliculogenesis, and steroidogenesis and in the creation of novel treatment modalities for PCOS disease in future.

P101

RNA-MEDIATED GENERATION OF INTEGRATION-FREE IPS CELL LINES FROM LATE-OUTGROWTH ENDOTHELIAL PROGENITOR CELLS (L-EPCS) DERIVED FROM HUMAN BLOOD

Eminli-Meissner, Sarah[1], Moon, Jung-Il[1], Poleganov, Marco[2], Beissert, Tim[2], Sahin, Ugur[2], Morrell, Nick[3], Huang, Chris[3], Rana, Amer Rana[3],
HAMILTON, Brad[1]
[1]Stemgent, Cambridge, MA, USA, [2]TRON-Translational Oncology, University Medical Center Mainz, Mainz, Germany, [3]University of Cambridge, Cambridge, United Kingdom

In 2010, it was first published that repeated transfection of fibroblasts with a cocktail of reprogramming mRNAs resulted in the generation of stable, integration-free human iPS cells. While many advancements have been made to refine this process on fibroblasts, to date no group has been able to demonstrate RNA-based reprogramming of a blood derived cell type. This limitation has been primarily due to the inability to efficiently and repeatedly deliver mRNA to cells originating from blood without inducing cytotoxicity. Peripheral blood provides easy access to adult human cell types for reprogramming purposes. As a result there are numerous studies utilizing blood derived cell types to generate patient and disease specific iPS cell lines for both research and clinical applications. Notably, late-outgrowth endothelial progenitor cells (L-EPCs) can be clonally isolated from both human peripheral blood and cord blood. The L-EPCs adherent nature and high proliferative capacity while maintaining their cell identity makes them highly desirable for repeated transfection with RNA, as non-adherent hematopoietic cells typically have limited expansion

POSTER ABSTRACTS

potential and exhibit maturation in culture. Lastly, the ability to generate clinical grade iPS cells from L-EPCs using RNA reprogramming technologies presents a unique therapeutic opportunity to treat myeloproliferative disorders in which the disease-causing somatic mutations are restricted to cells in the hematopoietic lineage. Here we present data demonstrating the cellular reprogramming of human L-EPC lines into stable and fully reprogrammed iPS cells via transfection with novel non-modified reprogramming mRNAs and reprogramming associated miRNAs.

P102

LGR5+ STEM CELLS ARE INDISPENSIBLE FOR THE MAINTENANCE OFEPITHELIAL HOMEOSTASIS IN THE GLANDULAR STOMACH

LEUSHACKE, Marc[1], de Sauvage, Frederic J.[2], Barker, Nick[1,3,4]
*[1]A*STAR Institute of Medical Biolog, Singapore, Singapore, [2]Department of Molecular Biology, Genentec, South San Francisco, CA, USA , [3] Centre for Regenerative Medicine, The University of Edinburgh, Edinburgh, UK, [4] Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*

The epithelium of the adult glandular stomach is constantly renewed throughout life. Limited reservoirs of adult stem cells located within numerous flask-shaped tubular glands fuel the process of repetitive tissue regeneration. Multiple Lgr5+ cells reside at the base of each pyloric gland. In-vivo lineage tracing analysis characterized these cells as self-renewing, multipotent adult stem cells involved in long-term renewal of the pyloric epithelium. Using the Lgr5-DTR-eGFP knock-in mouse model, we identified individual cell populations residing at the gland base of the pylorus and corpus epithelium that present high levels of Lgr5 driven DTR-eGFP expression. Such Lgr5-driven expression of DTR-eGFP selectively confers diphtheria toxin (DT) sensitivity on Lgr5+ cells. We aim to directly evaluate the contribution of Lgr5-expressing cells to long-term epithelial homeostasis in the stomach. For this, we specifically ablated Lgr5-expressing cells in Lgr5-DTR-eGFP transgenic mice. Ablation of Lgr5-DTR-eGFP-expressing cells in vivo caused severe tissue damage in a large proportion of the glandular epithelium. Interestingly, however, we also identified a minority of glands with an intact morphology, indicating a negligible effect of Lgr5+ stem cell loss within such glands. We now aim to evaluate a potential role for alternative gastric stem cell populations, such as Sox2-expressing stomach cells, during tissue regeneration.

P103

STROMA PROVIDES RSPO3 AND WNTS TO FORM INTESTINAL STEM CELL NICHE

GREICIUS, Gediminas[1], Kabiri, Zahra[2], Virshup, David M.[2]
[1]CMR, National Cancer Center, Singapore, Singapore, [2]Program in Cancer and Stem Cell Biology, Duke-NUS Graduate Medical School, Singapore, Singapore

Ex vivo reconstitution of tissue stem cells with their physiologic niche can provide important insights into the requirements for self-renewal and differentiation. The intestinal epithelium can self-renew and expand ex vivo via a continuous process of stem cell proliferation and differentiation. Purified intestinal epithelium requires Wnt3 secreted from Paneth cells, as well as supplemental factors including recombinant R-spondin1 (RSPO1). RSPO1 enhances Wnt responsiveness by inhibiting RNF43 and ZNRF3, ubiquitin ligases that otherwise target the Wnt receptor Frizzled for degradation.
While this system has been useful to expand the epithelium ex vivo, its physiologic relevance has been questioned. We and others have found that neither global knockout of intestinal epithelial Wnt secretion, nor loss of Paneth cells, disrupts intestinal homeostasis in mice. However, we find that epithelial cells from villin-cre/PorcnDel mice cannot form organoids ex vivo, unless they are co-cultured with stromal cells. The cultured intestinal myofibroblast enriched cells highly express RSPO3 as well as multiple Wnt ligands and can support epithelial

POSTER ABSTRACTS

organoid formation even without exogenous RSPO1 supplementation. Moreover, exogenous RSPO3 supports organoid culture as well as RSPO1, which is routinely supplemented in in vitro cultures. These results suggest that stromal cells secreting Wnts and RSPO3, but not RSPO1, form the niche for intestinal stem cells. To target stromal Wnts in vivo, we used the pharmacological Porcn inhibitor C59. C59 administration at high dose completely blocked stem cell marker expression and epithelial cell proliferation resulting in crypt degeneration. Thus, we propose that stromal-specific Wnts and RSPO3 are both necessary and sufficient to support the intestinal stem cell in vivo and in vitro.

P104

ACUTE EXPOSURE OF ASPIRIN ALLEVIATES GROWTH FACTOR ASSOCIATED GENES IN PERIODONTAL LIGAMENT STEM CELLS

ABDULLAH, Mariam[1], Abd Rahman, Fazliny[2], Govindasamy, Vijayendran[3], Abu Kasim, Noor Hayaty[2], Musa, Sabri[4]
[1]Department Of Restorative Dentistry, Faculty Of Dentistry, University Of Malaya, Kuala Lumpur, Malaysia, [2]Department Of Restorative Dentistry, University Of Malaya, Kuala Lumpur, Malaysia, [3]Hygieia Innovation Sdn.Bhd, University Of Malaya, Kuala Lumpur, Malaysia, [4]Department Of Children's Dentistry And Orthodontics, Faculty Of Dentistry, University Of Malaya, Kuala Lumpur, Malaysia

Aspirin is a type of non-steroidal anti-inflammatory drug (NSAIDs) which plays a role in a variety of systemic diseases such as diabetes mellitus, cardiovascular disease and premature low birth weight. Aspirin has also been intensively studied for the prevention of periodontal disease, mainly periodontitis. Periodontal ligament (PDL) stem cells are a useful source for periodontal and bone regeneration. Prior to the treatment for periodontal regeneration, aspirin was a well-known tolerable drug for periodontal diseases especially periodontitis. To date no research has been conducted to determine the role of growth factors after treatment with aspirin in PDLSCs. Therefore, the present study was undertaken to investigate the growth factors expression in periodontal ligament cells (PDLSCs) after exposure to aspirin as well as elucidating the effect of aspirin in periodontal regeneration. The healthy human periodontal ligament was extracted and isolated to periodontal ligament stem cell (PDLSCs). The aspirin was exposed to PDLSCs and the viability of cells was checked by MTT assay. The growth factor expression was studied after treatment with aspirin in PDLSCs. From the results, 13 of the known > 86 genes of growth factors were expressed more than twofold. The results show that several groups of genes, which are involved in tissue or cell regeneration, were upregulated including bone morphogenetic protein (BMP2, BMP3 and BMP10), fibroblast growth factor (FGF2, FGF7, and FGF14) and few genes involved in angiogenesis (VEGFA, VEGFC). Other upregulated genes, including IL10 and INHBB, are expected to play roles in modulating the tissue response. In conclusion, the aspirin encourages osteogenic differentiation as indicated by the up-regulation of several genes involved in bone formation.

P105

MESENCHYMAL STEM CELLS DERIVED CARDIOMYOCYTE FOR HEART TISSUE REGENERATION

RAYNAUD, Christophe, Ahmed, Idil, Bollensdorff, Christian
Qatar Cardiovascular Research Center, Doha, Qatar

For the treatment of heart failure, there are two potentially complementary cellular therapies:1- Replacement of damaged heart tissue with either fully functional cardiomyocytes or their immediate lineage committed progenitors. Towards this aim, methods for cytokine mediated directed differentiation of human embryonic stem cells (hESCs) toward cardiomyocytes have been widely described. These protocols result in low mature cardiomyocytes which hinder their translation to a clinical setting.2- The second approach is to use

POSTER ABSTRACTS

cells that will help functionally the heart and its self-regeneration. This is already clinically applied using mesenchymal stem cells (MSCs).To combine those two approaches, MSCs have been documented as presenting the potential to differentiate into cardiomyocytes. Our goal is to set up a robust differentiation protocol of MSCs into cardiomyocytes that could be used clinically. Alternatively, we will study by optical mapping the improvement brought by MSCs co-cultured with hESCs derived cardiomyocytes on maturity and functionality of those cells as well as pathophysiology and drug action. This strategy will allow the simultaneous use of the two different cell type proposed for cell-based therapy.

P106

EFFICIENT GENERATION OF IPSCS DERIVED FROM PARKINSON'S DISEASE (PD) STUDY PATIENT FIBROBLASTS USING CYTOTUNE®-IPS 2.0 SENDAI REPROGRAMMING KIT IN THE ESSENTIAL 8® FEEDER-FREE MEDIA SYSTEM

Piekarczyk, Marian[1], Hancock, Michael[1], Boucher, Shayne[1], Kuninger, David[1], Piper, David R.[1], Vogel, Kurt[1], Langston, J. William[2], Schuele, Birgitt[2]
[1]*Life Sciences Solutions Group, ThermoFisher Scientific, Madison, WI, USA,* [2]*The Parkinson's Institute, Sunnyvale, CA, USA*

The absence of cellular models for Parkinson's Disease (PD) represents a major bottleneck and unmet need in PD research. Patient-derived induced pluripotent stem cells (iPSCs) offer exciting potential in cell therapy and in vitro disease modeling. Efficient reprogramming of patient somatic cells to iPSCs in feeder-free conditions plays a key role in realizing this potential. Many reprogramming methods have been optimized for use with numerous cell lines, but lead to technical challenges for researchers in converting adult or disease somatic cells to iPSCs consistently and efficiently. The CytoTune®-iPS 2.0 Sendai Reprogramming Kit uses Sendai virus and polycistronic vectors to reprogram somatic cells into induced pluripotent stem cells (iPSCs) which provides a more robust reprogramming efficiency, lower cytotoxicity, and faster viral clearance to generate integration -free iPSCs in feeder-free conditions. In this study, fibroblasts from a skin biopsy of a Parkinson's disease (PD) study donor were reprogrammed in feeder-free conditions to iPSCs using Life TechnologiesTM CytoTune®-iPS 2.0 Sendai Reprogramming Kit. These iPSCs are transgene-free and karyotypically normal, express known pluripotency markers and are able to differentiate into embryoid bodies that present the three germ layer lineages: ectoderm, mesoderm, and endoderm. Given the efficiency, speed and ease of reprogramming of these adult, disease fibroblasts in feeder-free conditions, the CytoTune®-iPS 2.0 Sendai Reprogramming Kit can be applied to large scale reprogramming of multiple disease lines in an automated fashion to provide significant impact for researchers worldwide.

STUDENT TRAVEL FELLOWSHIPS

The Stem Cell Society (Singapore) supports postgraduate students to attend the symposium by providing Student Travel Fellowship (for overseas SCSS student members) and Student Fellowships (for local SCSS student members).

We are very grateful to Dr. Susan and Deepak Sharma for generously supporting these programs.

CONGRATULATIONS 2014 AWARDEES:

TRAVEL FELLOWSHIP PROGRAM

Rajendran Jagathesh Chandra Bose

Ravinder Kandi

Vidyanand Sasidharan

Nicholas Tan

Maximize Your Pluripotential

Reduce Variation With The Most Complete, Defined System For Pluripotent Stem Cell Culture

- **Reprogramming:** TeSR™-E7™
- **Maintenance and Expansion:** mTeSR™1, TeSR™2, TeSR™-E8™
- **Matrix:** Vitronectin XF™
- **Passaging:** ReLeSR™
- **Cryopreservation:** mFreSR™, FreSR™-S
- **Differentiation:** STEMdiff™

www.stemcell.com/hPSC

STEMCELL™ TECHNOLOGIES

Scientists Helping Scientists™ | WWW.STEMCELL.COM

STEMCELL TECHNOLOGIES INC.'S QUALITY MANAGEMENT SYSTEM IS CERTIFIED TO ISO 13485 MEDICAL DEVICE STANDARDS.

Copyright © 2014 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, STEMdiff, and ReLeSR are trademarks of STEMCELL Technologies Inc. mTeSR, TeSR, E8 and E7 are trademarks of WARF. All other trademarks are the property of their respective holders.

ABSTRACT INDEX

LIFE TECHNOLOGIES
STEM CELL RESEARCH

YOUR RESEARCH CAN CHANGE THE WORLD. WE CAN HELP.

CULTURE
ENGINEERING
DIFFERENTIATION
CHARACTERIZATION

Discover how our recent collaboration with The Parkinson's Institute is helping to develop systems that enable a more complete understanding of the puzzles scientists like you look to solve.

Explore our tools and resources now.
Go to **lifetechnologies.com/pdmodels**

life technologies
A Thermo Fisher Scientific Brand

For Research Use Only. Not for use in diagnostic procedures. © 2014 Thermo Fisher Scientific Inc. All rights reserved.
All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. C0240416 IPAC 0414

NOTES

NOTES

NOTES

StemMACS™ Media XF for xeno-free stem cell culture

Reliable culture designed with clinical translation needs in mind

StemMACS iPS-Brew XF, human

- **validated** and tested on ES/iPS cells
- enables high survival after thawing
- easy adaptation to feeder-free conditions

StemMACS MSC Expansion Media XF, human

- **efficient** expansion and enumeration of MSCs
- no attachment substrates required

StemMACS HSC Expansion Media XF, human

- **maintains** high levels of $CD34^+$ cells
- preserves broad differentiation potential
- best performance when supplemented with StemMACS HSC Expansion Cocktail

► **www.macs-stemcells.com**

macs@miltenyibiotec.com.sg +65 6238 8183

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use. MACS is a registered trademark of Miltenyi Biotec GmbH. Copyright © 2014 Miltenyi Biotec GmbH. All rights reserved. *From now till 15 December 2014.