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Schedule at a Glance

Day 1	October 1, Saturday	
08:00~16:50	**Registration**	**1F Lobby**
08:20~10:00	**Poster Set-up (Before AM 10:00)**	**B1 Lobby**
08:45~09:00	**Opening Remarks** Emcee: Heng-Yu Chang, Ph.D. (張亨仔教授) President Hong-Nerng Ho (TSSCR) (何弘能理事長) Dean Chii-Ruey Tzeng (TMU) (曾啟瑞院長)	**16F Hall**
Keynote Lecture	Moderators: Chii-Ruey Tzeng, M.D. (曾啟瑞院長), Bon-Chu Chung, Ph.D. (鍾邦柱教授)	**16F Hall**
09:00~09:40	**Various clinical applications of human induced pluripotent stem cells** Tatsutoshi Nakahata, M.D., D.M.Sci. *Deputy Director, Center for iPS Cell Research and Application, Kyoto University, Japan*	
09:45~10:25	**A functionalized scaffold for modulation of inflammation to permit stem cell survival in myocardial infarction** Abhay Pandit, Ph.D. *Director, Network for Excellence for Functional Biomaterials, National University of Ireland, Ireland*	
10:00~15:00	**TSSCR Board Member Election**	**16F VIP Room**
10:25~10:45	**Coffee Break**	**16F and B1 Lobby**
A: iPS and Pluripotent Stem Cells (I)	Moderators: Winston TK Cheng, Ph.D. (鄭登貴教授), Chung-Liang Chien, Ph.D. (錢宗良教授)	**16F Hall**
10:45~11:15	**Induction of pluripotency** Huck Hui NG, Ph.D. *Senior Group Leader & Associate Director, Genome Institute of Singapore, Singapore*	
11:15~11:45	**Pluripotent stem cells and the potential application in regenerative medicine** Qi Zhou, Ph.D. *Director, Center for Stem Cell Research and Regenerative Medicine, Institute of Zoology, Chinese Academy of Sciences, Beijing, China*	
11:45~12:15	**Utilization of iPS cells for retinal regenerative medicine** Masayo Takahashi, M.D., Ph.D. *Team Leader, Laboratory for Retinal Regeneration, Center for Developmental Biology, RIKEN Japan*	
12:15~12:45	**Group Photography**	**1F Outdoor Plaza**
12:45~13:15	**Lunch**	**B1 Food Court & Room 8001-8004**
13:00~14:00	**TSSCR Annual Meeting**	**4F Hall**
13:15~14:30	**Poster Sessions**	**B1 Lobby**

B: Cancer Stem Cells **16F Hall**

Moderators: Hong-Nerng Ho, M.D. (何弘能理事長), Wann-Hsin Chen, Ph.D. (陳婉昕研究員)

14:30~15:00 **Cancer stemness: activation of stemness genes in lung cancer cells**
Cheng-Wen Wu, M.D., Ph.D. (吳成文院士)
Academician, Academia Sinica
Distinguished Chair Professor, National Yang Ming University

15:00~15:20 **Significance of Twist1-Bmi1 axis in epithelial-mesenchymal transition, stem-like properties and movement of cancer cells**
Muh-Hwa Yang, M.D., Ph.D. (楊慕華副教授)
Associate Professor, Institute of Clinical Medicine, National Yang Ming University

15:20~15:40 **Cancer stem cells on head and neck cancer tumorigenesis**
Jeng-Fan Lo, Ph.D. (羅正汎副教授)
Associate Professor, Institute of Oral Biology, National Yang Ming University

15:40~16:00 **Identification of cancer stem cells from pancreatic adenocarcinoma with higher metastatic potentials**
Chia-Ning Shen, Ph.D. (沈家寧助研究員)
Assistant Research Fellow, Genomics Research Center, Academia Sinica

16:00~16:30 **Coffee Break** **16F and B1 Lobby**

C: Somatic Stem Cells and Regenerative Medicine (I) **16F Hall**

Moderators:Yung-Hsiao Chiang, M.D. (蔣永孝主任), Sheng-Mou Hou, M.D., Ph.D. (侯勝茂教授)

16:30~16:50 **Mesenchymal stem cells: plasticity and application**
Oscar K Lee, M.D., Ph.D. (李光申教授)
Professor, Institute of Clinical Medicine, National Yang Ming University

16:50~17:10 **Benefits of hypoxic culture on human mesenchymal stem cells**
Shih-Chieh Hung, M.D., Ph.D. (洪士杰教授)
Professor, Institute of Clinical Medicine, National Yang Ming University

17:10~17:30 **Wnt regulates neurotrophin-induced neuronal transdifferentiation of human bone marrow-derived mesenchymal stem cells via β-catenin signaling and non-canonical pathway**
Wen-Fu Lai, Ph.D. (賴文福教授)
Professor, Institute of Clinical Medicine, Taipei Medical University

17:30~17:50 **3D culture of adherent stem cells**
Bin-Ru She, Ph.D. (施冰如研究員)
Research Fellow, Industrial Technology Research Institute

18:30~20:30 **Banquet** **Far East Shangrila Hotel**

Day 2	October 2, Sunday	
08:00~16:50	**Registration**	**1F Lobby**
08:20~10:00	**Poster Set-up (Before AM 10:00)**	**B1 Lobby**

Keynote Lecture — **16F Hall**

Moderators: Ming-Jer Tang, M.D., Ph.D. (湯銘哲教授), Win-Ping Deng, Ph.D. (鄧文炳教授)

09:00~09:40 **Genetics in stem cells; From discovery to therapy**
Allan Bradley, Ph.D., F.R.S., F.Med.Sci
Director Emeritus, The Wellcome Trust Sanger Institute, United Kingdom

09:45~10:25 **Therapeutic consequences of cell-biomaterial interactions**
David Williams, Ph.D., D.Sc.
Director, Institute of Regenerative Medicine, Wake Forest Baptist Medical Center, USA
Editor-in-Chief, Biomaterials

10:25~10:45 **Coffee Break** — **16F and B1 Lobby**

D: iPS and Pluripotent Stem Cells (II) — **16F Hall**

Moderators: Nan-Chi Chang, Ph.D. (張南驥教授), Shui-Long Lee, Ph.D. (李水龍教授)

10:45~11:15 **Stem cell therapy for the regenerative medicine and anti-aging**
Kwang Yul Cha, M.D.
President of CHA Biotech, Korea

11:15~11:35 **Immunogenicity of human pluripotent stem cells and the derivatives.**
Hsin-Fu Chen, M.D. (陳信孚副教授)
Associate Professor, Graduate Institute of Clinical Medicine, National Taiwan University

11:35~11:55 **Novel sources of human stem cells: fetal-stage mesenchymal stem cells and induced pluripotent stem cells**
Betty Lin-Ju Yen, M.D. (顏伶汝副研究員)
Associate Investigator and Attending Physician, Institute of Cellular & System Medicine, National Health Research Institutes

11:55~12:15 **Pluripotency reprogramming beyond transcription factors**
Hung-Chih Kuo, Ph.D. (郭紘志助研究員)
Assistant Research Specialist, Institute of Cellular and Organismic Biology, Academia Sinica

12:15~12:45 **Lunch** — **B1 Food Court and Room 8001-8004**

12:45~14:15 **Mini Symposium: Nunc Cell Culture Surfaces for Stem cell Research**
Singa Hsu (Technical Specialist, Tseng Hsiang Life Science) — **B1 Room 8005**

12:45~13:45 **Mini Symposium: 1. 3D Quantitative Fluorescence Molecular Tomography; 2. Label-Free Technology for Cellular Interactions**
Hsiao Chu Tseng/ Hsing Tsen Lu (Application Specialist, J&H Technology) — **B1 Room 8006**

12:45~14:15 **Poster Sessions** — **B1 Lobby**

E: Somatic Stem Cells and Regenerative Medicine (II) — **16F Hall**

Moderators: Yao-Chang Chen, M.D. (陳耀昌教授), Lynn L.H. Huang, Ph.D. (黃玲惠教授)

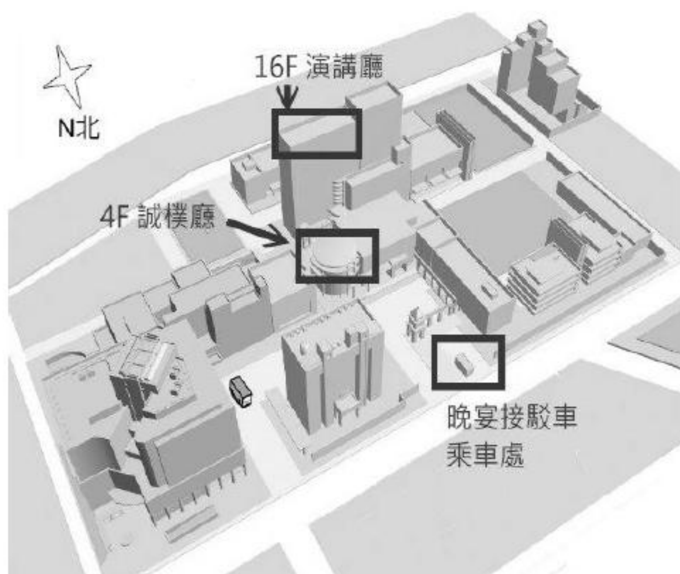
Time	Program
14:15~14:45	**Control of embryonic stem cell (ESC) differentiation by prostaglandin E_2 (PGE_2) and Rho-associated kinases (ROCK)** Kenneth Kun-Yu Wu, M.D., Ph.D. (伍焜玉院士) *Academician, Academia Sinica* *President and Distinguished Investigator, National Health Research Institutes*
14:45~15:05	**A novel neuronal differentiation system, combination of culture medium and biomaterial for neural stem precursor cells** Tai-Horng Young, Ph.D. (楊台鴻教授) *Professor, Institute of Biomedical Engineering, National Taiwan University*
15:05~15:25	**Acquire and nurture ESC-Like $CD34^+$ mesenchymal common progenitor cells ($CD34^+$ MCPCs) from human tissues for translational medicine** Daniel Tzu-Bi Shih, Ph.D. (施子弼教授) *Professor, Graduate Institute of Medical Sciences, Taipei Medical University*
15:05~15:45	**Dedifferentiation is a key to limb regeneration in salamanders** Hsuan-Shu Lee, M.D., Ph.D. (李宣書所長) *Professor & Director, Institute of Biotechnology, National Taiwan University*
15:45~16:05	**The opportunities and challenges in cardiac stem cell therapy** Patrick Ching-Ho Hsieh, M.D., Ph.D. (謝清河副教授) *Associate Professor, Institutes of Clinical Medicine, National Cheng Kung University*
16:05~16:30	**Coffee Break** — **16F and B1 Lobby**

F: Short Talk by TSSCR Best Poster Award Winners — **16F Hall**

Moderators: Chia-Ning Shen, Ph.D.(沈家寧助研究員), Wann-Hsin Chen, Ph.D. (陳婉昕研究員)

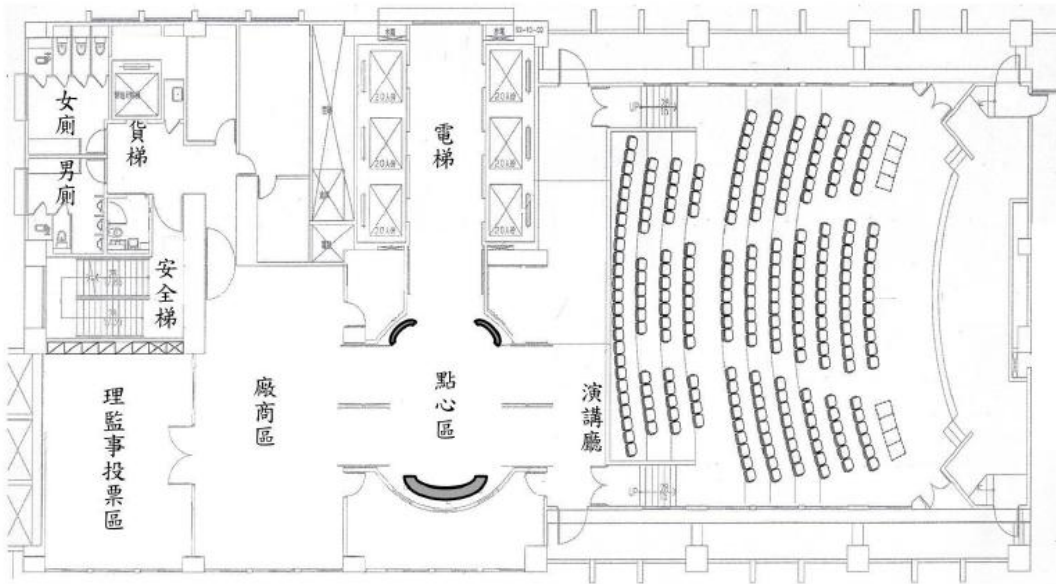
Time	Program
16:30~16:40	Best Poster Award_Second Runner Up
16:40~16:50	Best Poster Award_ Runner Up
16:50~17:00	Best Poster Award_ Champion
17:00~17:20	**Best Poster Award Ceremony and Closing Remarks** — **16F Hall** Emcee: Heng-Yu Chang, Ph.D. (張亨仔教授) Dean Chii-Ruey Tzeng (TMU) (北醫曾啟瑞院長) President Hong-Nerng Ho (TSSCR) (何弘能理事長)

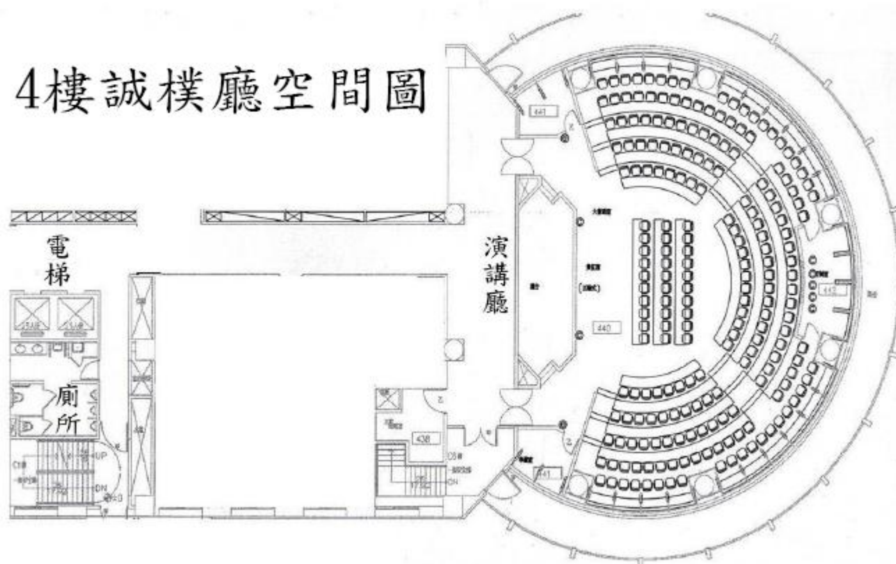
Map Guidance



- 主要演講廳於醫學綜合大樓**後棟**16F演講廳
- 同步演講廳於醫學綜合大樓**前棟**4F誠樸廳
- 10/1 13:00~14:30會員大會於醫學綜合大樓**前棟**4F誠樸廳
- 10/1晚宴接駁車於臺北醫學大學校門口警衛室旁乘車處
- 停車於醫學綜合大樓地下停車場之與會貴賓，請由B2、B3之醫學綜合大樓後棟搭乘電梯至1F報到處、16F演講廳。

Floor Plan





List of Sponsors

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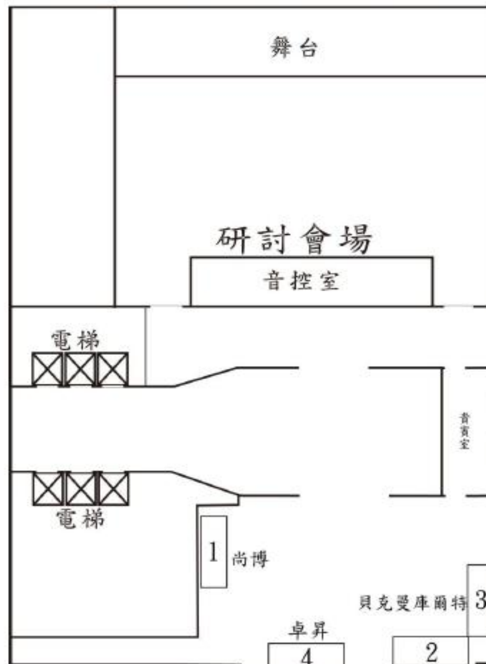
Exhibition Floor Plan

臺灣幹細胞學會2011年會
臺北醫學大學十六樓會議廳



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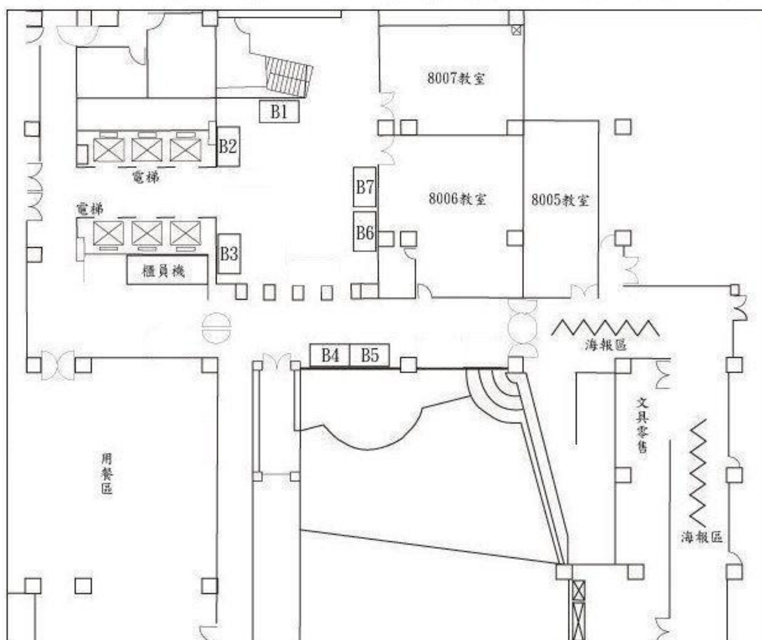

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Keynote Lecture I & II

Session Chairs:

Chii-Ruey Tzeng, M.D. (曾啟瑞院長)

Professor and Dean,
College of medicine,
Taipei Medical University

Bon-Chu Chung, Ph.D. (鍾邦柱教授)

Distinguished Research Fellow,
Institute of Molecular Biology,
Academia Sinica

Tatsutoshi Nakahata, M.D., D.M.Sci.

Professor and Deputy Director
Center for iPS Cell Research and Application
Kyoto University
E-mail: tnakaha@cira.kyoto-u.ac.jp

Recent Selected Publications:

1. Hemopoietic colony-forming cells in umbilical cord blood with extensive capability to generate mono- and multipotential hemopoietic progenitors. (with Ogawa,M.). J. Clin. Invest. 70:1324-1328, 1982.
2. Extensive proliferation of mature connective-tissue type mast cells in vitro. (with Kobayashi, T. et al) Nature 324:65-67, 1986.
3. Stimulation of mouse and human primitive hematopoiesis by murine embryonic aorta-gonad-mesonephros- derived stromal cells. (with Xu, M. et al). Blood 192:2032-2040, 1998.
4. Expansion of human NOD/SCID-repopulating cells by a combination of stem cell factor, Flk2/Flt3 ligand, thrombopoietin and a complex of interleukin-6 and soluble interleukin-6 receptor. (with Ueda, Y. et al). J. Clin. Invest. 105: 1013-1021, 2000.
5. NOD/SCIDγ_c^{null} mouse: an excellent recipient mouse model for engraftment of human cells. (with Ito, M. et al). Blood 100:3175-3182, 2002.
6. Generation of multipotent stem cells from postnatal mouse testis. (with Kanatsu-Shinohara M. et al). Cell 119:1001-1012, 2004.
7. Generatuion of functional erythrocytes from human embryonic stem cell-derived definitive hematopoiesis. (with Ma, F. et al.). Proc. Natl. Acad. Sci. USA 105:13087-13092,2008.
8. Disease-associated CIAS1 mutations induce monocyte death, revealing low-level mosaicism in mutation-negative cryopyrin-associated periodic syndrome patients. (with Saito M. et al). Blood 111:2132-2141,2008.
9. Frequent inactivation of A20 in B-cell lymphomas. (with Kato, M. et al.). Nature 459:712-716,2009.
10. Generation of transplantable, functional satellite-like cells from mouse embryonic stem cells. (with Chang H・et al）. FASEB J. 23:1907-1919,2009.
11. Generation of skeletal muscle stem/progenitor cells from murine induced pluripotent stem cells.(with Mizuno Y. et al). FASEB J. 24:2245-2253, 2010.
12. Neutrophil differentiation from human-induced pluripotent stem cells. (with Morishima T. et al). J. Cell. Physiol. 226(5):1283-1291,2011.
13. A novel serum-free monolayer culture for orderly hematopoietic differentiation of human pluripotent cells via mesodermal progenitors. (with Niwa A. et : PloS ONE in press.

Various Clinical Applications of Human Induced Pluripotent Stem Cells

Tatsutoshi Nakahata, M.D., Ph.D.
Center for iPS Cell Research and Application (CiRA), Kyoto University

In 2007, Drs. Takahashi and Yamanaka in our institute first demonstrated the generation of induced pluripotent stem (iPS) cells from adult human dermal fibroblasts by the retrovirus-mediated transduction of four transcriptional factor genes, *Oct3/4, Sox2, c-Myc,* and *Klf4.* Human iPS cells are similar to human embryonic stem (ES) cells in morphology, cell surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, telomerase activity, and differentiation capability into cell types of the three germ layers in vitro and in vivo. iPS cells are infinitely expandable and capable of differentiating into all types of somatic cells. Thus, it is our earnest desire to use iPS cells after induction into clinical grade somatic cells of the relevant cells in regenerative medicine.

The possibility of reprogramming mature somatic cells to generate iPS cells has enabled the derivation of various disease-specific pluripotent cells, thus providing unprecedented experimental platforms to model human disease. Disease-specific iPS cells would allow study of appropriated living cells generated from the patients with unknown genetic legion, providing insight into their interactions with other cell types, and their susceptibility to the environment that are considered to play an important role in the pathogenesis of various diseases. Disease-specific iPS cells would be useful to monitor drug safety and discover new therapeutic agents. In addition, the generation of patient-specific iPS cells may have a wide range of applications in cell and gene therapy. Recently, we succeeded the generation of various disease-specific iPS cells from patients with Duchenne muscular dystrophy (DMD), immune deficiency diseases, and hematopoietic disorders such as Kostmann syndrome and Fanconi’s anemia. Finally, I would like to introduce special disease-specific iPS cells generated from a CINCA (Chronic Infantile Neurological Cutaneous and Articular) syndrome patient who have disease-associated CIAS1 mutation as somatic mosaicism.

Abhay Pandit, Ph.D.

Director,
Network of Excellence for Functional Biomaterials,
National University of Ireland, Galway
E-mail: abhay.pandit@nuigalway.ie

Professor Pandit's research program hosts several patented technology platforms associated with the development of implantable materials for clinical applications. Functionality to these forms is achieved through custom chemistries which facilitate the attachment of surface tethered moieties or encapsulated therapeutic factors including drugs, genes and other active agents. NFB has developed the next generation of nanostructures that are more robust, biocompatible and responsive to their environment (temperature and pH sensitive) and thus triggers the smart release of the biomolecule facilitating targeted drug delivery to a specific site. This platform technology assigns a dual nature of diverse responsiveness and function to biomaterials and offers abundant possibilities in the fields of regenerative medicine, sensors and drug delivery. These platforms have been developed for musculoskeletal, cardiovascular, soft tissue repair and neural targets. His research is currently funded by Science Foundation Ireland, Enterprise Ireland, Health Research Board, Dystrophic Epidermal Bullosa Research Association, 7th EU Framework, European Molecular Biology Organisation and AO Foundation amongst others. He has generated research contracts from industry and government funding agencies totaling €22,093,630 in the last 9 years. He is the author of 2 patents with 13 other patent applications pending. He has published 93 papers in high-impact factor publications including Advanced Drug Delivery Reviews, European Cells and Materials, Molecular Therapy, Soft Matter, Trends in Biotechnology, Small, Biomaterials and Tissue Engineering and has authored 336 papers at both national and international conferences. He is a member of the editorial board of 9 journals including Biomaterials, Journal of Materials Science: Materials in Medicine, Journal of Tissue Engineering, Journal of Biomedical Materials Research – Part B, The Open Biomaterials Journal, Tissue Engineering, Recent Patents on Biomedical Engineering and European Cells and Materials.

A Functionalized Scaffold for Modulation of Inflammation to Permit Stem Cell Survival in Myocardial Infarction

Abhay Pandit, Ph.D.
Network of Excellence for Functional Biomaterials,
National University of Ireland, Galway

Cardiovascular disease is the leading cause of death in the developed world and is responsible for approximately 36% of Irish mortality. Myocardial infarction (MI), which is literally the death of cardiac tissue due to lack of oxygenation, accounts for the majority of deaths associated with cardiovascular disease. This death of cardiac tissue leads to a loss of cardiac function as the damaged area becomes a non-contractile scar. Reversal of this process is the main aim of regenerative cardiac strategies such as stem cell transplantation. While initial studies were promising, subsequent clinical trials yielded disappointing results. Stem cell therapy may be limited by the poor survival rate of the cells after implantation into the infarcted heart, which is likely due to the inflammatory response. Thus, anti-inflammatory gene therapy with interleukin-10 (IL-10) was proposed as a method to modulate the inflammatory response after implantation of a collagen scaffold seeded with rat mesenchymal stem cells (rMSCs). IL-10 is considered the most potent anti-inflammatory cytokine produced naturally and has been used in a number of studies to decrease or control inflammation. It was hypothesized that IL-10 gene therapy could be used to increase the retention rate of stem cells in a collagen scaffold when delivered to the ischemic myocardium. The primary objectives were to develop a controlled release scaffold-based gene therapy system suitable for stem cell delivery to the infarcted myocardium. The efficacy of this system was evaluated by assessing stem cell retention, overall cardiac function and the inflammatory response. A crosslinked collagen scaffold was developed and optimised for rMSC culture *in vitro*. Non-viral plasmid-dendrimer polyplexes were optimized for transfection in both two and three-dimensional culture. When cells were seeded into polyplex loaded scaffolds, relatively high levels of transgene expression were observed for up to three weeks of culture. When the polyplex-loaded scaffolds were implanted in rat skeletal muscle, increased retention of rMSCs was observed. This was associated with decreased inflammation and a change in macrophage phenotype from cytotoxic to regulatory. Similarly, when the polyplex-loaded scaffolds were implanted over the surface of infarcted rat hearts, rMSC retention was increased, the inflammatory and remodelling responses were modulated and, most importantly left ventricular ejection fraction – a measure of cardiac function – was significantly improved. Thus, combining biomaterial, gene and cell therapy improved functional outcomes after rMSC transplantation following MI. This combinatorial strategy can be utilised to provide functional efficacy in disease targets.

A: iPS and Pluripotent Stem Cells (I)

Session Chairs:

Winston TK Cheng, Ph.D. (鄭登貴教授)

Regius professor,
Department of Animal Sciences and Biotechnology,
Tunghei University

Chung-Liang Chien, Ph.D. (錢宗良教授)

Professor,
Department of Anatomy and Cell Biology,
College of Medicine,
National Taiwan University

Huck-Hui Ng, Ph.D.

President, Stem Cell Society of Singapore
Senior Group Leader &Associate Director,
Genome Institute of Singapore
E-mail: nghh@gis.a-star.edu.sg

Recent Selected Publications:

1. Jiang J, Ng HH. TGFbeta and SMADs talk to NANOG in human embryonic stem cells. Cell Stem Cell. 2008 Aug 7;3(2):127-8.
2. Chen X, Xu H, Yuan P, Fang F, Huss M, Vega VB, Wong E, Orlov YL, Zhang W, Jiang J, Loh YH, Yeo HC, Yeo ZX, Narang V, Govindarajan KR, Leong B, Shahab A, Ruan Y, Bourque G, Sung WK, Clarke ND, Wei CL, Ng HH. Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. Cell. 2008 Jun 13;133(6):1106-17.
3. Jiang J, Chan YS, Loh YH, Cai J, Tong GQ, Lim CA, Robson P, Zhong S, Ng HH. A core Klf circuitry regulates self-renewal of embryonic stem cells. Nat Cell Biol. 2008 Mar;10(3):353-60.
4. Feng B, Jiang J, Kraus P, Ng JH, Heng JC, Chan YS, Yaw LP, Zhang W, Loh YH, Han J, Vega VB, Cacheux-Rataboul V, Lim B, Lufkin T, Ng HH. Reprogramming of fibroblasts into induced pluripotent stem cells with orphan nuclear receptor Esrrb. Nat Cell Biol. 2009 Feb;11(2):197-203.
5. Feng B, Ng JH, Heng JC, Ng HH. Molecules that promote or enhance reprogramming of somatic cells to induced pluripotent stem cells. Cell Stem Cell. 2009 Apr 3;4(4):301-12.
6. Heng JC, Feng B, Han J, Jiang J, Kraus P, Ng JH, Orlov YL, Huss M, Yang L, Lufkin T, Lim B, Ng HH. The nuclear receptor Nr5a2 can replace Oct4 in the reprogramming of murine somatic cells to pluripotent cells. Cell Stem Cell. 2010 Feb 5;6(2):167-74.
7. Kunarso G, Chia NY, Jeyakani J, Hwang C, Lu X, Chan YS, Ng HH, Bourque G. Transposable elements have rewired the core regulatory network of human embryonic stem cells. Nat Genet. 2010 Jul;42(7):631-4.
8. Chia NY, Chan YS, Feng B, Lu X, Orlov YL, Moreau D, Kumar P, Yang L, Jiang J, Lau MS, Huss M, Soh BS, Kraus P, Li P, Lufkin T, Lim B, Clarke ND, Bard F, Ng HH. A genome-wide RNAi screen reveals determinants of human embryonic stem cell identity. Nature. 2010 Nov 11;468(7321):316-20.
9. Ng JH, Ng HH. LincRNAs join the pluripotency alliance. Nat Genet. 2010 Dec;42(12):1035-6.
10. Ng HH, Surani MA. The transcriptional and signalling networks of pluripotency. Nat Cell Biol. 2011 May;13(5):490-6.

Induction of Pluripotency

Huck-Hui, NG, Ph.D.

Genome Institute of Singapore

Embryonic stem cells (ESCs) are characterized by their ability to self-renew and remain pluripotent. A landmark work from Professor Shinya Yamanaka demonstrates that it is now feasible to reprogram somatic cells into induced pluripotent stem cells (iPSCs) using a simple method of expressing defined transcription factors. The ESCs and iPSCs hold tremendous clinical potential as they can be directed to differentiate into specialized cell-types of therapeutic importance. I will highlight our work on the development of alternative ways to reprogram somatic cells and on the understanding of human ESCs. I will also review the recent progresses on the characterization of iPSCs and their implications. Finally, the clinical applications of this remarkable technology will be discussed.

Qi Zhou, Ph.D.

Director, Center for Stem Cell Research and Regenerative Medicine
Institute of Zoology,
Chinese Academy of Sciences
E-mail: qzhou@ioz.ac.cn

Recent Selected Publications:

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Pluripotent Stem Cells and the Potential Application in Regenerative Medicine

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Stem Cell, with its property of unlimited proliferation and fully developmental potential, has become important cell resource in regenerative medicine research. For most researches focus on embryonic stem cells, the ethical issue has been an obstacle for its application. Induced pluripotent stem (iPS) technique is not limited by availability of donor cells or ethical objections, so it has been the hotspot of stem cell research and expected to have broad applications in regenerative medicine since it was invented in 2006. Up to now, ES cells and iPS cells have been successfully derived from mice, human, monkeys, rats and pigs and have been differentiated into many tissue specific cells. Recently, several progresses in stem cell research field have been achieved: Using the tetraploid complementation method, Zhao et al successfully produced viable mice from iPS cells, proven that fully reprogrammed iPS cells possessed similar pluripotency as ES cells; microarray and high-throughput sequencing data revealed that mouse ES and iPS cells with tetraploid complementation ability (4n-iPS cells) shared similar expression patterns, but were distinguishable from tetraploid complementation incompetent iPS cells in the Dlk1-Dio3 imprinting region; we also demonstrated that this marker region was also positively correlated with the pluripotent levels of 3F-iPS cells, indicating the feasibility of using this region as a marker to select full-pluripotent 3F-iPS cells; by comparing the development of embryonic and adult mice derived from iPS cells, we found it exhibited similar developmental features as ES cells, yet were prone to tumorigenesis.. These achievements will greatly promote the application of stem cell in both basic research and regenerative medicine.

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Utilization of iPS Cells for Retinal Regenerative Medicine

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Regenerative medicine has two strategies, one is cell replacement therapy and another is rescue of remaining cells by trophic factors. As for retinal pigment epithelial (RPE) cells, we can purify them from mixture of cells differentiated from iPS cells. By transplantation of these cells for wet type age-related macular degeneration, we can replace senescent RPE cells with young healthy RPE. Furthermore, auto-transplantation of mature RPE cells will not cause immune rejection.

The iPS cell-derived RPE cells show the characteristics of mature naïve RPE cells, such as brown polygonal shapes, expression of RPE specific genes, phagocytosis activity, secretion of various growth factors and tight junction formation.

We are now preparing the clinical level of culture methods and the clinical protocol. Of course we should precisely evaluate the safety of the end product in vivo and in vitro before clinical trial, yet the RPE cells derived from iPS cells are approaching to clinical trial.

B: Cancer Stem Cells

Session Chairs:

Hong-Nerng Ho, M.D. (何弘能理事長)

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Deputy Dean, College of Medicine,
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Cancer Stemness: Activation of Stemness Genes in Lung Cancer Cells

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Lung cancer is one of the leading causes of cancer-related deaths worldwide. In particular, LAC is the most common histological type. Its highly invasive and metastatic phenotypes are the major reasons for treatment failure and poor prognosis. Furthermore, a high failure rate and a low median survival rate are observed in patients undergoing chemoradiotherapy with recurrent, intractable LAC. To improve the patient survival, it is important to elucidate the regulatory mechanisms that control tumor-initiating and metastatic properties of LAC. Recently the cancer stem cell theory provides new insights in tumorigenesis. Increasing evidence from solid tumors and hematopoietic malignancies has strongly supported the concept that a subpopulation of cancer cells, termed cancer stem cells (CSCs) or cancer initiating cells, in each tumor has greater potential of cancer initiation and repopulation. This highly tumorigenesis and malignant population of cancer cells are thought to be responsible for the malignant progression and acquisition of drug-resistance ability of tumor. The cancer stem cells share some critical characteristics with normal stem cells, including the capacities for self-renewal, multi-lineage differentiation, and maintained proliferation, as well as expression of several stem cell-specific markers.

We started with the embryonic stem cell (ESC) transcription factors, Oct4, Nanog and Sox2, to analyze the mechanistic interplays between stemness pathway and tumorigenicity in lung adenocarcinoma cells. We found that Oct4 and Nanog alter the gene expression signature of lung cancer cells into a pattern similar to that of ESCs, and enhanced their CSC properties such as self-renewal, migration, and chemotherapy resistance. In animal model, Oct4/Nanog increases the tumor-initiating capacity and metastasis incident. High level of Oct4 and Nanog are correlated with poor patient survival outcome. Oct4/Nanog may serve as a potential target for tackling the stem-like population of cancer cells, as well as prediction marker. On the other hand, we discovered that another stemness factor, Sox2, forms a positive feedback regulatory loop with epithelial growth factor receptor (EGFR), the most commonly deregulated signaling pathway in lung cancer, and promotes cell proliferation and tumorigenesis. Our data demonstrated strong links between stemness factor-mediated regulations and cancer biology. Disregulated stemness pathways can induce cancer incidence or encourage cancer malignancy. Stemness factors act as a switch turning on stem-like properties in cancer and thus result in an obstacle for treatment. These factors might serve as prognostic markers to detect highly malignant lung cancer. Moreover, to target these miss placed stemness pathway would be a prospective direction for further therapeutic development.

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Significance of Twist1-Bmi1 Axis in Epithelial-mesenchymal Transition, Stem-like Properties and Movement of Cancer Cells

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Epithelial-mesenchymal transition (EMT) is a developmental process in which epithelial cells lose their polarity and acquire the migratory properties of mesenchymal cells. EMT has been shown to be the pivotal mechanism contributing to cancer metastasis. A recent breakthrough in metastasis research reveals that induction of EMT also generates cells with stem-like properties. However, the mechanism is not yet clear. We recently showed that the EMT inducer Twist1 directly regulated the transcription of *BMI1*, which is a member of polycomb repressive complex and maintains self-renewal of normal and cancer cells. Twist1 and Bmi1 were mutually essential to promote EMT and tumor-initiating capability. Twist1 and Bmi1 acted cooperatively to repress both *E-cadherin* and *p16INK4A*. Furthermore, our preliminary results demonstrated a novel mechanism in which Twist1 elicits cancer cell movement through activation of Rac1. Twist1 cooperates with Bmi1 to suppress microRNA *let-7i* expression, which results in upregulation of the Rac1 co-activators NEDD9 and DOCK3, leading to activating Rac1 and engendering mesenchymal-mode movement in three-dimensional environments. Meanwhile, suppression of *let-7i* contributes to Twist1-induced stem-like properties. Our results uncover an essential mechanism for understanding how Twist1 generates the motile stem-like cancer cells beyond the suppression of E-cadherin.

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Cancer Stem Cells on Head and Neck Cancer Tumorigenesis

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Head and Neck Cancer, including cancers arising from four basic areas: oral cavity, oropharynx, hypopharynx and larynx, represents the sixth prevalent malignancy worldwide, and the third most common cancer in developing nations. More than 95% of head and neck cancers are squamous cell carcinoma (HNSCC), including oral squamous cell carcinoma (OSCC). During 2010, OSCC has become the fifth most common type of cancer and ranked the fourth most lethal cause for male cancer patients in Taiwan, responsible for 1,794 deaths per year.

Disappointingly, despite of the improvement in diagnosis and management of OSCC, long-term survival rates have improved only moderately over the past decade. The prognosis of OSCC remains dismal because more than 50% of patients die of this disease or complications within 5 years under current therapies. The major problems come from limited ability on treatment to the heterogeneous nature of HNSCC. Therefore, prevention and early diagnosis, as well as an improved comprehension of the cellular–molecular events that regulates the cancer carcinogenesis processes, would help in reducing morbidity and increasing survival rates of HNSCC patients.

Tumor is composed of a heterogeneous population of cells, and it has been observed that a subpopulation of cells, so-called cancer stem cells (CSCs) or cancer initiating cells (CICs), within tumor tissues have stemness properties. CSCs are considered to be responsible for the initiation, propagation, metastasis of tumors. Importantly, the existence of CSCs might explain cancer recurrences, even after clinical treatment with either radiotherapy or chemotherapy on cancer patients. We have enriched oral cancer stem-like cells (OC-SLCs) from OSCC cells (OSCCs) or primary cultures of OSCC patients by tumor sphere formation approach. The isolated OC-SLCs possess both the characteristics of stem cells and malignant tumors. Additionally, elevated expression of stemness markers have been shown to negatively correlate with the survival prognosis of OSCC patients.

Further, we determined a candidate of stemness-maintaining molecule for HN-CICs the pro-inflammatory mediator S100A4, which is also known to be an inducer of epithelial-mesenchymal transition (EMT). Downregulation of S100A4 in HN-CICs reduced their self-renewal capability and their stemness and tumorigenic properties, both in vitro and in vivo. Conversely, S100A4 overexpression in HNSCC cells enhanced their stem cell properties. Mechanistic investigations indicated that attenuation of endogenous S100A4 levels in HNSCC cells caused inhibition of Notch2 and PI3K/pAKT along with up-regulation of PTEN, consistent with our biological findings. Immunohistochemical analysis of HNSCC clinical specimens showed that S100A4 expression was positively correlated with clinical grading, stemness markers and poorer patient survival. Together, we reveal a crucial role for S100A4 signaling pathways in maintaining the stemness properties and tumorigenicity of HN-CICs. Further, our findings suggest that targeting S100A4 signaling may offer a new targeted strategy for HNSCC treatment by eliminating HN-CICs.Overall, the enhancement of cancer stem-like cell properties in HNSCC should be warranted in the future translational oncology with the ultimate objective of improving anti-cancer therapy.

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6. Al-Adsani A., Z.D. Burke, D. Eberhard, K. L. Lawrence, C.N. Shen, A K. Rustgi, H. Sakaue, J. M. Farrant and D Tosh. 2010. Dexamethasone treatment induces the reprogramming of pancreatic acinar cells to hepatocytes and ductal cells. PLoS One 5(10): e13650.
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8. Liaw C.C., W.Y.Liao, C.S.Chen, Y.C.Wu, S.C. Jao, C.N. Shen*,and S.H. Wu. 2011. Calcium-Chelating Capability of Tetrahydrofuranic Moieties Modulates Cytotoxicity of Annonaceous Acetogenins. Angewandte Chemie Intl Ed 50(34): 7885-7891. *(*corresponding author)*
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Identification of Cancer Stem Cells from Pancreatic Adenocarcinoma with Higher Metastatic Potentials

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The evolution towards more aggressive form of tumor is often referred to as cancer progression which is thought to originate from the development of heterogeneous cancer population combined with the continuous selection toward more malignant cellular phenotypes. Indeed, metastasis is the major cause of death in pancreatic cancer patients, where most patients are diagnosed with metastatic disease and few show a sustained response to chemotherapy or radiation therapy. Recent identification of cancer stem cells might provide a solution to explain how cancer heterogeneity can be achieved. However, whether cancer stem cells existed and contribute to cancer metastasis need to be addressed further. Initially, we demonstrated that only rare population of pancreatic cancer cells are double-positive for CD24 and CD44. We further identified $CD44^+$ $ABCG2^+$ ($CD24^+$ or $CD24^-$) subpopulation not only had self-renewal capability and higher tumorigenicity, but they also had higher metastatic potentials. And this metastatic behavior was not seen in the sorted $CD44^+CD24^+ABCG2^-$ subpopulation or unsorted population suggesting the metastatic subpopulation are derived clonally and suppressed by existence of other cancer cell population. We also showed that $CD44^+CD24^+ABCG2^+$ cells displayed drug resistance, can be maintained constantly in culture, and were able to generate different subpopulations *in vitro*. Based on using extracellular flux analyzer, we revealed that the $CD44^+CD24^+ABCG2^+$ subpopulation has metabolic plasticity that produced higher levels of lactate and had higher oxygen consumption rate during differentiation suggesting this subpopulation can be selectively outgrow under extreme nutritional conditions. These finding provide novel insights into the metabolic features underlying pancreatic cancer progression and define a clonal integrity of cancer stem/initiating cell to contribute to pancreatic cancer metastasis.

C: Somatic Stem Cells and Regenerative Medicine (I)

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Sheng-Mou Hou, M.D., Ph.D. (侯勝茂院長)

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Recent Selected Publications:

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Mesenchymal Stem Cells: Plasticity and Application

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Mesenchymal stem cells (MSCs) were first defined as a group of cells which were of self-renewal ability, were able to be culture-expanded for a prolonged period of time, and are able to differentiate into various lineages of connective progenies originated from embryonic mesoderm including bone, cartilage and adipose tissues. These cells were first isolated from bone marrow; subsequently, MSCs isolated from other sources such as liposuction fat, synovial tissues and trabecular bone have been reported by other investigators. Recently, it was reported that certain population of stem cells in human bone marrow, although low in frequency, were able to differentiate into cells and tissues originated from not only mesoderm, but also ectoderm.

In our laboratory, we have successfully isolated and culture-expanded MSCs from human bone marrow and umbilical cord blood using negative immuno-selection and limiting dilution methods. Surface phenotype of these cells was performed using flow cytometry and surface marker phenotype was characteristic of MSCs. These MSCs were able to differentiate into progenies originated from all three germ layers including osteoblasts, chondrocytes, adipocytes, neuroglial cells and hepatocytes. In vitro functionality of these differentiated progenies was also demonstrated. Particularly, MSC-differentiated hepatocytes have been shown to be able to secrete urea and uptake of low density lipoproteins is also noted in these MSC-differentiated hepatocytes.

There are various applications of MSCs in biomedical research both *in vitro* and *in vivo*. The in vitro model of MSC culture can serve as an excellent model to study the control of differentiation as well as the cell fate in each lineage. Novel genes and proteins that control differentiation can also be explored in this model. Besides, it can also be used for screening of new drugs and compounds. Most important of all, MSCs are indispensable in the study of cell therapy, tissue engineering and regenerative medicine. In order to justify the possibility of applying MSC transplantation at the fetal stage for future therapeutic use, we have performed *in utero* transplantation of human BM-derived MSCs into fetal mice to investigate the possibility of MSC transplantation as a form of fetal therapy. The results showed that human BM-derived MSCs were found in tissues originated from all three germ layers. In summary, it is foreseeable that in the near future, MSCs will revolutionize the treatment of a variety of diseases. Therefore, more efforts should be made to further elucidate the basic science of MSCs so that such technologies can be applied from bench to bedside.

Shih-Chieh Hung, M.D., Ph.D. (洪士杰教授)

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Benefits of Hypoxic Culture on Human Mesenchymal Stem Cells

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Oxygen is essential for life, but cultivation of cells is usually performed under 21% O_2. Such a condition does not replicate the hypoxic conditions of normal physiological or pathological status in the body. Recently, the effects of hypoxia on bone marrow multipotent stromal cells or mesenchymal stem cells (MSCs) have been investigated. In a long term culture, hypoxia can inhibit senescence, increase the proliferation rate and enhance differentiation potential along the different mesenchymal lineages. Hypoxia also modulates the paracrine effects of MSCs, causing upregulation of various secretable factors, including the vascular endothelial growth factor and IL-6, and thereby enhance wound healing and fracture repair. Finally, hypoxia plays an important role in mobilization and homing of MSCs, primarily by its ability to induce stromal cell-derived factor-1 expression along with its receptor, CXCR4. After transplantation into ischemic limb, an environment combined of hypoxia and serum deprivation, can lead to apoptosis or cell death, which can be overcome by the hypoxic preconditioning of MSCs. More importantly, the mechanism involved in the increase of MSC properties by hypoxic culture is through the upregulation of hypoxic inducible factor-1 signaling pathway.

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Recent Selected Publications:

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Wnt Regulates Neurotrophin-induced Neuronal Transdifferentiation of Human Bone Marrow-derived Mesenchymal Stem Cells via β-catenin Signaling and Non-canonical Pathway

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Previous studies reported neurotrophins (NT) and retinoic acid (RA) triggered a transdifferentiation of human mesenchymal stem cells (hMSCs) toward neuronal progenitor-like cells. The Wnt is crucial during embryonic development and adult tissue homeostasis. In the nervous system, Wnt regulates the neurogenesis, such as neurite and synapse formation, of neural stem or progenitor cells. Thus, we hypothesize that Wnt regulates the terminal neuronal differentiation of hMSCs. Stimulation of hMSCs with neurogenic differentiation medium (NDM) resulted in expression of Wnt1, 7a, and 7b. Wnt7a showed an up-regulated effect on neuronal markers expressed by hMSCs. In addition, incorporated neurotrophin with Wnt7a or lithium hMSCs expressed Synapsin1. The induction was furhter inhibited by Wnt inhibitors by rhDKK-1, sFRP-4, anti-human Frz-5 and anti-mouse Frz-9 antibody. Moreover, Wnt7a triggered formation of cholinergic, dopaminergic, GABAergic and serotonergic neurons. Conclusively, canonical Wnt7a is crucial to synapse formation and neuron type determination in hMSCs. These findings are pivotal in stem-cell therapy for nerve regeneration.

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Recent Selected Publications:

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3D Culture of Adherent Stem Cells

Bin-Ru She, Ph.D.

Tissue Regeneration Product Technology Division, Biomedical Technology and Device Research Labs, Industrial Technology Research Institute

The pharmaceutical industry is highly interested in mesenchymal stem cells (MSC) for their potential in semi-mass production and allogenic applications. However, cell attachment area of 5-10 $x10^6$ cm^2 is likely to be required for producing 10^{11} MSC, possibly 1000 doses for treatment such as GVHD. For conventional 2D culture, this requirement presents great difficulties in space, equipment, labor and quality control. 3D culture provides opportunity to overcome the difficulties. Currently 3D cell culture is used in pharmaceutical industry to produce vaccines and protein drugs. In tissue engineering, 3D scaffolds are often used to create tissue-like structure. These technologies will require major adaptation if they are to be used for the production and harvesting of high quality cells in the form of single-cell. We aimed to build a 3D culture system for producing therapeutic mesenchymal stem cells. In the system, cells are grown in serum-free medium on thin 3D scaffolds coated with synthetic polymers. We developed a serum-free medium which allows the stem cells derived from bone marrow, adipose tissue and umbilical cord to proliferate equally to or faster than they do in FBS-containing medium. No abnormality of karyotype was detected for the cells grown in this medium. Cells were frozen and thaw in this medium supplemented with DMSO without losing viability. In collaboration with Altrika Inc. in UK, through the HTP screening by polymer chips we discovered several synthetic polymers on which cells attached and grew well. The polymers may replace ECM coating on culture surface for various applications. It is also demonstrated that the thin 3D scaffold provided advantages in producing and harvesting large number of cells.

Keynote Lecture III & IV

Session Chairs

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Distinguished Professor,
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National Cheng Kung University

Win-Ping Deng, Ph.D. (鄧文炳教授)

Director and Professor,
Institute of Biomedical Materials and Engineering Director &
Stem Cell Research Center,
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Allan Bradley, Ph.D., F.R.S., F.MedSci.

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Recent Selected Publications:

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Genetics in Stem Cells; From Discovery to Therapy

Allan Bradley, Ph.D.

The Wellcome Trust Sanger Institute

Allan is former Director of the Wellcome Trust Sanger Institute, where he holds the title of Director Emeritus. He leads the Mouse Genomics Team, which uses the mouse as a model system to investigate the function of individual gene.

In 1984 Allan Bradley and Liz Robertson demonstrated that embryonic stem (ES) cells could be transmitted through the germ line of mice and two years later reported that ES cells could be used to generate mice with mutations in endogenous genes. This work contributed to the award of the Nobel Prize in Physiology or Medicine to Martin Evans in 2007. In 1987, Allan took up an appointment as an Assistant Professor at Baylor College of Medicine, Houston Texas. He was appointed a Howard Hughes Medical Institute Investigator in 1993 and was promoted to full Professor in 1994. At The Baylor College of Medicine, his laboratory played a seminal role in developing the techniques, technology and tools for genetic manipulation in the mouse. As a result mice can now be generated with changes as subtle as an alteration in a single nucleotide or as massive as the deletion, duplication or inversion of millions of base pairs, a technology that has become known as chromosome engineering. Allan Bradley's laboratory have used ES cell technology extensively, generating and analysing many of the first generation of mouse knockouts as well as helping numerous other laboratories to utilize this technology. This work has provided key functional information on many genes with an emphasis on cancer, DNA repair and embryonic development. In 2000, Allan returned to the United Kingdom as Director of the Sanger Centre, now called the Wellcome Trust Sanger Institute. He has established a new direction for the Institute - genetic analysis of gene function, which includes among other projects the largest systematic gene knockout project ever attempted in ES cells funded by the European Union (EUCOMM) and National Institutes of Health (KOMP).

David F. Williams, Ph.D., D.Sc.

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Professor Williams started his career in biomaterials science, in 1967. He worked alongside some of the early pioneers in implant surgery, including Charnley, the inventor of total hip replacement, and quickly realized that too little was known about the host response to implanted biomaterials for there to be any reasonable hope of routine success. He wrote the first monograph on this subject in 1973 in order to place the existing scientific knowledge into the perspective of the amazing potential that reconstructive surgery offered. He then set out to develop *in vitro* and in *vivo* systems to explore the subject of biocompatibility, encompassing phenomena such as polymer biodegradation, physiological metallic corrosion, the role of the immune response in the biocompatibility of synthetic biomaterials and mechanobiology. Crucially, he developed systems for the quantification of the host response to biomaterials, using, for the first time, the combination of immunohistochemistry and image analysis. He went on to study, and introduce into clinical practice, some high performance engineering biomaterials, including new titanium alloys, silver-based products, highly biostable polyurethanes, biodegradable polymers such as the PHB family and thermoplastics such as PEEK. He went on, through directing two of the major collaborative programs in tissue engineering in Europe to identify the essential specification parameters for tissue engineering biomaterials.

During the last decade, Professor Williams has worked extensively on issues of balancing risk and innovation in medical technology, and has written several Opinion Papers on which new European legislation has been based, including public health issues concerned with latex, PVC, dental amalgams and so on. In recent years he has devoted more time and energy to the development of new research strategies and the promotion of biomaterials science and regenerative medicine, and has advised governments and institutions around the world on these matters. His recent publications on the nature of biomaterials and the mechanisms of biocompatibility are already seen as seminal works in the evolution of biomaterials science. His primary focus has been as Editor-in-Chief on the journal *Biomaterials*, which he has now taken to the top position in the world's journals in this area. He travels extensively, especially in Asia, to promote excellence in scientific research and writing. In the pursuit of the globalization of excellence in biomaterials research, he has been elected to the position of President-elect of TERMIS, the international society concerned with regenerative medicine, and is actively promoting the application of biomaterials science in Asia and Africa. Professor Williams is currently finishing a single-author textbook, on Essential Biomaterials Science, to be published by Cambridge University Press, nearly 40 years after publication of his first book.

Therapeutic Consequences of Cell-Biomaterial Interactions

David F. Williams, Ph.D., D.Sc.

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Much has been written about the potential for cell therapies to treat many conditions for which there are few existing clinical options. For many reasons, few procedures have delivered on this potential. This is in spite of the many significant advances have been made in our understanding of the science of cell biology, both with respect to the stem and progenitor cells and fully differentiated cells that ideally form the basis of regenerative medicine procedures. Both cell therapies and tissue engineering procedures depend on cell signaling processes, and these are guided by combinations of mechanical and molecular cues. It is suggested here that we have yet to understand and exploit the synergistic effects that these two separate types of signaling processes have on cell behavior within the regenerative mode. This is especially true with respect to the role of biomaterial characteristics on cell behavior. This lecture will explore the science of mechanotransduction and mechanical signaling and discuss the significance of superior specifications for scaffolds and matrices with respect to the persuasion on cells to generate new tissue.

D: iPS and Pluripotent Stem Cells (II)

Session Chairs:

Nan-Chi Chang, Ph.D. (張南驥教授)

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Kwang Yul Cha, M.D

President of CHA Biotech,
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Dr. Kwang Yul Cha is the Founder and Chancellor of CHA HEALTH SYSTEMS and CHA University in Seoul, Korea. Dr. Cha received his medical degree from the prestigious Yonsei University graduating summa cum laude and performed his postdoctoral fellowship in endocrinology and infertility at the University of Southern California. Dr. Cha also served as a visiting professor at Columbia University where he established and operated CHA Columbia Fertility Center in collaboration with Columbia Presbyterian Hospital.

An internationally renowned medical expert and one of the most recognized authorities on reproductive endocrinology and infertility, Dr. Cha pioneered the world's first successful pregnancy and birth through in vitro maturation from immature oocytes (1987) and also the world's first successful birth with frozen oocytes utilizing the technique of oocyte vitrification using grid (1999). His landmark accomplishment received worldwide media coverage on NBC, CBS, BBC, The Wall Street Journal, TIME Magazine, People Magazine and many others. And in 2001, Dr. Cha yet again became the first in the world to establish a commercial ovum bank, located in Los Angeles, which has naturally become the archetype for all future commercial ovum banks opened throughout the world.

Among all of his countless accomplishments, accolades and appointments, Dr. Cha also holds the following distinctions:

- Author of numerous books, medical reviews, and over 200 articles & papers published in medical journals
- Recipient of more than 10 Prize Paper Awards conferred by the American Society for Reproductive Medicine, the International Federation of Fertility & Sterility, and the European Society of Human Reproductive Endocrinology for his authorship of selected journal articles & papers
- Distinguished guest speaker at over 80 international medical conferences & symposiums
- Founder & President of Asia's first and most notably recognized society for fertility – Pacific Rim Society for Fertility & Sterility

Stem Cell Therapy for the Regenerative Medicine and Anti-aging

Kwang-Yul Cha, M.D.
CHA Stem Cell Institute, CHA University College of Medicine,
CHA Health Systems

Stem cell therapies are one of the most promising areas in medicine, and many clinical studies are in progress to develop therapies for chronic degenerative diseases by industrial and academic groups. Stem cells have two important properties that distinguish themselves from other types of cells; they can both proliferate without changing their phenotypes indefinitely, and they also can differentiate into one or more new kind of cells depending on the culture conditions. Thus, stem cell therapy could be most effective to treat the diseases that are marked by the loss of cells.

CHA Stem Cell Institute (CHA Health Systems, Korea) is developing a feasible and applicable hESC driven stem cell therapies for various clinical studies with leading research institutes in ACT. Inc, USA. In our previous studies with a Robert Lanza's group at ACT. Inc, USA, we showed that the generation of RPE (Retinal Pigmented Epithelial) was reproducible; batches of RPE derived from NIH-approved hES cells (H9) were tested and shown to rescue host photoreceptors and attenuate loss of visual function in animals. Furthermore, we described an efficient and reproducible method for generating large numbers of these bipotential progenitors-known as hemangioblasts-from human embryonic stem (hES) cells using an in vitro differentiation system. Our data suggest that hES-derived blast cells (hES-BCs) could be important in vascular repair. In addition, CHA Health Systems developed CHAdiForm^{TM}, a product made of unexpanded pure adipose stem cells for autologous transplantation, which has shown to be effective in anti-aging.

As the source of stem cells used to treat diseases varies and the number of clinical trials in regenerative medicine increases, two main issues are important to consider. First, it is necessary to resolve immunological problems after implantation of allogeneic stem cells in patients. There are several available solutions such as SCNT-hESC generation, HLA-matched stem cell bank, single blastomere or parthenogenetic stem cells generation, and development of iPSCs. Second, the safety issue of stem cell therapy in clinical trials needs to be evaluated. We need to evaluate the toxicity and the risk of tumor formation in clinical trials by checking if the undifferentiated cells are completely eliminated and the stem cells are induced to fully differentiated cells in the pilot stage. CHA Health Systems have established comprehensive human stem cell banks such as germ cell bank (oocyte, sperm, germline stem cells), fetus stem cell bank (amniotic fluid stem cell, placenta, Wharton's jelly stem cell), embryo bank (hES cell, ES cell lines from blastomere, somatic cell cloning), and adult stem cell bank (bone marrow-, adipocyte-, cord blood-derived stem cells). Furthermore, CHA Health Systems also have managed the CHA BIO Human BANK which includes and operates the oocyte bank, umbilical blood bank (ICORD^{TM}), human ESC bank for several years, and developed placenta stem cell bank. ICORD^{TM} is one of the largest public and private cord blood stem cell banks in Korea.

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Recent Selected Publications:

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Immunogenicity of Human Pluripotent Stem Cells and the Derivatives

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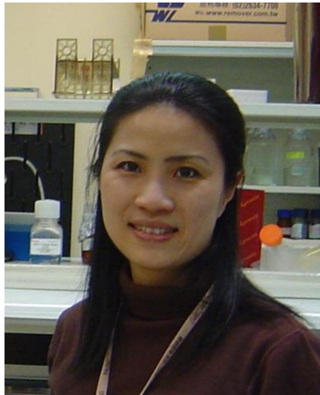
Pluripotent stem cells, including human embryonic stem (hES) and human induced pluripotent stem (hiPS) cells, have been regarded as potentially useful sources for cell-based transplantation therapy. However the immunogenecity of these cells remains the major determinant for successful future clinical application. We therefore reported the derivation of a hiPS cell line (iDPC3) and the examination of multiple hES cell lines (H9 and NTU1) and hiPS cell lines (iGra1, iGra2, iDPC3, iCFB46 and iCFB50) and their derivatives (including stem cells-derived hepatocyte-like cells) mostly derived by us for major histocompatibility complex (MHC) expression, human leukocyte antigen (HLA) haplotyping, expression of immune-related genes and T cell activation, in comparison with somatic cells. Flow cytometric analysis showed lower levels (percentages and fluorescence intensities) of MHC class I (MHC-I), β2-microglobulin and HLA-E in undifferentiated pluripotent stem cells, but the levels were increased to near those of somatic cells after co-treatment with interferon gamma and/or *in vitro* culture. The levels of antigen presenting cell markers (CD11c, CD80 and CD86) and MHC-II molecules (HLA-DP, DQ and DR) however remained low throughout the treatment. Activation of responder lymphocytes by the pluripotent stem cells was significantly lower than that by allogeneic lymphocytes in mixed lymphocyte reactions, but comparable to that by syngeneic lymphocytes. Real-time reverse transcription polymerase chain reaction data showed significant differential expressions of immune privilege genes (*TGF- 2, Arginase 2, Indo1, GATA3, POMC, VIP, CACLA, CACLB, IL-1RN, CD95L, CR1L, Serpine 1, HMOX1, IL6, LGALS3, HEBP1, THBS1, CD59* and *LGALS1)* in pluripotent stem cells/derivatives compared to somatic cells. It is concluded that pluripotent stem cells and their progeny show some evidences of immune privilege *in vitro* when compared to somatic cells. However further studies will be needed to determine whether they will also behave in a way significantly different from somatic cells after transplantation.



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Novel Sources of Human Stem Cells: Fetal-stage Mesenchymal Stem Cells and Induced Pluripotent Stem Cells

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Despite the isolation of human embryonic stem cells (hESCs) and the more recently discovered induced pluripotent stem cells (iPS), many critical issues still preclude prevalent clinical use of these pluripotent stem cells (PSCs), including the ethical concerns of hESC derivation and tumorigenic potential of both these PSCs. Increasing reports of plasticity for many adult stem cells have brought excitement and hope for broad therapeutic application, but invasive procedures are required to obtain these are rare cells which further decrease in number with age. Thus, our laboratory has turned to using fetal extraembryonic tissue for isolation of fetal-stage stem cells. The placenta and associated membranes are temporary organ with fetal contributions which are discarded after birth, obviating ethical problems and invasive procedures for procurement. We have isolated human term placenta-derived multipotent cells (PDMCs), which are multilineage cells that exhibit adult bone marrow (BM) MSCs surface markers as well as some hESC markers. Differentiation into lineages from all three germ layers including multiple mesodermal lineages, an endodermal hepatocytic phenotype, and ectodermal neural-related lineages can be achieved with these cells. Moreover, PDMCs are strongly immunosuppressive and are much more proliferative than adult MSCs, both important issues in clinical application. The higher proliferative potential of fetal cells in general have allowed us to successfully reprogram fetal umbilical venous endothelial cells (HUVEC) into iPS with just the 2 non-oncogenic transcription factors, Oct4 and Sox2. Thus, fetal extraembryonic tissues are a good source for isolation and derivation of novel human stem cells for therapeutic use.

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Pluripotency Reprogramming beyond Transcription Factors

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Recently, both mouse and human somatic cells have been successfully reprogrammed into iPSCs with defined transcription factors such as OCT4, SOX2, KLF4 and MYC (OSKM). Previous studies indicated that iPSCs resemble ESCs in morphology, growth requirements, proliferation, expressions of markers and genes, and epigenetic and functional features. Thus, iPSCs have been regarded as an attractive contender for patient-specific regenerative medicine as they circumvent the immuno-rejection problem and the ethical issues associated with ESCs. Despite these advantages, the low efficiency of iPSC derivation is one of the major challenges in the field of somatic cell reprogramming. Recently, several studies have put forward methods by which reprogramming efficiency can be improved, including: 1) the use of chemicals modifying chromatin structures such as valproic acid, 5-aza-cytidine, and a combination of BIX and BayK 2) the use of inhibitors of signaling pathways such as TGF-β inhibitors and a combination of MEK and GSK3 inhibitors; 3) the use of antagonists of senescence, such as suppressors of p53 and vitamin C; 4) hypoxia; and 5) the use of Cdh1 (E-cadherin). These diversified approaches have revealed that reprogramming is a complex process involving many levels of sub-cellular biochemical and molecular events, and implicated that efficient reprogramming may be achieved by effective manipulation at several of these levels.

The purpose of this study was to determine whether membrane proteins that form a complex with EpCAM in some cancer cells also form a complex with EpCAM in ESCs and iPSCs, and whether components of this complex play a role in enhancing the efficiency of fibroblast reprogramming in the hope of advancing the search for a more effective means of generating iPSCs. Through the analysis of EpCAM-associated proteins in ESCs, iPSCs, and fibroblasts that underwent reprogramming, we have shown that EpCAM and Cldn7 were required for reprogramming of mouse somatic cells. Moreover, we found that overexpression of EpCAM or EpICD during the process of reprogramming further enhanced the reprogramming efficiency of MEFs, probably through EpICD-mediated activation of *Oct4* and downregulation of *p53*. Therefore, our results revealed that EpCAM complex proteins are not merely a passenger gene that is upregulated during reprogramming but a player in a positive feedback loop among key factors that propel the reprogramming machinery. Thus, our studies highlight the importance of signaling events from the extracellular milieu to the nucleus in the reprogramming process.

E: Somatic Stem Cells and Regenerative Medicine (II)

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Control of Embryonic Stem Cell (ESC) Differentiation by Prostaglandin E_2 (PGE_2) and Rho-associated Kinases (ROCK)

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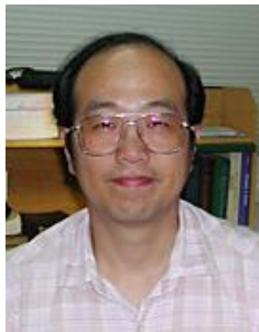
ES cell renewal and differentiation are regulated by multiple factors and signaling pathways. Our work uncovers PGE_2 and its downstream signaling molecules as important players in controlling murine ESC differentiation. Our experimental results indicate that PGE_2 protects murine ESC survival against oxidant stress through EP2 receptor-mediated PI-3K/Akt. On the other hand, PGE_2 prevents murine ESC differentiation by an EP3 receptor-mediated PKCδ activation. In search for downstream signaling pathways, we discovered that rho-associated kinases (ROCKs) are involved. Inhibition of ROCKs with Y-27632 results in loss of renewal factors and appearance of nestin-positive neural progenitor cells (NPC). ROCK-1 or ROCK-2 siRNA independently induced murine ESC differentiation to nestin-positive NPCs. Our preliminary data reveal that ROCKs signal through GSK-3β/β-catenin in a 14-3-3 dependent manner. These results suggest that PGE_2 and ROCK signaling pathway play an important role in maintaining ESC survival and renewal.



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Recent Selected Publications:

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A Novel Neuronal Differentiation System, Combination of Culture Medium and Biomaterial for Neural Stem Precursor Cells

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It is known that many proliferation- and differentiation-promoting molecules are present in the serum, which has great effects on the behaviors of neural stem/precursor cells (NSPCs). Regulating neural lineage differentiation from NSPCs is a challenge of neuroscience research. In previous study, when culture medium was in the presence of serum, the more differentiation proportions of NSPCs cultured on biomaterials are astrocytes. It is shown that serum might have strong influence between NSPCs and substrates. However, serum is a complex mixture containing a variety of components with different molecular weights. Up to now, serum components have never been systematically identified how they influence NSPC phenotypic choice. In our published study, we found using low molecular weight serum fractions to culture NSPCs could increase neuronal differentiation of NSPCs and could be applied for general biomaterials.

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Acquire and Nurture ESC-Like CD34$^+$ Mesenchymal Common Progenitor Cells (CD34$^+$ MCPCs) from Human Tissues for Translational Medicine

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While pluripotent embryonic stem cells (ESCs) and tissue cells induced pluripotent stem cells (iPSCs) studies are widely and actively conducting, searching sources of safe and effective multi-potent stem cells for repairing and renewing damaged tissues have also been extensively investigating. In this presentation, we are describing a newly identified and characterized clonogenic multipotent progenitor cells from human neonatal placenta and adult tissues. These tissue progenitor populations present typical mesenchymal stem cell (MSC) markers (CD29, CD44, CD73, CD90, CD105, EGFR and integrin α2β1) with co-express a hematopoietic CD34$^+$ membrane antigen but negative to the epethilail cytokeratin 18 marker expression. These progenitor cells strongly co-expressed early ESC genes (Oct-4$^+$, Nanog$^+$, Rex-1$^+$, SOX-2$^+$, and SSEA3, 4) and somatic stem cell (CD117, CD133, CD146, CD54) markers. We named them as CD34$^+$ Mesenchymal Common Progenitor Cells (CD34$^+$ MCPCs) due to their unique multi-transdermal differentiation potentials. CD34$^+$ MCPCs exhibit neurogenic differentiation potentials with highly expressions of GFAP, GalC, tyrosine hydroxylase, and Tuj1. In cardiomyogenic differentiation, CD34$^+$ MCPCs generated mature MHC$^+$/ Troponin$^+$ cardiomyocytes. In hepatic transdifferentiation, CD34$^+$ MCPCs derived hepatocytes produce functional albumin protein. CD34$^+$ MCPCs also possess superior efficiency in myotube and vascular tube formation. A further functional genomic profiling analysis supportive is to the above biological observations.These results suggested that CD34$^+$ MCPCs in human somatic tissues may be acquired as an ideal resource for cell therapeutic and tissue regenerative medicine. Further in vivo animal studies are undergoing.

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Recent Selected Publications:

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Dedifferentiation is a Key to Limb Regeneration in Salamanders

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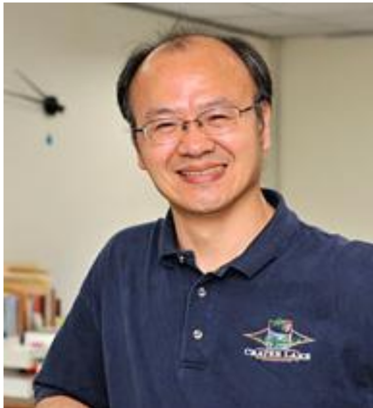
Unique among vertebrates, salamanders are able to perfectly regenerate their lost limbs and other body parts after amputation. This amazing ability has already been known since Aristotle over 2000 years ago. However, only in recent decades have the scientists been able to study their limb regeneration at molecular and cellular levels. They found this regeneration begins from a clump of cells called a blastema formed at the tip of a lost limb a few days after amputation. From the blastema comes the muscle, bone, skin, blood vessels and nerves, and ultimately organizing precisely into a limb identical to the one prior to amputation. The blastema is a self-organizing system that keeps its positional memory after amputation at any level on proximodistal axis. Its formation is obviously nerve-dependent as denervation not only inhibits its appearance but also the regeneration.

The blastemal cells are currently believed to arise by dedifferentiation from adult mesenchymal cells at the plane of amputation. However, the molecular mechanisms underlying their generation and properties remain largely unknown. To obtain the full spectrum of molecules involved in this mysterious process, we analyzed and compared the transcriptomes by *de novo* next generation sequencying from the usual blastemas and the stumps with their proximal nerves cut off on *Ambystoma mexicanum* (axolotl), a salamander species coming from Mexico. The obtained reads were assembled and annotated with gene descriptions, gene ontology and clusters of orthologous group terms. The majority of the differentiated transcriptomes were related to regeneration processes in addition to many anatomical structure and development- associated genes. Many new candidate gene sequences were recorded and these will contribute greatly to the understanding of the underlying molecular mechanisms. Among them is pax-7 gene, a muscle progenitor satellite cell marker. *In situ* hybridization on pax-7 showed significant signals on the terminal and slender muscle fibers close to the stump and on some discrete cells in the blastema. These findings suggested the muscle fibers around the stump end had undergone dedifferentiation to their progenitors which might contribute to individual blastemal cells.

C2C12 cells, a murine muscle satellite cell line, may be easily differentiated into myotubes in the Petri dish. Original cells are highly proliferative, while myotubes are not. Proteins extracted from blastemas were shown to induce disintegration from the myotubes of single cells which quickly proliferated into colonies. These results directly showed certain substances in the regenerating blastema were capable of inducing dedifferentiation of even mammalian mature muscle cells. This platform may be used to search for the contributing substances in the salamanders' regenerating tissues and this knowledge may in the long run help people to regenerate efficiently.



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The Opportunities and Challenges in Cardiac Stem Cell Therapy

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Although the field of cardiac stem cell therapy has significantly advanced over the past years, the premise of regenerating or replacing diseased human myocardium with functional tissue remains unsatisfied. The overall objective of our research is to promote myocardial stem cell therapy through nano-/micro-environmental engineering the intramyocardial stem cell niche for endogenous cardiac stem cells to reactivate, proliferate, differentiate and repopulate the diseased myocardium. Using a cardiac specific inducible Cre-Lox transgenic mouse model, we have found that an endogenous cardiac stem cell pool may contribute to ~15% of new cardiomyocytes in the peri-infarcted area, saturated as early as within the first 2 weeks after infarction. Accordingly, we focus on (1) animal models to identify the source of this cardiac stem cell pool, and (2) methods to engineer the cardiac nano-/micro-environments for augmenting the regenerative signaling for spontaneous repair. We have employed a team approach which brings together people from a broad variety of backgrounds, including stem cell biology and biochemistry, biophysics and biomechanics, materials science and controlled drug/gene delivery, synthetic and biochemical engineering, and clinical medicine. Our progress towards this goal will be presented and the future perspectives will be discussed.

F: Short Talk by TSSCR Best Poster Award Winners

Session Chairs:

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2011 International Symposium on Recent Advances in Pluripotent Stem Cells & 7th Annual Meeting of Taiwan Society for Stem Cell Research

Poster Abstract

A01

Endothelial Progenitor Cells in Primary Aldosteronism: a Biomarker of Severity for Aldosterone Vasculopathy and Prognosis

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Context: Primary aldosteronism (PA) is associated with a higher incidence of cardiovascular events, probable through mineralocorticoid receptor (MR)-dependent endothelial cell dysfunction, in comparison to essential hypertension (EH).
Objective: To investigate the number and function of endothelial progenitor cells (EPC) in PA and the relationship with arterial stiffness and disease progression.
Design: A prospective study of the change of EPC number and outcome of PA patients after treatment.
Setting: A tertiary medical center
Primary Outcomes: Changes in arterial stiffness and EPC number after treatment, and the curability of hypertension.
Patients: A total of 113 PA patients (87 patients diagnosed of aldosterone-producing adenoma, 26 with idiopathic hyperaldosteronism) and 55 patients with EH.
Results: PA patients had higher arterial stiffness than EH patients ($p = 0.006$), with a lower numbers of circulating EPC and endothelial colony forming units (CFU) ($p<0.05$). The differences were ameliorated at six months after unilateral adrenalectomy or treatment with spironolactone. Expression of MR was identified in the EPC. The number of circulating EPC was inversely correlated with the plasma aldosterone concentration ($p = 0.021$), arterial stiffness ($p=0.029$) and serum high-sensitivity C-reactive protein ($p=0.03$). High dose aldosterone (10^{-5} and 10^{-6}M) attenuated EPC proliferation and angiogenesis *in vitro*. Among the 45 patients who underwent unilateral adrenalectomy, 32 (71%) were cured of hypertension. The preoperative number of EPC (Log[EPC number%] > -3.6) predicted the curability of hypertension after adrenalectomy ($p=0.003$).
Conclusions: The relative deficiency of EPC in PA patients may contribute to the aldosterone vasculopathy, which can reversed by adrenalectomy and spironolactone. High aldosterone level attenuated EPC proliferation and angiogenesis. Circulating EPC number may be a valuable biomarker to identify PA patients with a high incidence of arterial stiffness and to predict postoperative residual hypertension of APA.

Key Words; Primary aldosteronism, endothelial progenitor cells, colony forming units, arterial stiffness, C-reactive protein, oxidative stress.

A02

Systemic Human Orbital Fat-derived Stem Cell Transplantation Ameliorates Acute Inflammation in Lipopolysaccharide-induced Aculte Lung Injury

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Acute lung injury (ALI) results in acute respiratory distress syndrome (ARDS). There is no standard therapy for ARDS but supportive care. Stem cells offer a new therapeutic potential for tissue regeneration due to the self-renewal, multipotency, and paracrine effect. The objective of this study is to investigate the effects and the mechanisms of systemic human orbital fat-derived stem/stromal cell (OFSCs) transplantation on lipopolysaccharide (LPS)-induced ALI. In this study, twenty-five μg LPS in 50 μl sterile saline or 50 μl of sterile saline was delivered into male BALB/c mice via intra-tracheal injection. Twenty minutes later, the animals were further randomized into subgroups that received either a tail vein injection of 3×10^5 OFSCs in 50 μl of phosphate buffer solution (PBS) or 50 μl of PBS. We demonstrated that systemic OFSC transplantation did not trigger an immune response in BALB/c mice. OFSCs significantly reduced LPS-induced pulmonary inflammation, which was evidenced by a decrease in total protein concentration and neutrophil counts in alveolar fluid via bronchoalveolar lavage (BAL), which reduced endothelial and alveolar epithelial permeability, as well as neutrophil (Ly6G expressing cells) and macrophage (CD68 expressing cells) infiltration. The LPS-induced expression of CD14, inducible nitric oxide synthase (iNOS), and transforming growth factor-β (TGF-β) in lung tissue was significantly inhibited by OFSCs. OFSCs not only reduced the circulation numbers of macrophages and neutrophils (CD11b expressing cells), but also decreased systemic macrophage-released pro-inflammatory chemokine levels such as macrophage inflammatory protein-1-gama (MIP-1γ), B-lymphocyte chemoattractant (BLC), interleukin-12 (IL-12), and subsequent circulation helper T cell (CD4 expressing cells) numbers. Moreover, few human OFSCs were detectable in the recipient lung after acute inflammation subsided. Taken together, systemic OFSC transplantation was effective in modulating inflammation during acute lung injury. The therapeutic effect was mainly attributed to the inhibition of the macrophage-mediated inflammatory response.

Key Words: orbital fat stem cells (OFSCs); acute lung injury; lipopolysaccharide; macrophage; inflammation

A03

Immunological Aspects of Syngeneic Mesenchymal Stem Cells-mediated Modulation in Animal Model of Autoimmune Diabetes

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Mesenchymal stem cells (MSCs) have shown their capacities in treating various autoimmune diseases; however the application of syngeneic MSCs treatment in autoimmune diabetes has not clarified yet. Thus, we obtained the bone marrow MSCs from autoimmune diabetic mouse model and directly assayed the immunomodulatory regulations in syngeneic or allogeneic MSCs coculture systems. Our results demonstrated that cell-cell contact is critical for MSCs-mediated immunomodulatory effects and it is mouse strain independent. Regulatory pathways of iNOS, proinflammatory cytokines and PD-L1 were involved in the MSCs-mediated immune none-responsiveness. In addition, the inhibitory effects of MSCs were modulated through the induction of regulatory cells, including regulatory T cells and myeloid-derived suppressor cells, and reduction of dendritic cell maturation and T cell development. Besides, an enhancive adhesion between MSCs and hematopoietic cells was also one of the participated mechanisms. Notably, treated syngeneic MSCs were homing to draining lymph node and provided a significant delay of disease onset in NOD/SCID recipients that were pre-transferred with pathogenic lymphocytes and also efficiently prolonged the graft survival in islet transplantation. In summary, we demonstrate that the immune non-responsiveness potential of syngeneic MSCs is similar to allogeneic MSCs. Administration of syngeneic MSCs efficiently exerts the induction of regulatory T cells toward delayed diabetic process and survival of islet grafts. Our data support the potential of autologous MSCs-mediated therapeutic application in autoimmune diabetes.

Keywords: autoimmune diabetes, mesenchymal stem cells

A04

3D Matrix Compliance Induce Human Mesenchymal Stem Cells towards Neuronal Fate

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Stem Cells are known with its unlimited cell proliferation and its magnificent multipotency to differentiate into various tissue cells. Previous studies have found that the induction of stem cells differentiation can be controlled by mechanical cues including matrix compliance. It was suggested that substrate's elastic modulus less than 1kPa, stem cells have the potential to differentiate into neural-like cell, while at about 10kPa, they may be differentiate into glial-like cell. By knowing the interaction between stem cells and biomimic microenvironment, we may modify and manipulate stem cell's differentiation lineage. In this study, we used hyaluronic acid (HA) combined with type I collagen (Col) to synthesize a three dimensional uniform porous structure with interconnected pores and high porosity by using freeze-drying technique. Different N-(3-Dimethylaminopropyl)-N'-ethyl carbodiimide hydrochloride (EDAC) crosslinking concentration was used to modify the stiffness of substrate. We had successfully modified our matrix's stiffness in the ranges less than 1kPa as soft substrate and at about 10kPa as stiff substrate. Human mesenchymal stem cells (hMSCs) were selected to culture on different matrix's stiffness 3D substrate for 2 weeks to investigate hMSCs behavior. We found that after 7 days of differentiation, immunohistochemistry results showed that hMSCs expressed the neuronal early marker, nestin and βIII-tubulin, while mid/late neuronal marker MAP2 was expressed after 14 days of culture. hMSCs also revealed that they tend to differentiate into neuron-like cells on soft substrate and astrocyte-like cells on hard substrate after 14 days induction. By using HA-Col porous scaffold with variable stiffness, we can manipulate mesenchymal stem cells differentiation towards neuronal lineage and this may be beneficial for clinical application in neuroregeneration.

Keywords: Mesenchymal stem cell, matrix compliance, neuronal differentiation, scaffold

A05

In vitro Differentiation of Glioblastoma Stem-like Cells into Endothelial-like Cells

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Glioblastoma multiforme (GBM) is a highly angiogenetic malignancy, which accompanies with microvascular proliferation and extensive necrosis. Therefore, inhibition of angiogenesis seems to be an ideal treatment for such highly vascularized tumors as GBM. However, current clinical evidence demonstrates that antiangiogenetic therapy only confers marginal survival benefit partly because some tumor-derived endothelial-like cells (TDEC) can not only rebuild vascular channels but also show resistance to conventional antiangiogenetic agents. Inhibition of TDEC formation may become a promising therapeutic option, and detailed characterization of TDECs will therefore be mandatory. In our present study, we aim to generate TDECs *in vitro* either from a human GBM cell line, U-87 MG, or from U87MG-derived cancer stem-like cells. Their efficiency of TDEC formation will be compared. Tube formation assay and immuofluorescent staining will also be conducted to characterize the functional and phenotypical differences of TDEC of two different origins.

Keywords: glioblastoma multiforme, angiogenesis, stem-like cells and tumor-derived endothelial cells

A06

Rho A Signalling Contributes to Statin-induced Osteogenesis in Bone Marrow Mesenchymal Stem Cells

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Statins, 3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, reduce cholesterol synthesis and prevent cardiovascular disease. They also have been found to inhibit prenylation of Rho proteins in recent decade. Previous reports showed that statins inhibited protein prenylation and decreased the active form of Rho A in osteoclasts. Others reports indicated that statins inhibited protein prenylation but increased the active form of Rho A in human erythroleukemia cells. Therefore, the role of statins regulate Rho A activity remains unclear. Rho GTPases act as molecular switches to regulate mesenchymal stem cell differentiation. Previous study showed that transfected constitutively active-form of RhoA into human mensenchymal stem cells (hMSCs) which leaded differentiation of hMSCs into osteoblasts. On the other hand, dominant negative RhoA leaded differentiation of hMSCs into adipocytes. According to the description above, we want to investigate whether Rho A signaling contributes to statin-induced osteogenesis in BMSCs. Pluripotent mesenchymal cells, D1, which were cloned from Balb/c mouse bone marrow cells and purchased from ATCC. For all experiments, cells were seeded at a density of 80% confluence and treated with or without simvastatin. The mRNA expression of Rho A was detected and quantified by real time PCR. And the mineralization effect on rat bone mesenchymal stem cells (rBMSCs) was tested by Alizarin Red S Staining. The Alizrin red S staining assay showed that the simvastatin were potentially enhanced the cell mineralization on BMSCs. The data of mRNA expression of Rho A showed that simvastatin 1uM significantly increased Rho A gene expression on first day. However, there were no significantly different between control and treatment group on third and fifth days. The Rho A activity under SIM 1uM treatment was significantly increased on day 2. From these results, we suggest that simvastatin enhanced rat bone mesenchymal stem cells (rBMSCs) mineralization may through Rho A signaling.

Keywords: Statins, Rho A, mesenchymal stem cells, osteogenesis

A07

Human Umbilical Cord Blood-derived CD34+ Cells Attenuate Inflammations but Stimulate both Angiogenesis and Neurogenesis after Traumatic Brain Injury in Rats

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Background: Human umbilical cord blood contained <0.2% $CD34^+$ cells have been shown to be beneficial in reducing the neurological deficit in the rat after traumatic brain injury (TBI). This study aimed to generate human cord blood-derived $CD34^+$ cells (>95%) and to investigate the mechanisms underlying their beneficial effects in treating TBI in rats.
Materials and Methods: Rats were divided into three groups: (1) sham operation; (2) TBI + $CD34^-$ cells (5×10^5 human cord blood lymphocytes and monocytes that contained <0.2% $CD34^+$ cells); and (3) TBI + $CD34^+$ cells (5×10^5 human cord blood lymphocytes and monocytes that contained 95% $CD34^+$ cells). Behavioral dysfunction and brain infarction, inflammation, apoptosis, angiogenesis, and neurogenesis were evaluated 4 days after TBI onset.
Results: As compared to sham operation controls, $CD34^-$-treated TBI rats had motor and cognitive dysfunctions and cerebral infarction, apoptosis, and inflammation. TBI-induced neurological dysfunction, and cerebral infarction, apoptosis, and inflammation could be significantly attenuated by $CD34^+$ cells therapy. In addition, $CD34^+$ cells migrated to the injured brain regions and significantly promoted both angiogenesis and neurogenesis in the injured brain.
Conclusions: The results indicate that human umbilical cord blood-derived $CD34^+$ cells therapy may be beneficial in attenuating the TBI-induced cerebral infarction and apoptosis and behavioral dysfunction via stimulating both brain angiogenesis and neurogenesis but inhibiting activated inflammation in a rat model.

Keywords: Traumatic brain injury, $CD34^+$ cells, apoptosis, inflammation, angiogenesis, neurogenesis.

A08

Identify ApoA-1 as a Positive Regulator in Osteogenesis

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Mesenchymal stem cells (MSCs) are the prominent source for regenerative medicine. MSCs not only have the ability to proliferate *in vitro* and *in vivo*, MSCs also have the potential to differentiate into osteo-, adipo- or chrondro-lineage cells. However, the detail mechanism of osteogenic differentiation is unclear. We utilized an unbiased cDNA library that contains 12000 human ORF clones to screen for the candidate genes promote human bone marrow-derived MSCs (hBM-MSCs) differentiate into osteoblasts. Base on the results of alkaline phosphatase (ALP) activity, an early marker in osteogenesis, nine candidate genes were obtained from the gain-of-function screen. Furthermore, by a loss-of-function study performed with at least two independent short hairpin RNAs (shRNAs), we demonstrated that these genes are also essential for the process of osteogenesis.

Apolipoprotein A-I (ApoA-I), a major component of human high-density lipoproteins (HDLs), is one of candidate genes from our preliminary screen. In the last decades, the reduced of ApoA-I expression in plasma was found to be associated with cardiovascular disease, obesity, or other metabolic syndrome. Heart disease and osteoporosis are both elevated in menopause women. However, the function of ApoA-1 in osteogenesis is unknown. In this study, we found ApoA-I not only can enhance the expression of early osteogenesis marker (ALP activity) but also stimulate the expression of late osteogenesis marker (Alizarin Red staining). In addition, we also discovered the c-Jun N-terminal kinase (JNK) pathway is involved in ApoA-I-mediated function. Inhibition of JNK pathway by SP600125, a JNK inhibitor, blocked the enhancement of osteogenesis markers and the mineralization of bone by ApoA-1. Taken together, our results demonstrate that ApoA-I stimulates ALP activity and mineralization through JNK in the osteogenesis process of hBM-MSCs. This study shows that ApoA-1 is possible to be a therapeutic target in bone diseases.

Keywords: Mesenchymal stem cells, Osteogenesis, ApoA-1

A09

Effects of Bisphenol A on Cell Proliferation and Epithelial-mesenchymal Transition (EMT) in Human Breast Epithelial Stem Cells

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There is evidence indicated that exposure to bisphenol A (BPA) during early development may increase breast cancer risk later in life. In this study, a SV40-immortalized cell line from normal human breast epithelial cells with stem cells and luminal characteristics (M13SV1) was used to investigate whether BPA can induce epithelial–mesenchymal transition (EMT) and cause cell number increase. The results clearly showed that BPA was able to induce colony formation in an inverted U-shaped dose–response manner. Furthermore, BPA induced EMT based on morphological change which concomitant with increased protein expression of mesenchymal markers, and decreased expression of epithelial markers. In summary, BPA, one of the main environmental endocrine disruptors, investigated the effect on the proliferation and EMT of human breast epithelial stem cell.

Keywords: bisphenol A; breast cancer stem cell; epithelial–mesenchymal transition

A10

Estrogen Induces Cell Invasion and Tumorigenesis in Human HER2-overexpressing Breast Cancer Stem Cells

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Cancer cells are believed to be derived primarily from stem cells. In human breast cancer, $CD44^+/CD24^-$ have been identified as markers for human breast cancer stem cells. Although, estrogen plays an important role in the pathogenesis and recurrence of breast cancer, the mechanisms of action on tumor development are not completely understood. We have previously reported a tumorigenic cell line, R2N1d, was developed from this cell type following SV40 large T-antigen transfection, X-ray irradiation, ectopic expression of HER2/C-erbB2/neu and under the growth factor/hormone-deprived condition. In this study, we tested the hypothesis that estrogen may enhance cell invasion and tumorigenicity in R2N1d cells. Indeed, estrogen increased cell migration and invasive ability. Furthermore, these cells gained estrogen responsiveness in tumor development. These results will help us to explore the detailed understanding of estrogen effects on tumor biology of HER2-overexpressing breast stem cells.

Keywords: estrogen; breast cancer stem cell; tumorigenesis

A11

Assessing the *in vivo* Developmental Potential of Reprogrammed Naive Monkey Pluripotent Stem Cell in Inter-specific Chimeras

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Recently, it has been demonstrated that pluripotent stem cells can be derived from epiblast of post-implanted mouse embryos. These epiblast derived pluripotent stem, namely epi-stem cells, possess pluripotent characteristics of mouse ESCs (mESCs). However, they are different in several aspects, including growth requirements, status of X chromosome inactivation, the germline potential, and especially, in the ability to contribute to in vivo development after injecting into preimplantation blastocysts. These difference, thus defined two pluripotent status, naïve and primated pluripotency, which belong to ICM-derived mESCs and epiblast-derived Epi-stem cells in mouse, respectively. Unlike mouse ESCs, both human and monkey ESCs resemble mouse Epi-stem cells closely in many aspects. Pluripotent stem cells that exhibit features of mESCs have been generated by overexpression of defined transcription factors in human ESCs. However, the in vivo developmental potential of human ESCs and their mouse ESC-like counterparts is still unknown. Since monkey pluripotent stem cells resemble human pluripotent stem cells closely in many aspects, they can serve as a primate model for *in vivo* studies involve interspecies chimera in which it practice is largely prohibited in human due to the bioethic issues. In this study, we have established mESC-like monkey pluripotent stem cells and investigated and compared their *in vitro* and *in vivo* developmental potential in interspecies chimeric embryos created by engrafing both monkey pluripotent stem cell types into mouse preimplantation blastocysts. Our results showed that monkey ES cells and mESC-like pluripotent stem cells were preferentially allocated to inner cell mass (ICM) of mouse. Immunofluorescence analysis demonstrated that the progeny of mESC-like monkey stem cells present in the fetuses that were transiently implant into surrogate mice up to E7.5 whereas no monkey ESC derivatives was detected in the mouse fetuses. Collectively, our studies demonstrate, for the first time, that the reprogrammed mESC-like monkey pluripotent stem cells are able to contribute to in vivo development in monkey/mouse chimera. The superior ability of the reprogrammed monkey mESC-like pluripotent stem cells to participate in vivo development will provide an excellent opportunity for generation of transgenic monkeys through creation of chimeric non-human primates.

Keywords: naïve, monkey pluripotent stem cell, inter-specific chimeras

A12

Proliferative Decline and Differentiation Alteration in Senescent Human Fetal Mesenchymal Stem Cells are independently Regulated by Protein Kinase C (PKC)-p21 and H_2O_2

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Increasing data suggest that mesenchymal stem cells (MSCs) are excellent sources for cell therapy, given their multilineage differentiation capacity and immunomodulatory properties. However, prolonged *in vitro* culture of MSCs leads to senescence, which results in a decline in proliferative and differentiation capacities. We therefore investigated the mechanisms mediating senescence in human placenta-derived multipotent cells (PDMCs), an ethically compliant source of highly proliferative fetal-stage MSCs. PDMCs are more proliferative than adult bone marrow MSCs but still become senescent with prolonged *in vitro* culture. Compared to early-passage PDMCs, late-passage PDMCs showed evidence of senescence, with positivity for β-galactosidase staining, decreased SIRT1 expression, increased p21 expression, cell cycle arrest at G0/G1 phase, and increased levels of H_2O_2. Moreover, senescent PDMCs showed a differentiation bias toward adipogenesis. Mechanistically, the proliferative decline was not due to elevated H_2O_2 levels nor mediated by the tumor suppressor gene p53. Instead, inhibition of protein kinase C (PKC) -α and -β in senescent PDMCs decreased p21 expression. The detrimental effect of senescence on PDMC differentiation potential was mediated by H_2O_2 since scavenging of H_2O_2 restored the differentiation capacity. Our data suggest that the two biological consequences of senescence—proliferative decline and differentiation alterations—are distinctly regulated by the PKC-p21 and H_2O_2 pathways, respectively. These findings not only show the profound effect of senescence on MSCs, but also reveal mechanistic insights into possibly reversing the detrimental effects of replicative senescence.

Keywords: Mesenchymal stem cell, senescence, PKC, p21, ROS

A13

SENP1 Regulates OCT4 under Hypoxia: Effect of Sumoylation on Protein Stability and Drug Susceptibility of Human Pluripotent Germ Cell Tumors

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The well-differentiated testicular germ cell tumors (TGCTs) are more drug insensitivity and poor prognosis than the pluripotent germ cell tumors. However, mechanism underlying the differentiation regulation of pluripotent seminomas/embryonic carcinomas (ECs) still remains largely unknown. Herein, we demonstrated an important role of SUMO1/sentrin specific peptidase 1 (SENP1) in sumoylation and stability of OCT4 protein in hypoxic EC's drug sensitivity. Hypoxia decreases the OCT4 protein level, but not the mRNA level, in pluripotent ECs. Hypoxia challenge or SUMO1gg overexpression increased HA-OCT4 sumoylation and protein instability in HEK293T cells. Further mutation at the specific OCT4 Lysine 123 (HA-OCT4-K123R) effectively suppressed the OCT4 sumoylation and protein instability, demonstrating the important role of specific lysine 123 sumoylation in OCT4 protein stability of hypoxic ECs. Consistently, overexpression of SUMO1gg decreased endogenous OCT4 half-life in hypoxic-NCCITs. The band-shift of endogenous SUMO1-OCT4 in hypoxic NCCITs was demonstrated using western blot analysis. SENP1 effectively suppress the OCT4 sumoylation and increases the OCT4 stability and drug sensitivity in hypoxic NCCITs *in vitro*. Furthermore, overexpression of SENP1 in hypoxic NCCITs not only decreases the tumor size but also increased drug sensitivity in nude mice model, demonstrating the up-stream regulation of SENP1 in OCT4 sumoylation, protein stability, and drug susceptibility of NCCITs. In summary, this study demonstrated that sumoylation regulator SENP1 positively regulates the OCT4 stability and drug sensitivity in pluripotent EC cells through sumoylation. Findings in this study provide the insights of sumoylation regulation in pluripotent TGCT differentiation, which facilitate potential pharmacological development for effective therapeutic targeting.

Keywords: OCT4, SENP1, sumoylation, hypoxia, embryonal carcinoma

A14

Human Placenta Chorion Mesenchymal Stem Cells and HLA-G Molecules Expression

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Placenta derived mesenchymal stem cells had been isolated by using different protocols. Here, we demonstrated a new novel serum-free selection culture condition to isolate MSCs from the chorionic membrane of the human term placenta, named pcMSC. In our studies, pcMSCs presented the therapeutic potential to treat acute injury diseases, such as paraquat induced acute lung injury and severe total body irradiation in animal models. After the therapy, the results showed that the survival increased, however, other therapies did not increase the survival rates. By flow cytometry analysis, the results showed that pcMSC was positive for MSC markers CD70, CD90, CD105, CD29, and CD44 but negative for hematopoietic lineage markers including CD14, CD34, and CD45. In addition to the above markers, pcMSC was positive for HLA-G. In further study, RT-PCR results indicate pcMSC express different isoforms of HLA-G, including membrane forms (HLA-G1, -G2, -G3, -G4) and soluble forms (HLA-G5, and –G6). Moreover, immunohistochemistry (IHC) and immunofluorescence (IFC) results of the human term placenta show that HLA-G was strongly expressed on the chorionic membrane and this protein was co-localized at the position where CD105 was expressed, which was on the chorionic membrane of the human term placenta. Additionally, the studies also indicated that pcMSC could down-regulate the migration of differentiated HL-60 cells, a neutrophil-like cells; and a neutralizing antibody which blocked HLA-G molecular could restore the effects. Hence, we hypothesize that pcMSC can regulated neutrophils infiltration in acute injury condition, and pcMSC may be used as a model to further understand the immunomodulatory effects between HLA-G and neutrophils. Furthermore, these results show that pcMSC may be a promising cell used in cell therapies for diseases with acute injury.

Keywords: placenta, mesenchymal stem cell, serum free culture, HLA-G

A15

DLK Suppression of Mouse Embryonic Stem Cell Renewal is Inhibited by Akt

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Mouse embryonic stem cells (mESCs) have the properties to proliferate indefinitely and to maintain cell pluripotency at the same time. To find kinases involving in the self-renewal of mESCs, we performed a high-throughput screen with 4801 short hairpin RNA (shRNA). We found that shRNAs target dual-lucine-zipper-bearing kinase (DLK) can increases the growth rate of mESCs. Consistently, the overexpression of DLK decreases the growth rate of mESCs. In the C-terminal of DLK, there are two Akt putative phosphorylation sites. Akt has been proved to maintain the self-renewal state of mESCs. To investigate whether Akt can modulate DLK activities, the phosphorylation sites of DLK are disrupted and transfected into mESCs. These mutated constructs is more effective in the growth suppression of mESC than wild type DLK. Besides, we confirm that DLK interacts with Akt by co-immunoprecipitation experiment, and overexpression of Akt diminishes the effect of DLK on mESCs growth. These results implicate that as a positive regulator of mESCs self-renewal, Akt can restrain DLK to suppress mESC growth.

Previously, Akt has been found to regulate Nanog expression. When DLK is down-regulated, the expression amount of Nanog protein increases. It implies that Akt might regulate Nanog expression through inhibition of DLK. The result also shows that overexpression of DLK will reduce the Akt-mediated Nanog expression. In conclusion, our finding suggests that DLK reduces the self-renewal of mESCs and Nanog expression. However, in undifferentiated state of mESCs, the activities of DLK are constitutively suppressed by Akt. The discovery of DLK, a novel suppressor in Akt-Nanog pathway, would therefore benefit for the manipulation of ESCs and the understanding self-renewal mechanism.

Keywords: mouse embryonic stem cells, dual-lucine-zipper-bearing kinase, Akt, Nanog

A16

Resveratrol Impedes the EMT, Stemness, and Metabolic Reprogramming of Cancer Stem Cells by Inducing miRNAs in Nasopharyngeal Carcinoma through p53 Activation

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To investigate the metabolic signatures of cancer stem cells (CSCs) in nasopharyngeal carcinoma (NPC), we employed a triple-selection strategy to isolate CSC via 3 pivotal CSC properties of radioresistance, chemoresistance, and tumor sphere formation. Furthermore, we generated induced cancer stem cells (iCSCs) by ectopically expressing epithelial-to-mesenchymal transition (EMT) gene including *TWIST* and 4 stemness genes *OCT4*, *SOX2*, *KLF4*, and *c-MYC* (known as Yamanaka factors). These iCSCs displayed CSC properties and underwent metabolic shift to predominately rely on glycolysis for energy supply. Resetting in the distribution and biogenesis of mitochondria also occurred in CSCs. Intriguingly, we found that the natural polyphenol resveratrol turned off the metabolic switch, increased the ROS level, and depolarized mitochondrial membranes. These alterations in metabolism also led to the suppression of malignant phenotype such as the resistance to therapy, the self-renewal capacity, tumor initiation capacity, and metastatic potential in NPC CSCs. Particularly worth mentioning is that resveratrol tackled the nexus of NPC CSCs by down-regulation of the expressions of stemness, EMT, and CSC surface markers. The anti-Warburg regulator p53 was re-activated after treatment with resveratrol. We also noticed that miR-145 and miR-200c were down-regulated in CSCs but up-regulated after resveratrol treatment. We thus proposed that p53 may suppress the stemness and EMT genes through enhancing the expression of miR-145 and miR-200c. Herein, we demonstrated that CSCs employed the p53/miR-145 pathway in regulating stemness and metabolic reprogramming. Development of the methods to regulate the p53/miR-145 pathway may lead to a wealth of novel therapies for cancer treatment through targeting to CSCs.

Keywords: Cancer stem cell, nasopharyngeal carcinoma, resveratrol, metabolic shift

A17

Generation of a Cre-activable *R26* Dual Fluorescent Protein Reporter Mouse

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Green fluorescent protein (GFP) and its derivatives are the most widely used molecular reporters for live cell imagining. Recent development of organelle-specific fusion fluorescent proteins improves the labeling resolution to a higher level. Here we generate a *R26* dual fluorescent protein reporter mouse, activated by Cre-mediated DNA recombination, labeling target cells with a chromatin-specific EGFP and a plasma membrane-anchored monomeric cherry fluorescent protein (mCherry). This dual labeling allows the visualization of mitotic events, cell shapes and intracellular vesicle behaviors. We expect this reporter mouse to have a wide application in developmental biology studies as well as cancer/stem cell lineage tracing.

Keywords: Cre, dual fluorescent, lineage tracing, *R26* reporter mouse

A18

Cryopreservation of Human Embryonic Stem Cells by Cell Alive System (CAS)

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Human embryonic stem cells (hESCs) are pluripotent cells with unlimited proliferative potential and have capability to differentiate into all kinds of lineage cells of three germ layers. Up to now, there are three FDA-approval clinical trials undergoing in the US using hESC-derived cells. Due to the nature of hESC growing characteristics, these cells always grow as clumps to maintain their stemness. Therefore, there is always an obstacle in cultivation of hESCs：low survival and recovery rate after cryopreservation.

The Cell Alive System (CAS) is a technology that generates a special magnetic field around a subject material by using pulsed magnetic field, low-frequency wave and several types of weak energy. By Combine the technology with refrigerating process, water molecules within cells or tissues are frozen instantaneously from its super-cooled state. Different from the conventional cryopreservation, the system make cells or tissues frozen in vitrification method and minimize the damage due to crystallisation. Vitrification also can be achieved by applying open pulling straw (OPS) method with non-equilibrium solution. But OPS method is time-consuming, operator-dependent and limitation on small volume. Most scrupled is possible contamination due to the direct exposure to liquid nitrogen

Therefore we expect improvement on the recovery rate of hESCs by CAS. In our study, comparing with conventional slow cooling method, hESCs (TW1) frozen by CAS show obviously higher attachment rate after thawing. We also inspect whether the cells frozen by CAS remain pluripotency. Surface marker assay by flow cytometry and immunofluoroscence suggest the cells express markers of hESCs such as SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81. The results of embryoid body forming assay also reveal the cells can exactly differentiate into different cells of three germ layers *in vitro*.

Keywords: human embryonic stem cells, cryopreservation, cell alive system

A19

Dedifferentiation of Acinar Cells Induced by Sphere Culture and Oncogenic Kras-mediated Transformation

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Dedifferentiation is a cellular process in which a terminally differentiated cell reverts to a simpler, more embryonic, unspecialized form. Dedifferentiation usually occurs as a part of a regenerative process, but it can also occur in the development of neoplasm. Acinar-to-ductal or acinar-to-hepatic metaplasia has been considered as either an initial event or risk factors for development of pancreatic neoplasm which is believed to be mediated by initial dedifferentiation of mature exocrine cells to generate a population of nestin- or ABCG2-positive intermediate precursors. However, the molecular and cellular basis controlling the process is less clear. Initially, we revealed that both primary mouse acinar cells and exocrine AR42J cell lines were able to form spheres in suspension cultures. Further analysis of acinar cell spheres revealed some acinar cells dedifferentiate into fetal cell status as judged by expression of Pdx-1, Ptf1a, Hes1 and ABCG2. These cells were plastic and can differentiate to hepatocytes upon treatment with glucocorticoids. Since activated Kras mutations were the first genetic changes detected in the earliest stage of pancreatic intraepithelial neoplasia and over 95% of pancreatic adenocarcinoma, we further investigate the role of Kras mutant in acinar cell reprogramming and found that overexpression of activated Kras mutant in acinar cells significantly downregulated exocrine phenotype and largely enhanced formation of dedifferentiated precursor population if glucocorticoid and insulin were added in the culture. In combination of activated Kras mutant with TGF-β treatment can further alter the acinar cell fate toward ductal lineage cells *in vivo*. Our current work indicates that either sphere culture or activated Kras signaling can dedifferentiate pancreatic acinar cells into precursor-like status. Moreover, the metaplastic state and cell fate commitment can possibly be determined by microenvironmental factors.

Keywords: dedifferentiation; acinar cell; Kras

A20

A shRNA Functional Screening in Embryonic Stem Cells Reveals Nme Signaling is Crucial for Stem Cell Renewal

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In contrast to the somatic cells, embryonic stem cells (ESCs) are characterized by its immortalization ability, pluripotency, and oncogenicity. Revealing the underlying mechanism of ESC characters is important for the application of ESCs in clinical medicine. We performed systematically functional screening in ESCs with 4801 shRNA against 929 kinase and phosphatase and identified 132 candidate genes which regulate both ESC expansion and stem cell marker expression. Among these genes, the knockdown of 27 genes induces morphological change. We further demonstrate non-metastatic cells 6 (Nme6, also named as NM23-H6) and non-metastatic cell 7 (Nme7, also designated as NM23-H7) are important for the regulation of Oct4, Nanog, Klf4, cMyc, Telomerase, Dnmt3B, Eras and Nanog expression. This implies the importance of Nme6 and Nme7 in ESC renewal. The knockdown of the Nme6 and Nme7 reduce the formation of embryoid body and teratoma. The overexpression of Nme6 and Nme7 can rescue the stem cell marker expression and the embryoid body formation in the absence of leukemia inhibiting factor (LIF). This finding not only increases our understanding in the embryonic stem cells, but also contributes to build the foundation of the signaling roadmap of ESCs.

Keywords: embryonic stem cells, self renewal, teratoma, embryoid body

A21

Nanofibrous Scaffolds Combined with VEGF Delivery Creates an Intramyocardial Microenvironment for Arteriogenesis and Cardiac Regeneration

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Angiogenic therapy is a promising approach for tissue repair and regeneration; however, current clinical trials often fail to prove therapeutic effects. Recent studies have suggested that for effective revascularization it relies primarily on a durable microvasculature termed arteriogenesis, which requires not only angiogenic factors but also a microenvironmental support for recruiting mural cells to envelope the nascent endothelial tubes. Accordingly, we carries out experiments to test if combined delivery of self-assembling peptide nanofibers (NFs) with VEGF may create an intramyocardial microenvironment with prolonged VEGF releasing to improve post-infarction neovascularization in rats.

We performed coronary ligation to induce myocardial infarction in rats followed with direct intramyocardial injection of saline (as control treatment), NFs, VEGF or NFs + VEGF in the infarct through 3 directions ($n \geq 10$). Our data showed that VEGF delivery was sustained within the myocardium for up to 14 days when injected with NFs, and the side effect of systemic edema and proteinuria was significantly reduced to the same level of control. Importantly, NF/VEGF injection not only allowed controlled local delivery, but also created a favorable microenvironment for recruiting endogenous myofibroblasts and vascular progenitor cells in the injected sites. Consequently, NF/VEGF injection significantly improved angiogenesis (2.8 folds increase in capillary density), arteriogenesis (4.9 and 4.4 folds increase in small and large artery density, respectively) and cardiac performance (24% increase in left ventricular ejection fraction) 28 days after myocardial infarction. Strikingly, the engineered vascular niche further attracted a new population of cardiomyocyte-like small cells homing to the injected sites, suggesting cardiomyocyte regeneration.

To our knowledge, this is the first study reporting the engineering of intramyocardial microenvironment *in vivo* with therapeutic efficacy and safety. We believe that these unprecedented results represent a new strategy for cardiovascular regeneration which can be translated into clinical therapy in the foreseeable future.

Keywords: Cardiac tissue engineering, VEGF, microenvironment, angiogenesis, arteriogenesis

A22

Diverse Effects of Type II Collagen on Osteogenic and Adipogenic Differentiation of Mesenchymal Stem Cells

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Type II collagen is known to modulate chondrogenesis of mesenchymal stem cells (MSCs). In this study, MSCs from human bone marrow aspirates were used to study the modulating effects of type II collagen on MSCs differentiation during early stages osteogenesis and adipogenesis. With osteogenic induction, MSCs cultured on the type II collagen-coated surface showed an enhanced calcium deposition level with increasing mRNA expressions of RUNX2, osteocalcin and alkaline phosphatase. A synthetic integrin binding peptide which specifically interacts with the I-domain of α1β1 / α2β1 integrins significantly blocks the mineralization-enhancing effect of type II collagen. MSCs attached on the type II collagen-coated plates exhibited expanded cell morphology with increasing spreading area, and the pretreatment of cells with integrin α1β1 or α2β1 blocking antibody reduced the effect. The phosphorylation levels of FAK, ERK and JNK significantly increased in the MSCs that attached on the type II collagen-coated plates. On the contrary, the mineralization-enhancing effect of type II collagen was reduced by JNK and MEK inhibitors. Furthermore, type II collagen blocked the adipogenic differentiation of MSCs, and this effect is rescued by JNK and MEK inhibitors. Finally, type II collagen-coated HA/TCP (hydroxyapetite/tricalcium phosphate) scaffold was shown to enhance BMSC mineralization better than either non-coated or type I collagen-coated scaffolds. In conclusion, type II collagen facilitates osteogenesis and suppresses adipogenesis during early stage MSC differentiation. Such effects are integrin-binding dependant and mediated through FAK-JNK and / or FAK-ERK signaling cascades. These results inspire a novel strategy encompassing type II collagen in bone tissue engineering.

Keywords: mesenchymal stem cell, type II collagen, endochondral ossification, osteogenesis, adipogenesis

A23

Variable Neural Differentiation Potency in Mouse Embryonic Stem Cells and Inducible Pluripotent Stem Cells

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Embryonic stem cells (ESCs) possess powerful ability to self-renew and to differentiate into all cell types of three germ layers. It was reported that induced pluripotent stem cells (iPSCs) from somatic fibroblasts could be generated through delivering four transcription factors including Oct4, Sox2, Klf4 and c-Myc. It is known that iPSCs gain similar abilities like ESCs such as self-renewal and pluripotency. Therefore, iPSCs might provide advantages in various applications, such as developmental studies, pharmaceutical screening, and autologous cell transplantation.

In this study, we studied the neural differentiation potency of mouse ESCs and iPSCs with the neural inducer"retinoic acid (RA)" treatment. We demonstrated the mouse ESCs and iPSCs derived neural stem/progenitor cells and various neural lineage cells *in vitro*. Besides, the differential expression of early neural genes between ESCs and iPSCs was assayed by flow cytometry and quantitative PCR. Different neural differentiation patterns were also observed during early and late stages of RA induction by immunocytochemical approaches. To examine whether RA-Fgf/Erk pathway was involved in differences of neural induction from ESCs and iPSCs, quantitative PCR was applied to analyze gene expression of RA receptors and RA-metabolizing enzymes and Western blotting was performed to assess the phosphorylation level of Erk1/2. We found that the RA receptors, RA-metabolizing enzymes and phosphorylation of Erk1/2 were expressed at significantly different levels between ESCs and iPSCs. Our results suggest that neural differentiation potency of ESCs is higher than that of iPSCs, and the RA-Fgf/Erk pathway may play an important role in neural differentiation of both ESCs and iPSCs.

Keywords: embryonic stem cells, induced pluripotent stem cells, neural differentiation, retinoic acid, Fgf/Erk pathway

A24

Trends in Liver Cancer Stem Cells – Identification, Markers and Assays

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The stem cell theory of cancer hypothesizes that 'stem-like' cancer cells are responsible for the generation of tumors and for sustaining tumor growth. Cancer stem cells (CSCs) are generally dormant or slowly cycling tumor cells that have the ability to reconstitute tumors. They are thought to be involved in tumor resistance to chemo/radiation therapy and tumor relapse and progression. However, neither their existence nor their identity within many cancers has been well defined. In previous investigation, we evaluated most common CSC markers in hepatocellular cancer cells, including the CD133, epithelial cell adhesion molecule (EpCAM), aldehyde dehydrogenase (ALDH), CD90, CD24, c-kit, global-H and stemness genes. And the results do not suggest good correlation between reported markers in liver cancer stem cells. However, by using developed suspension culture method, we successfully enrich and collect the cell with cancer initiating characteristics population from the cancer cells. In this study, over thirty clinical samples collected from hepatocellular carcinoma patients were further evaluated through indicated culture condition, spheroid cell grew and had been isolated, which perform CSC-like properties. Among those well-diagnostic clinical samples, HCC-16, which isolated from a HCV-infected patient is considered as a HCV-correlated liver cancer cell line. HCC-16 performs high EMT signals and secret high angiogenic factors with cancer stem cell properties, including spheroid growth, drug resistance, stemness gene expression and grow in mice in 1000 cells. We are now establishing further identifications, markers and assays for HCC-16 and more clinical HCC samples directly. Unmet treatment and diagnosis development for HCV-related liver cancer and liver CSC are both our aim to study.

Keywords: cancer stem cell (CSC), hepatocellular carcinoma cells(HCC), HCC-16, EMT

A25

Effects of Hypoxia on Human Mesenchymal Stem Cells

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Mesenchymal stem cells (MSCs), which can be isolated from bone marrow and other somatic tissues, are residing in an environment with relative low oxygen tension. The purpose of this study is to investigate the effects of short-term hypoxia on MSCs, and we hypothesize that oxygen concentration regulates the intricate balance between cellular proliferation and commitment towards differentiation. In this study, human bone marrow-derived MSCs were cultured under hypoxia with 1% O_2. The proliferation rate of MSCs was significantly increased after a 7-day hypoxic culture period. Migration assay showed that hypoxia enhanced migration capabilities of MSCs. Moreover, expression of stemness genes including Oct4, Nanog, Sall4 and Klf4 was increased under hypoxia. Furthermore, the differentiation ability of MSCs under hypoxia favored osteogenesis while adipogenesis was inhibited during a 4-week induction period. Cytokine antibody array analysis showed that a number of growth factors were up-regulated after a 7-day hypoxic incubation and the differential expression of growth factors may account for the increased proliferation and osteogenic potentials of MSCs under hypoxic condition. Taken together, hypoxia provides a favorable culture condition to promote proliferation as well as osteogenesis of MSCs through differential growth factor production.

Keywords: mesenchymal stem cells, hypoxia, proliferation, differentiation, migration

A26

Endothelialization Capacity of Mesenchymal Stem Cells in Collagen-nanogold Composites

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This project is to use an extracellular matrix (collagen I, Col-I) incorporated with small amount (≈ 43.5 ppm) of gold nanoparticle (approximately 5 nm) as a material (Col-Au) to promote mesenchymal stem cells (MSCs) in damaged vessels in the endothelialization of the repair capacity, and to clarify the related molecular mechanism. The surface morphology of Col-I and Col-Au was exhibited that the average domain size of Col was 20.6 nm and the Col-Au was 25.1 nm. It can be observed the chemical composition of Col-Au had exhibited more NH group cross-link on original Col-I by using the FTIR analysis. First, intended to be test the biocompatibility of the Col-Au (proliferation, monocyte activation, free radical scavenging ability and platelet activation). Second, we also assesses on Col-Au induction of MSCs in the ability to repair damage vessels. Indeed, the molecular mechanism of stromal-derived factor (SDF-1α)/CXCR4 and vascular endothelial growth factor (VEGF) also evaluate for the interaction of the MSCs on Col-Au by in vitro study. The higher levels of eNOS/p-Akt protein and focal adhesion kinase (FAK)/metalloproteinase-2 (MMP-2) protein expression was induced by Col-Au. It was suggested that the Col-Au can be related through the activation of this signaling pathway promote MSCs proliferation and migration. We expected that Col-Au can be offer as novel biomaterials of endothelialization for vascular tissue engineering.

Keywords: collagen-nanogold composites (Col-Au), mesenchymal stem cells (MSCs), endothelialisation

A27

Engineered Nanogold-based Carrier for Disease Application

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Gene therapy has long been mentioned a promising treatment for many diseases. Successful gene therapy is thus dependent on the development of an efficient delivery vector. Two major vehicles have been used for gene delivery: viral and nonviral vectors. Typically based on nanoparticles delivery are preferred because of more safety concerns than viral vectors. Recently, we have developed a nanogold-based carrier, while conjugated to FITC. Nano-sized gold nanoparticles were modified by building self-assembling. We focus on this nanogold-based carrier, which are classified as multifunctional hybrid nucleic acid vectors or peptide lead to a delivery system and consider further prospect for clinical application. Nanogold-based carrier had no significantly toxicity effect in three different cells (mesenchymal stem cells, endothelial cells and fibroblast) has been observed by MTT assay. Meanwhile, the cellular uptake ability was enhanced upon treatment of cells with nanogold-based carrier conjugated with peptide, and the enhancement was especially remarkable for 30 min after the uptake. The uptake ability of nanogold-based carrier quantified as the fluorescence intensities by flow cytometry also demonstrated such tendencies. Overall, this study demonstrates that nanogold-based carrier is feasible and effective which may enable serve as a novel delivery strategy of biological molecules directly to the target tissue.

Key word: nanogold-based carrier

A28

Isolation and Characterization of Multipotent Stem Cells from Human Orbital Fat Tissue

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Loss of corneal epithelial cells results in visual problems. Stem cells isolated from the limbal area of the ocular surface are able to replenish lost corneal epithelial cells. However, destruction of the healthy limbus tissue is inevitable. Theoretically, orbital fat should be an excellent source to isolate stem cells for regenerating ocular tissues as the orbital connective tissues share the same embryonic origin with the ocular proper in early organogenesis. The purpose of this study is to isolate stem cells from the human orbital fat and to explore their differentiation potentials into epithelial cells. It was found that spindle-shaped, fibroblast-like cells with extensive proliferation potentials could be isolated from orbital fat tissues. These orbital fat-derived stem cells (OFSCs) possessed multi-lineage differentiation potential to become osteoblasts, chondrocytes and adipocytes. Upon mix-culture with corneal epithelial cells, OFSCs changed their morphology to round, polygonal epithelial-like cells. Loss of CD105 expression and increased expression of epithelial cell markers including epithelial specific antigen and zonal occludin-1 were found upon mix-culture with corneal epithelial cells. Moreover, corneal epithelial differentiation was evidenced by the expression of CK-19 and CK-3 after mix-culture with corneal epithelial cells while human adipose-derived stem cells from subcutaneous fat were unable to differentiate into corneal epithelial cells under the same induction condition. We further found that direct contact with corneal epithelial cells was essential for OFSCs to commit to corneal epithelial cells. Taken together, orbital fat tissues are a novel source for multi-potent stem cells which possess the potential to differentiate into corneal epithelial lineage. OFSCs are therefore a potential candidate for cell therapy and tissue engineering of corneal epithelium.

Keywords: orbital fat stem cells (OFSCs), corneal epithelial differentiation, cell-cell contact

A29

Meiotic Competent Human Germ Cell-like Cells Derived from Human Embryonic Stem Cells Induced by BMP4/WNT3a Signaling and OCT4/EpCAM Selection

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The establishment of an effective germ cell selection/enrichment platform from *in vitro* differentiating human embryonic stem cells (hESCs) is crucial for studying the enigmatic molecular, signaling and epigenetic processes governing human germ cell specification and development. In this study, we demonstrated the first successful enrichment of a germ cell population from spontaneously differentiating hESCs by an OCT4-EGFP reporter system. We further demonstrated that a surface marker, EpCAM, is expressed in the human fetal gonads. By itself, EpCAM is an effective selection marker for germ cell enrichment from differentiating hESCs. Combining OCT4 and EpCAM double selection can further enrich a meiotic competent germ cell population. With the percentage of $OCT4^+/EpCAM^+$ cells as readout, we demonstrated the synergistic effect of WNT3a/β-CATENIN and BMP4/pSMAD1/5/8 in promoting hESCs toward germline fate. Finally, we showed that combining WNT3a /BMP4 induction and OCT4/EpCAM selection can significantly increase the quantity and quality of the germ cell-like population. Importantly, co-transplantation of these cells with dissociated mouse neonatal ovary cells into SCID mice resulted in a more homogenous germ cell cluster formation *in vivo*. The stepwise platforms established in this study provide a solid foundation towards illuminating the detailed molecular mechanisms governing human germ cell development, which has implications in not only human fertilities but regenerative medicine in general.

A30

The Study of Role of Pulmonary Stem Cell (PSCs) in Hyperoxia Induced Lung Injury

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Hyperoxic ventilation has been widely used to support respiratory function for preterm infants. However, such treatment causes injuries and diseases. BPD (bronchopulmonary dysplasia) is the most predominant chronic lung disease among children after hyperoxia ventilation treatment. Its syndrome includes inflammation, alveolar hypertrophy, lung development impairment and respiratory malfunction among the entire lifespan. It is believed that adult stem cells play critical roles in tissue development and injury repair. In previous studies, we have isolated pulmonary stem/progenitor cells (mPSCs) via primary culture of lung tissues. Under induction condition, the mPSCs underwent differentiation into type-1 pneumocytes. Although various therapies were proposed, there was little information about the behavior of pulmonary stem/progenitor cell in this treatment. In our study, hyperoxic condition (90% O_2) arrested differentiation of mPSCs into alveolar lineage by inducing cell apoptosis. In different hyperoxic condition, a significant reduction of apoptotic cells numbers was observed in treatment with 40 % O_2, which indicate the marginal level of hyperoxic tolerance for mPSCs. Nevertheless, treatment of hyperoxia on differentiating mPSCs resulted in an apoptotic cells area between stem/progenitor cells and terminal differentiated type-1 pneumocytes, where a differentiation switch was believed to be turn on. Apoptosis of mPSCs could be rescued by treatment with NAC (N-acetyl cysteine). This phenotype could be reproduced by treating the cells with H_2O_2 , which generates enormous ROS. Taken together, we proposed that mPSCs undergoing an alveolar differentiation lineage had a weaker resistance to ROS hazards; compared to stem/progenitor cells incorporated terminal differentiated type-1 pneumocytes.

A31

Cell Contact Accelerates Replicative Senescence of Human Mesenchymal Stem Cells Independent of Telomere Shortening and P53 Activation: Role of Ras and Oxidative Stress

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Mesenchymal stem cells (MSCs) are of great therapeutic potentials due to their multi-lineage differentiation capabilities. Before transplantation, *in vitro* culture-expansion of MSCs is necessary to get desired cell number. We observed that cell contact accelerated replicative senescence during such process. To confirm the finding as well as to investigate the underlying mechanisms, we cultured both human bone marrow- and umbilical cord blood-derived MSCs under non-contact culture (subculture performed at 60-70% of confluence), or contact culture (cell passage performed at 100% of confluence). It was found that MSCs reached cellular senescence earlier in contact culture, and the doubling time was significantly prolonged. Marked increase of senescence-associated beta-galactosidase positive staining was also observed as a result of cell contact. Cell cycle analysis revealed increased frequency of cell cycle arrest after contact culture. It was noted, however, that the telomere length was not altered during contact-induced acceleration of senescence. Moreover, cell cycle checkpoint regulator P53 expression was not affected by cell contact. Marked increase in intracellular reactive oxygen species (ROS) and a concomitant decrease in the activities of anti-oxidative enzymes were also observed during contact-induced senescence. Importantly, increased $p16^{INK4a}$ following Ras up-regulation was found after contact culture. Taken together, cell contact induced accelerated senescence of MSCs, which is telomere shortening- and p53-independent. ROS accumulation due to defective ROS clearance function together with Ras and $p16^{INK4a}$ up-regulation play an important role in contact-induced senescence of MSCs. Over-confluence should therefore be avoided during in vitro culture-expansion of MSCs in order to maintain their qualities for clinical application purposes. Besides, contact-induced senescence model reported in this study will serve as a useful model system that allows further study of the molecular mechanisms of senescence in MSCs.

Key words: mesenchymal stem cells (MSCs), senescence, Ras, oxidative stress, $p16^{INK4a}$

A32

Cardiac Injury Protection from Mouse Bone Marrow Stromal Cells with In Utero Transplantation Followed by Secondary Postnatal Boost

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Limited donor-cell engraftment to the injured tissue restricts therapeutic efficacy of stem cell transplantation. Herein, we proposed an alternative strategy by using *in utero* transplantation (IUT) to create mixed-chimerism environment in recipients and to facilitate donor-cell engraftment followed by postnatal secondary boost with same cells. Mouse bone marrow stromal cells (BMSCs) were used as the xenogenic donor cells and given into rat fetus as an early exposure of IUT treatment. The engraftment potential was analyzed as the presence of BMSCs by flow cytometry or PCR in recipient tissues. The function of cardioprotection, was tested by given 1×10^6 cells to rat IUT hearts with ischemia/reperfusion (IR) injury that was induced by a 45 min of left coronary ligation and released for 72 h. Mouse BMSCs demonstrated an immunosuppressive effect when mixed with mouse or rat lymphocytes. IUT treatment only causes few BMSCs engrafted to fetal (embryonic day 20) and adult (4 weeks after birth) rat organs including heart, but engraftment was increased in hearts of the IUT rats after second boost. This was coincided with attenuation of cardiac injury caused by IR. Interestingly, an up-regulation of CXC chemokine receptor type 4 (CXCR4) was seen when exposed BMSCs to hypoxia. This indicates that enhanced engraftment of mouse BMSCs to post-ischemic rat hearts possibly is dependent on CXCR4. Moreover, results of flow cytometry demonstrated that the presence of $CD34^+$ cells in rat IUT hearts with IR injury were increased. These observations suggest that enhanced engraftment of donor BMSCs to rat IR hearts by CXCR4 may recruit endogenous $CD34^+$ cells of recipients which in turn protects heart against IR. This also supports the notion of fetal preconditioning with BMSC enhances the efficiency of progenitor cell-mediated organ protection after postnatal second boost in xeno-transplantation.

Keywords: In utero transplantation, Preconditioning, Bone marrow stromal cells, ischemia/reperfusion

A33

Calcium Phosphate Cement Chamber as Immune-isolative Device for Bioartificial Pancreas: an *in vivo* Study on Diabetic Canine

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Bioartificial pancreas (BAP) may resolve the issues of immune rejection and insufficient donor supply to provide a new treatment for type 1 diabetes. However, fibrous tissue outgrowth and hypoxia are the major restrictions for the application of BAP. Accordingly, a calcium phosphate cement (CPC) chamber which satisfied the criteria of immunoisolation is utilized as an immunoisolative device, and the intramedullary cavity is proposed as an implant site. Mouse insulinoma cells were encapsulated in agarose gel and then enclosed in a CPC chamber to fabricate a BAP. BAPs were implanted in the femoral intramedullary cavity of diabetic dogs. Pre- and postprandial blood glucose levels were monitored perioperatively. Blood samples were collected for the analysis of C-peptide level, and physiological conditions were observed at pre-determined intervals. BAPs were retrieved at 12 weeks post-operatively for histological examination. Preprandial blood glucose level of diabetic dogs decreased from 420±25 to 223±47 mg/dl at one day post-operatively and was maintained in the range of 259±36 mg/dl for 12 weeks. As serum C-peptide level increased from 5.3±2.8 to 105.7±19.4 pmol/l, the rate of decrease of postprandial blood glucose was accelerated. Histological examination revealed that recipient bone tissues were binding to the surfaces of BAPs directly; there was no development of fibrous tissue. Immunohistochemical stain was positive for insulin in the enclosed insulinoma cells. This study demonstrated that BAPs implanted into the intramedullary cavity functioned well during the experimental period. Thus, the intramedullary cavity can serve as an implant site for BAPs.

Keywords: Xenotransplantation; Immunoisolation; Bioartificial pancreas; Calcium phosphate cement.

A34

Serum-free Production of Erythroid Progenitor cells from Hematopoietic Stem Cells

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Hematopoietic stem cells (HSCs) are multipotent stem cells that give rise to all the blood cell types, including the myeloid and lymphoid lineage cells. We had previously developed the serum-free HSC (SF-HSC) expanding medium and the induction system of nature killer cells, megakaryocytes and plateles derived from HSCs. In this study, we develop a novel serum-free culture system that could induce HSCs into erythroid progenitor cells by one-step protocol. Firstly we used the systematic procedure with factorial design and steepest ascent method to optimize the cytokine cocktail and chemical combination that could facilitate erythropiesis. Using the quantitative real-time PCR, the erythropoieis markers, including GATA-1, α-hemoglobin, β-hemoglobin and γ-hemoglobin could be detected to highly express in induced cells. The morphology of cells showed the hemoglobin accumulation obviously. In addition, we also found that over 80% of the induced cells are $CD71^+/GlyA^+$ cells by using FACS analysis. In conclusion we combined the SF-HSC expansion medium with the novel serum free induction system to make erythroid progenitor cells differentiated from HSCs with high efficiency, and this culture system could provide the alternative supply for clinical trails.

Keywords: hematopoietic stem cell, serum-free, erythroid progenitor cell

A35

The Roles of DNMT3L in Germ Line Homeostasis and the Biological Functions of Germ Line Stem Cells

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Epigenetic regulation is crucial for proper cellular function. DNA methyltransferase 3-like (DNMT3L), an epigenetic modifier tightly associated with transcriptional repression, is an essential regulator for gametogenesis. The *Dnmt3l*-knockout (*Dnmt3l*-KO) experiments showed that ablation of DNMT3L results in retrotransposon reactivation, imprinting defect, male germ cells arrest at meiotic prophase and gradual germ cell loss in adult mice. The presence of Sertoli cells only in *Dnmt3l*-KO adult testes implies that DNMT3L may have a function in the maintenance of spermatogonial progenitor cells (SPCs). In the testes of neonatal *Dnmt3l*-KO mice, we found a reduction in cells positive for PLZF which predominantly marks the slow cycling quiescent population of SPCs. Consistent with this decrease in the quiescent cell population, loss of *Dnmt3l* in SPCs was associated with (1) the disappearance of perinuclear staining pattern of heterochromatin (H3K9me3) characteristic of quiescent SPCs, (2) an increase in proliferating SPCs and (3) decreased expression of piRNA-associated factors that are essential for retrotransposon silencing. Furthermore, we performed germ cell transplantation to test the stem cell capacities of *Dnmt3l*-KO SPCs. It showed that early postnatal *Dnmt3l*-KO germ cells failed to repopulate busulfan treated recipient testes, while germ cells from their wild-type littermate controls displayed obvious colonization activity. These results indicate that DNMT3L exerts an epigenetic effect on germline homeostasis by influencing the stem cell capacity of germline stem cells.

Keywords: DNMT3L • Germline stem cell • Quiescence • Transplantation

A36

Dual Regeneration of Muscle and Nerve by Intravenous Administration of Human Amniotic Fluid Mesenchymal Stem Cells Regulated by SDF-1α in a Sciatic Nerve Injury Model

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Background: Human amniotic fluid mesenchymal stem cells (AFS) have been shown to promote peripheral nerve regeneration. The expression of stromal derived factor 1 α (SDF-1α in the injured nerve exerts a trophic effect by recruiting progenitor cells that promote nerve regeneration. In this study, we investigated the feasibility of intravenous administration of AFS according to SDF-1α expression time profiles to facilitate neural regeneration in a sciatic nerve crush injury model.
Material and Methods: Peripheral nerve injury was induced in 63 Sprague-Dawley rats by crushing the left sciatic nerve using a vessel clamp. The animals were randomized into one of three groups: Group I: crush injury as the control; Group II: crush injury and intravenous administration of AFS ($5x10^6$ cells for 3 days) immediately after injury (early administration); and Group III: crush injury and intravenous administration of AFS ($5x10^6$ cells for 3 days) 7 days after injury (late administration). Evaluation of neurobehavior, electrophysiological study, and assessment of regeneration markers were conducted every week after injury. The expression of SDF-1α, neurotrophic factors, and the distribution of AFS in various time profiles were also assessed.
Results: SDF-1α increased the migration and wound healing of AFS *in vitro*, and the migration ability was dose dependent. Crush injury induced the expression of SDF-1α at a peak of 10-14 days either at nerve or muscle, and this increased expression paralleled the expression of its receptor CXCR-4. Most AFS was distributed to the lung during early or late administration. Significant deposition of AFS in nerve and muscle only occurred in the late administration group. Significantly enhanced neurobehavior, electrophysiological function, nerve myelination, and expression of neurotrophic factors and acetylcholine receptor were demonstrated in the late administration group.
Conclusion: AFS cells can be recruited by expression of SDF-1α in muscle and nerve after nerve crush injury. The increased deposition of AFS paralleled the expression profiles of SDF-1α and its receptor CXCR-4 either in muscle or nerve. AFS administration led to improvements of neurobehavior and expression of regeneration markers. Intravenous administration of AFS may be a promising alternative treatment strategy in peripheral nerve disorder.

Keywords: Amniotic fluid mesenchymal stem cells, SDF-1α, nerve regeneration, sciatic nerve crush injury

A37

Human Chromosome-21 Derived microRNAs are Overexpressed in Neuron Progenitor Cell Derived from Trisomy-21 iPS cells

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Trisomy 21 is a common chromosomal abnormality caused by an extra copy of chromosome 21 that contributes to the cognitive impairments associated with Down Syndrome. Recent reports demonstrated that human chromosome 21 (Hsa21) harbors five miRNA genes; let-7c, miR-99a, miR-125b-2, miR-155 and miR-802 (Hsa21-miRNAs). In order to investigate neuronal progenitor cells carrying triplicates of chromosome 21, we generated Trisomy-21 iPS cells from human second trimester amniotic fluid stem cells by lentiviral delivery of 4 Yamanaka factors (Oct4, Sox2, Klf4 and c-Myc). We found that the expression of Hsa21-miRNAs in neuron progenitor cells derived from Trisomy-21 iPS cells (T21 AF-iPS-NPC) are overexpressed, contributing to decreased expression of specific target proteins and to features specific to neuronal development in Trisomy 21 patients. In this study, we report that T21 AF-iPS as a disease model to further understand the role of Hsa21-miRNAs in neuron development of individuals with Down Syndrome.

Keywords: Amniotic fluid**;** Induced pluripotent stem cells**;** Feeder free; Alkaline phosphatise

A38

Characterization of CD34 Positive Mesenchymal Stem Cells Derived From Human Endometrium Tissue (CD34$^+$ EnMSCs)

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Human endometrium is a highly regenerative tissue of growth, differentiation, and shedding during women's reproductive years. In this communication, characteristics of an embryonic like clonogenic adult endometrial CD34$^+$ mesenchymal stem cell population (EnMSCs) with co-expressions of somatic and hematopoietic CD34 stem/progenitor markers, irrespective to their tissue origin, are described.

Comparing FACS-sorted CD34$^+$ EnMSCs to the CD34$^-$ cells, we found both populations present typical mesenchymal stem cell markers (CD29, CD44, CD73, CD90, CD105, EGFR and integrin α2β1) but negative to the cytokeratin 18 epithelial marker. CD34$^+$ EnMSCs co-expressed stronger early embryonic genes (Oct-4$^+$, Nanog$^+$, Rex-1$^+$, SOX-2$^+$, and SSEA3,4) and somatic stem cell markers (CD117, CD133, CD146, CD54) than the CD34$^-$ EnMSC population EnMSC. CD34$^+$ EnMSCs exhibit neurogenic differentiation potentials with highly expressions of GFAP, GalC, tyrosine hydroxylase, and Tuj1. In cardiomyogenic differentiation, CD34$^+$ EnMSCs generated mature MHC$^+$/ Troponin$^+$ cardiomyocytes. In hepatic transdifferentiation, CD34$^+$ EnMSCs derived hepatocytes present more albumin production beside CD34$^-$ EnMSCs. CD34$^+$ EnMSCs also possess superior efficiency in myotube and vascular tube formation. A further functional genomic profiling analysis supportive is to the above biological observations.

These results suggested that CD34$^+$ EnMSCs in endometrium tissue may be acquired as an ideal resource for cell therapeutic and tissue regenerative medicine. Further precilinical animal study in vivo for their engraftment efficiency is undergoing.

Keywords: endometrium, CD34$^+$, MSCs, biomaterial, cytotherapy, myoangioblast, cardio-myogenic, neurogenic, and hepatic, regenerative medicine.

A39

Glucocorticoids Modulate Acinar-to-ductal Transdifferentiation in Pancreatitis

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Ductal reprogramming has been considered as an initial event of pancreatic intraepithelial neoplasm (PanIN), a precursor lesion of pancreatic ductal adenocarcinoma (PDAC). Recent reports demonstrated that acinar cells can directly transdifferentiate into ductal-like cell during chronic pancreatitis. However, the mechanism is less clear. We hypothesize that certain inflammation-induced factors may responsible for induction of acinar-to-ductal transdifferentiation. Indeed, glucocorticoids play an important role in pancreatic cell fate determination and in modulation of inflammatory responses. In current work, activation of glucocorticoid receptor was initially identified in patients of chronic pancreatitis. We also observed activation of glucocorticoid receptor in the pancreas of mice that treated with caerulein . Moreover, the level of ductal markers such as DBA and CK19 were increased in these mice that received pancreatitis inducer. Initially, we bred Elas-CreER mice to UBC-floxedDsRedT3-emGFP mice for lineage-tracing experiments and demonstrated some GFP-labeled acinar cells can directly transdifferentiated to ductal cells in the pancreas of mice that received 3-week caerulein treatment. Moreover, we also found that Dexamethasone treatment increased ductal tissue formation in the pancreas of adult mice. To further determine the role of glucocorticoid receptor, Elas-CreER;GR $^{flox/flox}$ mice was utilized and confirmed glucocorticoid receptor was a key regulator of acinar-to-ductal transdifferentiation. Indeed, pancreatitis is a risk factor of pancreatic cancer, and our findings suggest that activation of glucocorticoid pathways in chronic pancreatitis maybe a key step of ductal reprogramming which possibly leads to generation of ductal lesion in pancreatic carcinogenesis.

Keywords: Glucocorticoids, acinar-to-ductal transdifferentiation, pancreatitis

A40

Integrative Omics Study on Epigenetic Modulators in Colorectal Cancer Initiating Cells during Cancer Progression

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Epigenetic alterations such as aberrant chromatin structure changes play important roles in the establishment of tumor suppressor gene silencing during tumorigenesis and metastasis. Heterogeneous epigenetic abnormalities at the levels of DNA methylation and core histone modifications are found to coexist with genetic alterations in most of the colorectal cancer specimens. The interactions between histone H3 lys9 methylation and the methylation of the nearby genomic DNA currently adapt a model whereby these two changes form a mutually reinforced silencing loop and contribute to the epigenetic inactivation of particular genes, such as tumor suppressor genes, cyclin-dependent kinase inhibitors, DNA repair genes, apoptosis mediators, nuclear receptors, transcription factors, and cell adhesion molecules, during the development of colorectal cancer cells. However, the function of the histone H3 lys9 methyltransferases in colorectal cancer initiating cells remains largely unclear. Euchromatin histone lysine N-methyltransferase 2 (EHMT2) is a major H3 lys9 methyltransferase which adds mono- and di-methyl groups to the lysine 9 of histone 3 in euchromatin regions. The result of EHMT2 activity ends in the transcriptional repression of its downstream targets, including epithelial cell adhesion molecule (EpCAM), an important molecule involved in preventing tumor invasion and metastasis. Here we not only use the existing colon cancer model, the ApcMin mouse, as a resource to provide materials for “omics” studies, we also use an Lgr5-EGFP-IRES-CreERT2 allele to manipulate epigenetic modulators, EHMT2, to provide colon stem cells and cancer initiating cells specificities to dissect their roles with a specific focus on the epigenetic regulatory networks in colorectal cancer progression in vivo.

Keywords:, cancer initiating/stem cell, colorectal cancer, epigenetic modulators, systems biology, mouse model for human disease

A41

HBx-mediated Transformation of Adult Liver Stem Cells to Tumor Initiating Cells

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Chronic hepatitis B virus (HBV) infection is the major risk factor for hepatocellular carcinoma (HCC); however, the molecular mechanisms that HBx induces hepatocarcinogenesis have not been fully elucidated. Recent data obtained from the clinical studies indicated that in an area endemic for HBV infection, some HCC cells were positive for stem cell markers and displayed the characteristics of cancer stem cells. We therefore assume that liver cancer caused by HBV infection is a disease of stem cells as bipotential liver stem/progenitor cells are activated during hepatitis and persist in the injured tissue for a sufficient length of time that allows acquiring the requisite number of genetic changes for neoplastic development. Initially, we determined if trans-activating factors derived from the HBV genome-HBx antigens would cause direct transformation of liver stem/progenitor cells. Previously, we demonstrated that, when fetal liver cells were grown on polyvinyl alcohol (PVA) coated glassware, fetal liver stem/progenitor cells was enriched in spheroids. We applied the same system to isolate and grow liver stem/progenitor cells to enrich liver stem/progenitor cells. These cells expressed a panel of markers of stem cells including CD133, EpCAM, CD49f, AFP, CK19 and Oct-4 and can differentiate into mature hepatocytes expressed albumin and CYP3A1. We further r ectopically expressed HBx in adult liver stem/progenitor cells and found it would cause cells transformation as judged by (1) increased colony-forming ability and proliferation rate of liver stem/progenitor cells, and (2) transformed liver stem/progenitor cells could be continuously passaged without signs of senescence. Most importantly, orthotropic transplantation of HBx-transformed liver stem/progenitor cells to NOD-SCID mice would enable to form HCC-like tumor nodules. In summary, our findings indicate HBx has the capability to cause direct transformation of liver stem/progenitor cells to self-renew HCC-initiating cells.

Keywords: adult liver stem cells, HBx, transformation, tumor tumor initiating cells

A42

Stem Cells from Human Exfoliated Deciduous Teeth Differentiate into Neuron-like Cells by Chitosan conduit

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Stem cells from human exfoliated deciduous teeth (SHED) can identify as a novel population of postnatal stem cells capable of differentiating into many mature cells. This study investigated the differentiation efficacy of SHED into neural cells by chitosan conduit. The cells isolated from the exfoliated deciduous teeth following purification, and expressing mesenchymal surface molecules ($CD34^-$, $CD105^+$, $CD73^+$ and $CD90^+$) by flow cytometry assay. SHED can proliferate greatly in α-MEM medium (including 10% FBS，5 ng/ml bFGF and 100 μM L-ascorbic acid 2-phosphate).

SHEDs seeded into 4% chitosan conduit with Neurobasal A medium (including B27 supplement、20 ng/ml EGF and 40 ng/ml bFGF) for neuronal differentiation. The cellular morphology is slender spindle that is similar to the shape of mesenchymal stem cell by SEM and confocal microscope observation. However, cells would cluster together on chitosan conduit under dynamic culture, and the slender morphology decreased evidently. After 34 days incubation, the cultivated cells can express Neuron Specific Enolase that distributed over neuron, peripheral nerve and nerve internal secretion organization in vivo. Our data indicate that SHEDs potentially differentiate into neural cells.

Keywords：SHED、neural cell、differentiation、chitosan conduit

A43

Cytokeratin-19 Lineage Tracing in the Liver Provides Direct Evidence that Hepatic Oval Cells are Originated from Bile Ducts and are Capable of Differentiating into Hepatocytes

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It has been known that liver has amazing regenerative capability after acute injury or partial hepatectomy. In these occasions, restoration of the lost liver mass is accomplished by proliferation of mature hepatocytes. However, during several liver diseases that hepatocyte proliferation is impaired, there emerges a population of so called “oval cells” that were believed to come from terminal bile ductules and to give rise to both hepatocytes and bile duct cells. Although histological studies have shown that the oval cells expanding into liver parenchyme were connecting the bile ductules at one end and hepatocytes at the other, direct evidence showing their origin and their differentiating cell fate was still lacking. The present study by tracing cytokeratin-19 (K19)-positive lineage in the liver is to provide the direct evidence of above scenario. K-19 is a well-established marker for both bile duct cells and oval cells. K19LacZ mice were generated by crossing tamoxifen-inducible K19CreERT mice with Rosa26f/f-LacZ mice. They received liver toxins diethylnitrosamine or 3, 5-diethoxycarbonyl-1, 4- dihydrocollidine to induce liver injury and tamoxifen to induce Cre expression. The liver sections were subjected to staining of β-galactosidase (gal) activity and K19 immunohistochemistry. In mice receiving only tamoxifen treatment, the X-gal staining was restricted to bile duct cells indicating a highly specific labeling. When liver toxins were administered following tamoxifen treatment, dual-labeling of β-gal and K19 showed that β-gal labeling was shown in K19+ oval cells expanding from portal tracts into liver parenchyma. These observations showed direct evidence that the hepatic oval cells were derived from bile duct cells. Furthermore, in mice with β-gal labeling at hepatic oval cells (tamoxifen treatment was preceded by liver toxin administration), β-gal+ hepatocyte clusteres were identified in the parenchyma, providing evidence that some of the oval cells were differentiated into hepatocytes. In conclusion, the present K19 lineage tracing studies proved that oval cells are derived from bile duct cells and some of the oval cells are able to differentiate into hepatocytes.

Keywords: liver regeneration, lineage tracing, oval cell

A44

Inflammation Enhances the Transformation of Epithelial-like Endometrium Stem Cells and Initiates Endometriosis

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Endometriosis is the growth of endometrial tissues eutopically and/or ectopically. This disease affects nearly 10% of reproductive aged women with infertility. However, mechanism involved in endometriosis initiation still remains largely unknown. Stem cells in endometrial basalis layer have been hypothesized to mediate endometrium regeneration and endometriosis initiation. In clinic, chronic inflammation is tightly associated with endometriosis. In this aspect, we hypothesized the role of inflammatory cytokines in stemness gene expression and epithelial-mesenchymal transition (EMT) for endometriosis initiation. To test this hypothesis, endometrium tissues of normal endometrium (n= 3), myoma (n= 4), hyperplasia (n= 37), adenomyosis (n= 20), and chocolate cyst (n= 49) were collected for stemness-related gene and CA125 analysis. Real-time PCR analysis showed high OCT-4 and NANOG expression and CA125 level in endometriosis tissues (adenomyosis and chocolate cyst) which were in inflammation situation. Immunohistochemical staining showed the OCT-4 protein expression was localized in luminal epithelial cells of human endometriosis tissues. Further experiments using a serum-free culture system to generate mouse epithelial-like endometrium stem cells (mEESCs) which expressed alkaline phosphatase activity, stem cell markers (such as Oct-4, SSEA-1, CD49f, CD63, and CD34), and IGF-1/IGF-1R (stemness regulatory signaling). Interestingly, real-time PCR analysis reveals that proinflammatory cytokines elevated the stemness expression (Oct-4, Sox2, c-Myc, Klf4, and Igf-1) and EMT of mEESCs. This result was further demonstrated that proinflammatory cytokines increased the OCT-4 expression in human endometrium RL-95-2 cells using OCT-4 promoter luciferase assay. Additionally, overexpression of OCT-4 in human endometrium RL95-2 and HEC-1A cells significantly increased the cell EMT process and cell migration. In conclusion, here we demonstrated that proinflammatory cytokines might promote EMT process to initiate endometriosis by transformation of endometrium epithelial stem cells. This observation may provide the possible therapeutic targeting of infertility and tumorigenesis.

Keywords: inflammation, endometrium stem cells, endometriosis, IGF-1R signaling, EMT

A45

Endothelial Cells Promote the Maturation and Cell Therapy of Embryonic Stem Cell-derived Cardiomyocytes

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Embryonic stem (ES) cells and induced pluripotent stem (iPS) cells have the potential to supply an unlimited source of cardiomyocytes for cardiac cell therapy. However, freshly isolated ES/iPS cell–derived cardiomyocytes are immature; they lack mature sarcomeres and gap junctions, and do not contract synchronously, raising the risk of arrhythmia when used in cardiac stem cell therapy.

Here, we demonstrate both *in vitro* and *in vivo* that co-culturing with endothelial cells but not with fibroblasts significantly increases the maturity indices of ES-derived cardiomyocytes (ES-CMs), including cell size, axis alignment, sarcomere organization, contraction rate and the expression of the gap junction protein connexin-43. This effect was not observed in conditioned medium from endothelial cell culture or in a transwell culture system, indicating that direct cell–cell contact is necessary. Investigation of the molecular mechanisms regulating this endothelial effect revealed that the Notch signaling was involved. In particular, upon coculturing, Notch downstream effector genes Hes1, Hey1 and Hey2 and also the activated form of Notch1 were downregulated. The sole addition of a γ-secretase inhibitor to ES-CM cultures was sufficient to increase ES-CM maturity similar to endothelial cell coculture. Finally, co-transplantation of ES-CMs with endothelial cells improved ES-CM engrafting and growth, ameliorated cardiac functions and reduced infarct size in a mouse model of myocardial infarction.

Our findings indicate that coculturing ES-CMs with endothelial cells promotes its maturation through direct cell contact. Additionally, co-transplantation of both cells into the infarct heart rescues cardiac function after myocardial infarction, suggesting its potential to enhance the effectiveness of cardiac stem cell therapy.

Keywords: heart failure, cardiac stem cell therapy, arrhythmia; Notch.

A46

Hypoxia Mediates the PGC-like Cell Migration through Cooperative Interaction of IGF-1/IGF-1R-Akt and SDF1/CXCR4 Signaling

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Recent studies have demonstrated the important role of G-protein-coupled receptor CXCR4, and its ligand Stromal cell-derived factor-1 (SDF1) in mediating the migration of primordial germ cells (PGCs) which are under niche hypoxia. However, little is known about hypoxia-interacting endocrinal signals which are cooperative with SDF1/CXCR4 signaling in regulating PGC migration. For this purpose, we have previously generated mouse PGC-like alkaline-phosphatase-positive germline stem cells (PGC-like AP^+GSCs) *in vitro* using a serum-free culture system (Huang et al, FASEB J, 2009). By using this serum-free culture system, we demonstrated that hypoxia (5% O_2) greatly increased the cell proliferation (*Cyclin D1*/*c-Myc* gene expression, BrdU+ incorporation), stemness-related gene level (*Oct-4*, *Sox2*, *Nanog*, *Klf-4*), and cell migration of AP^+GSCs. Proteomic approach demonstrated that hypoxia significantly enhanced the SDF1 protein level of AP^+GSCs up to 78 folds. Interestingly, hypoxia also increased IGF-1 as well as the SDF1 and CXCR4 expression level of AP^+GSCs. PPP (IGF-1R phosphorylation inhibitor) and LY294002 (PI3K inhibitor) significantly suppressed the hypoxia-induced the cell migration of AP^+GSCs. In summary, here we demonstrate the niche hypoxia affects the PGC-like AP^+GSC migration might be through the cooperative interaction of IGF-1/IGF-1R-Akt and SDF1/CXCR4 signaling. This finding provides insights into the niche endocrinology underlying the early pluripotent germ cell development.

Keywords: hypoxia, primordial germ cells, germline stem cells, IGF-1R signalling

A47

Inflammation Initiates Oct-4/Nanog Expression through IL-6-IGF-1R Signaling Activation and is Associated with Early Recurrence of HBV-related HCC

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Inflammatory microenvironment is a well-known promoter of hepatocellular carcinoma (HCC), a cancer with high rates of postoperative recurrence and poor prognosis. However, mechanisms underlying the inflammation-induced HCC promotion remain largely unknown. Here we demonstrate the effect of proinflammatory cytokine IL6 on IGF-1R signaling activation which initiates OCT4/NANOG expression and is associated with HBV-related HCC (HBV-HCC) recurrence. In this study, real-time quantitative PCR analysis demonstrated a high correlation between IGF-1R and OCT4/NANOG transcriptional expression in human HCC frozen tissues (N=119, R=0.08097 for OCT4; R= 0.8375 for NANOG). Interestingly, the OCT4, NANOG, or IGF-1R level in HBV-HCC was significantly higher than that in non HBV-HCC. Cultivation of cells in inflammation condition medium (Inflam-CM) increased OCT4/NANOG level and side population cell percentage in $HBV^{+}HBsAg^{+}$ cells (HepG2.2.15 and Hep3B), but not in $HBV^{+}HBsAg^{-}$ (HA22T) or $HBV^{-}HBsAg^{-}$ cells (HepG2 and Huh7). Epifluorescence/luciferase assay further demonstrated that Inflam-CM increased the GFP+ cell population as well as the luciferase activity of OCT4 promoter-GFP/luciferase-HepG2.2.15 cells. Importantly, Inflam-CM significantly increased the IGF-1/IGF-1R transcriptional level and activated the IGF-1R signaling (phospho-IGF-1R and phospho-Akt) in HepG2.2.15 and Hep3B. Blockage of IGF-1R phosphorylation by picropodophyllin (PPP) dramatically decreased the OCT4/NANOG and ABCG2 transcriptional activity. IL-6 stimulated the autocrinal IGF-I and IL-6 expression whereas AG490 (a phospho-STAT inhibitor) decreased the IGF-1R phosphorylation. The significant clinical association between early and overall postoperative recurrence and IGF-1R/OCT4/NANOG expression in both gene (real time RT-PCR) and protein

(immunohistochemical staining) levels were demonstrated. When etiologic differences were considered, HBV-positive patients have the trend towards early HBV-HCC recurrence. ***Conclusion*:** Niche inflammatory stress might activate an autocrinal IL-6-IGF-I/IGF-1R-Akt signaling and OCT4/NANOG expression which is associated with early HBV-HCC recurrence. These results provide potential targets for individualized adjuvant therapy for HBV-HCC.

Keywords: HCC, IGF-1R, inflammation/IL-6, NANOG, OCT4, HBV

A48

Ciliogenic RFX Transcription Factors Regulate FGF1 Promoter and Aurora-A Kinase Activation in Human Glioblastoma Stem Cells

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Ciliogenesis regulated by ciliogenic RFX transcription factors is initiated as cells enter quiescence, whereas Aurora-A kinase-mediated cilium resorption precedes mitosis. Fibroblast growth factor 1 (FGF1) has been suggested as a mitogenic factor for many cell types and a survival factor for non-dividing quiescent cells. Human *FGF1* gene 1B promoter (-540 to +31)-driven green fluorescent protein (F1BGFP) could recapitulate endogenous *FGF1* gene expression. It can also be used to isolate neural stem/progenitor cells (NSPCs) and glioblastoma stem cells (GBM-SCs) from mouse brains and human glioblastoma tissues, respectively. In this study, we investigate the role of FGF1 in the balance of ciliogenesis and cell proliferation through Aurora-A kinase activation. We showed that F1BGFP reporter could be used to isolate F1BGFP(+) GBM-SCs with higher levels of cell proliferation rate and Aurora-A kinase activation than F1BGFFP(-) cells in serum-free culture conditions either with or without EGF and FGF stimulation. Furthermore, we demonstrated that ciliogenic transcription factors RFX1, RFX2 and RFX3 could directly bind the 18-bp *cis*-element (-484 to -467) of F1B promoter, and regulate *FGF1* promoter and F1BGFP expression. In addition, protein kinase inhibitors, staurosporine and rottlerin, could decrease the percentage of F1BGFP(+) cells, the amount of RFX2/3 complex and the efficiency of neurosphere formation. The reduction in neurosphere forming efficiency could be rescued by addition of exogenous FGF1. In conclusion, this study suggests, for the first time, that the combinatorial effects of ciliogenic RFX transcription factors may adjust the balance between cell proliferation and ciliogenesis through the differential modulation of *FGF1* gene expression.

Keywords: glioblastoma stem cells, FGF1, primary cilia, RFX, Aurora-A

A49

The Characterization of Bone Marrow-derived Mesenchymal Stem Cells from Dogs after Euthanasia

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Compared to embryonic stem cell, bone marrow-derived mesenchymal stem cells (BMMSCs) are more suitable for clinical cell therapy due to the low immunogenicity, low tumorigenic risk and better accessibility. In this study, we successfully purified mononuclear cells with spindle shape derived from canine bone marrow after euthanasia due to various clinical conditions. This population of cells can be cultured *in vitro* and induced differentiation into at least three lineages: osteocytes, adipocytes and chondrocytes. The flowcytometry of the cell-surface cluster of differentiation makers showed consistent profiles as BMMSCs-specific marker ($CD34^-$, $CD44^+$, $CD45R^-$, $CD90^+$). Telomerase activity remained stably low through at least passage 6 with no significant variation between individuals implying low tumorigenic risk. These results suggested that BMMSCs could be harvested form dogs after euthanasia, and the body donation of dogs after euthanasia may effectively expand and maintain the stem cell inventory for biomedical research and potential clinical applications.

Keywords: bone marrow-derived mesenchymal stem cells, BM-MSCs, MSCs, canine, dog.

A50

Cyclin D1 Negatively Regulates Pluripotent Reprogramming through Inhibiting Mesenchymal-to-epithelial Transition

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Pluripotent embryonic stem cells (ESCs) express epithelial features and exhibit a unique cell cycle structure characterized by a shorter G1 phase and higher proportion of cells located in S phase. During pluripotent reprogramming of fibroblasts, epithelial features and the fidelity of pluripotent cell cycle control can be re-established via expressing Yamanaka factors (Oct3/4, Sox2, Klf4, c-Myc). However, the molecular events that associated with this reestablishment remain largely unknown. We initially separated early reprogramming cells into three subpopulations based on SSEA1 expression and Oct4 promoter activity. We found only SSEA1$^+$/Oct4-GFP$^+$ cells that expressed epithelial features and exhibited cell cycle structure closely resemble ESCs can give rise to fully reprogrammed induced pluripotent stem cells although SSEA1$^+$/Oct4-GFP$^-$ or SSEA1$^-$/Oct4-GFP$^-$cells can proliferate quickly and expressed a number of pluripotent characteristics.

The further analysis of the profile of cell cycle regulators identified that SSEA1$^+$/Oct4-GFP$^+$ cells had lower levels of Cyclin D1 compared to partially reprogrammed cells and somatic cells. We further demonstrated that Cyclin D1 play a dual role in pluripotent reprogramming. Although Cyclin D1 was required to form colonies in the initial phase, however, higher level expression of Cyclin D1 affected mesenchymal-to-epithelial transition which involved in the progression of the intermediate cells to become fully-reprogrammed pluripotent cells. The suppression was due to Cyclin D1 would inhibit pluripotent reprogramming through repressing nanog promoter via down-regulating endogenous Klf4. The further analysis revealed that Cyclin D1 could repress Klf4 and upregulate Slug expression via activation of Notch/Hes1 pathways, which therefore led to inhibition of mesenchymal-epithelial transitions during pluripotent reprogramming. In conclusion, it has been shown that mesenchymal-to-epithelial transition is an essential event for successful progression through intermediate transition to fully reprogrammed pluripotent cells, and our findings indicate that down-regulation of Cyclin D1 is a critical step in re-establishment of the fidelity of pluripotent cell cycle control and epithelial features.

Keywords: induced pluripotent stem cells, Cyclin D1, mesenchymal-to-epithelial transition

A51

Human Placental Multipotent Mesenchymal Stromal Cells may Modulate Trophoblast Migration via Rap1 Activation

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Multipotent mesenchymal stromal cells were known to produce a wide array of growth factors and cytokines, but little is known about the interaction between human placental multipotent mesenchymal stromal cells (hPMSCs) and trophoblasts. Here we examined the contribution of the Rap1 regulatory pathway in integrin β1 activation of human trophoblasts via hepatocyte growth factor (HGF) expressed by hPMSCs. We have isolated hPMSCs from term placenta. HGF induces the second messenger cAMP production in trophoblasts. The downstream pathways mediating through actions of PKA and Epac1 induced trophoblast Rap1 and integrin β1 activation. Treatment of trophobalsts by HGF, PKA-specific (6-Bnz-cAMP) and Epac1-specific (8-pCPT-2′-O-Me-cAMP) agonists increased trophoblast cell adhesion and migration, and a higher scratch wound closure rate. The total trophoblast cell integrin β1 protein did not change, but the cell surface active form of integrin β1 was significantly increased. These findings were inhibited by PKA-specific inhibitor H89 or silencing Epac1 and Rap1 using siRNA. Conditioned medium of hPMSCs induced trophoblast Rap1 activation. HGF expression by hPMSCs reduced when cell cultured in hypoxia ($<1\%$ O_2). hPMSC conditioned medium enhanced trophoblast migration, which was decreased by c-Met blocking antibody or conditioned medium from hPMSCs cultured in hypoxia. Furthermore, the expression of HGF and cAMP was decreased in preeclamptic placentas compared to gestational age-matched controls. Flow cytometry revealed the SSEA4-positive hPMSC number was decreased in preeclamptic placentas compared to controls. Thus, trophoblasts may acquire signals for migration depending on villous stromal hPMSCs. hPMSCs expressed HGF and increased trophoblast cAMP production. cAMP effector PKA and Epac1 act in concert to modulate adhesion and migration of trophoblasts via signaling to the Rap1 and to integrin β1.

Keywords: Epac1; integrin β1; PKA; placental multipotent mesenchymal stromal cells; Rap1

A52

A Graphene-Based Platform for Induced Pluripotent Stem Cells Culture and Differentiation

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Induced pluripotent stem cells (iPSCs) hold great promise as a cell source for regenerative medicine yet its culture, maintenance of pluripotency and induction of differentiation remain challenging. Here we report on that grapheme (G) and grapheme oxide (GO) can support the iPSCs culture and allow for spontaneous differentiation. Intriguingly, G and GO surfaces led to distinct cell proliferation and differentiation characteristics. In comparison with the glass surface, iPSCs cultured on the G surface exhibited similar degrees of cell adhesion and proliferation while the cells on the GO surface adhered and proliferated at a faster rate. Moreover, G favorably maintained the iPSCs in the undifferentiated state while GO expedited the differentiation. The iPSCs cultured on both G and GO surfaces spontaneously differentiated into ecdodermal and mesodermal lineages without significant disparity, but G suppressed the iPSCs differentiation towards the endodermal lineage whereas GO augmented the endodermal differentiation. These data collectively underscored that the different surface properties of G and GO governed the iPSC behavior

Keywords: induced pluripotent stem cells, graphene, graphene oxide, differentiation, cell culture

A53

Epithelial Cell Adhesion Molecule (EpCAM) Complex Proteins Promote Transcription Factor-mediated Pluripotency Reprogramming

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Epithelial cell adhesion molecule (EpCAM) is a transmembrane glycoprotein that is highly expressed in embryonic stem cells (ESCs) and its role in maintenance of pluripotency has been suggested previously. In epithelial cancer cells, the activation of EpCAM surface-to-nucleus signaling transduction pathway involves a number of membrane proteins. However, their role in somatic cell reprogramming is still unknown. Here we demonstrate that EpCAM and its associated protein, Cldn7, play a critical role in reprogramming. Quantitative RT-PCR analysis of Oct4, Sox2, Klf4, and c-Myc (OSKM) infected mouse embryonic fibroblasts (MEFs) indicated that EpCAM and Cldn7 were upregulated during reprogramming. Analysis of numbers of alkaline phosphatase- and Nanog-positive clones, and the expression level of pluripotency-related genes demonstrated that inhibition of either EpCAM or Cldn7 expression resulted in impairment in reprogramming efficiency, whereas overexpression of EpCAM, EpCAM plus Cldn7, or EpCAM intercellular domain (EpICD) significantly enhanced reprogramming efficiency in MEFs. Furthermore, overexpression of EpCAM or EpICD significantly repressed the expression of p53 and p21 in the reprogramming MEFs, and both EpCAM and EpICD activated the promoter activity of Oct4. All these observations suggest that EpCAM signaling may enhance reprogramming through upregulation of Oct4 and possible suppression of the p53-p21 pathway. In vitro and in vivo characterization indicated that the EpCAM-reprogrammed induced pluripotent stem cells (iPSCs) exhibited similar molecular and functional features to the mouse ESCs. In summary, our studies provide additional insight into the molecular mechanisms of reprogramming and suggest a more effective means of iPSC generation.

Keywords: Cell adhesion, Embryonic stem cells, Induced pluripotent stem (iPS) cells, EpCAM, p53, Oct4, Transcription factors

A54

Fate Regulation of Human Mesenchymal Stem Cells by Nano-topography of Silicon Nanowires

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Extracellular stimuli imposed on stem cells initiate mechanotransduction signaling and play an important role on the regulation of stem cell fates. Physical cues such as the distinct size and shape of nano-topographic matrices affect stem cell adhesion, morphology, proliferation and lineage commitment. Furthermore, less research has explored how physical cues of three-dimensional (3-D) nano-topography controls stem cell fates. The aim of this study is to further delineate the effects of 3-D nano-topography and the relevant physical properties on the fate regulation of human mesenchymal stem cells (hMSCs). We hypothesize that silicon nanowires (SiNWs) with a 3-D nano-topography affects stem cell physiology through cell adhesion-associated pathways and subsequently regulates hMSC lineage specification.In this study, we developed a novel substrate, in the form of SiNWs to investigate the interaction between cell adhesion and different physical properties of matrix nano-topography. It was found that hMSC cell viability was similar on all 3-D SiNW groups, but was lower than 2-D silicon wafer. Cell protrusions and spreading area were decreased as the length of SiNWs increased, and spreading area of hMSCs cultured on SiNWs were significant smaller than on silicon wafer. Expression of alpha2 integrin was higher on shorter SiNWs, while 3-D SiNWs was higher than 2-D silicon wafer. Protein level of focal adhesion related molecules such as phosphorylated focal adhesion kinase and vinculin was higher on shorter SiNWs. Expression of specific osteogenesis transcription factors including Runt-related transcription factor 2 and type I collagen alpha1 were significantly increased on SiNWs with shorter lengths compared to those on SiNWs with longer lengths, and slightly increased compared to 2-D silicon wafer.

Taken together, hMSCs cultured on shorter SiNWs have a tendency to differentiate into osteoblasts through cell morphology changes that are correlated by alpha2 integrin and focal adhesion molecular expression. In summary, the fate of hMSCs is dependent on 3-D nano-topography, and the findings in this study may shed new light on the design of nano-topographical structure in tissue engineering of bone regeneration.

Keywords: silicon nanowire, mesenchymal stem cell, three-dimensional nano-topography, osteogenesis

A55

Rapid Generation of Mature Hepatocyte-like Cells from Human Induced Pluripotent Stem Cells by an Efficient Three-Step Protocol

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Liver transplantation is the only definitive treatment for end-stage cirrhosis and fulminant liver failure but the lack of available donor livers is a major obstacle to liver transplantation. Recently, induced pluripotent stem (iPS) cells, derived from the reprogramming of somatic fibroblasts, have been shown to resemble embryonic stem (ES) cells in that they have pluripotent properties and the potential to differentiate into all cell lineages *in vitro*, including hepatocytes. Thus iPS cells may provide a favorable cell source for a wide range of applications, including drug toxicity testing, cell transplantation, and patient-specific disease modeling. Here we describe an efficient and rapid three-step (endodermal induction step, hepatic commitment step and maturation step) protocol that can rapidly generate hepatocyte-like cells from human iPS cells. This occurs because the endodermal induction step allows for more efficient and definitive endoderm cell formation. We showed that hepatocyte growth factor (HGF), which synergizes with Activin A and Wingless-type MMTV integration site family, member 3A (Wnt3a), elevates the expression of the endodermal marker Foxa2 (*Forkhead box a2*) by 39.3% compared to when HGF is absent (14.2%) during the endodermal induction step. In addition, the iPS cell–derived hepatocytes had a cuboidal morphology, expressed liver-specific markers, and exhibited a similar gene expression profile to mature hepatocytes. Importantly, the hepatocyte-like cells exhibited cytochrome P450 3A4 (CYP3A4) enzyme activity, secreted urea and had the ability to store glycogen.

Conclusion: We have established a rapid and efficient differentiation protocol that is able to generate functional hepatocyte-like cells from human iPS cells. This may be a potentially important step in the use of iPS cells for pre-clinical and clinical applications.

Keywords: Liver transplantation, epithelial-to-mesenchymal transition, definitive endoderm, hepatocytes, E-cadherin

A56

Somatic Stem Cells Adopt the Phenotype of Differentiated Cells by Cell Fusion rather than Differentiation

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Intra-uterus transplantation (IUT) is an approach to cure the inherited genetic defect for the fetus during the gestation period of the pregnant female. The researchers could not prove that the donor cells were given rise to the specific cell or proceed the cell fusion mechanism to recover the illness. As a result, the aim of this study is to investigate the bio-distribution and engraftment of mice amniotic fluid derived stem cell (mAFSCs) after IUT and further determine the fate of the donor cells through EGFP-harboring mAFSCs inject into Ds-red harboring fetus of the pregnant mice. The 13.5 days pregnant mice were performed the IUT surgery by mAFSCs injection. As to organ section method, the EGFP signal and RFP signal were co-expressed and discovered in certain organ, and merge figure present the yellow color that is EGFP signal and RFP signal fused together. It implies that EGFP-labeled donor stem cell fused with RFP-labeled recipient mice. Furthermore, flow cytometry data shown here revealed that co-express EGFP signal and RFP signal, and no any only EGFP cells were detected. This consequence indicate that through the IUT approach EGFP-labeled stem cell migrate to certain tissue to proceed cell fusion instead of differentiation or maintain the undifferentiated status retaining in the tissue. The outcomes given demonstrate that mAFSCs could be detected in a couple of organs such as intestine, kidney, muscle, skin, bladder, heart and stomach ect. The phenomena of donor cell fused with recipient cell was detected. The effect of these findings may supply the information of pre-clinical trial of IUT for those who have inherited genetic disorder in the gestation period.

Keywords: Intra-uterus transplantation, amniotic fluid derived stem cell, EGFP, RFP.

A57

Multiple Intravenous Transplantation of Mesenchymal Stem Cells Effectively Restore Long-term Blood Glucose Homeostasis by Hepatic Engraftment and beta Cell Differentiation in Streptozotocin-induced Diabetic Mice

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Depletion of pancreatic beta cells results in insulin insufficiency and diabetes mellitus (DM). Single transplantation of mesenchymal stem cells exhibits short-term effects in some pre-clinical studies. Here, we further investigated the long-term therapeutic effects of multiple intravenous MSCs transplantations. In this study, multiple human MSCs transplantations (4.2 x 10^7 cells/kg each time) were performed intravenously at two-week intervals into streptozocin (STZ)-induced diabetic mice for 6 months. Blood sugar, insulin, renal function, cholesterol and triglyceride levels were monitored. We demonstrated that compared to single intravenous transplantation, which only transiently decreased hyperglycemia, multiple MSCs transplantations effectively restored blood glucose homeostasis. Systemic oxidative stress levels were reduced from the 7th week of treatment. From the 11th week, production of human insulin was markedly increased. When MSCs transplantation was skipped after blood sugar level returned to normal at the end of 15th week, a sharp rebound of blood sugar occurred, and was then controlled by subsequent transplantations. At the end of 6 months, histopathology examination revealed MSCs specifically engrafted into liver tissues of the recipients. Fifty-one percent of human cells in the recipient liver co-expressed human insulin, especially those surrounding the central veins. Taken together, intravenous MSCs delivery was safe and effective for blood glucose stabilization in this pre-clinical DM model. Multiple transplantations were essential to restore and maintain glucose homeostasis through decreasing systemic oxidative stress in the early stage and insulin production in the late stage. Liver engraftment and differentiation into insulin-producing cells account for the long-term therapeutic effects of MSCs.

Key Words: Mesenchymal stem cells, multiple intravenous transplantations, blood glucose homeostasis, beta cell depletion, liver engraftment

A58

In vitro Estimate the Differentiation Potential of Wharton's Jelly Derived Mesenchymal Stem Cells on Chitosan-gold Composite Films for Nerve Tissue Engineering

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 # Contribution is equally to this correspondence.

In this study, we have employed biomimetic strategies to develop a novel chitosan-composite films (Chi-Au) that is incorporated with various amounts (12.5, 25, 50 and 100 ppm) of gold nanoparticles (Au) (approximately 37 nm) of differentiating human wharton's jelly derived mesenchymal stem cells (WJCs). To understand the aspects of the Chi-Au composite films that induced spheroid formation on WJCs, chemical and physical properties of the Chi-Au composite films were examined. WJCs were first cultured on Chi-Au composite films, and spheroid formation of WJCs was observed, especially at 25~50 ppm of Au. These changes may contribute to the enhanced nerve tissue differentiation potential of WJCs. Furthermore, WJCs on Chi-Au were induced by nerve induction condition medium and their nerve tissue differentiation potential was enhanced by assessing the expression of nerve related genes at both mRNA and protein levels by real-time PCR and immunostaining respectively. We supposed that spheroid formation by Chi-Au composite films helped to increase and maintain the expression of nerve stemness genes in WJCs. In summary, the surface biomimetic Chi-Au composite films of WJCs may as a critical biological cue to promoting WJCs differentiate into nerve cells in vitro.

Key words: chitosan-gold composite films, wharton's jelly derived mesenchymal stem cells, nerve tissue differentiation

A59

C-Jun N-terminal Kinase 1 Negatively Regulates Osteroblastic Differentiation Induced by BMP-2 via Phosphorylation of Runx2 at Ser104

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Runx2 plays a crucial role in the osteoblastic differentiation, which can be upregulated by bone morphogenetic proteins 2 (BMP2). Mitogen-activated protein kinase (MAPK) cascades, such as extracellular signal-regulated kinase (ERK) and p38, have been reported to be activated by BMP2 to increase Runx2 activity. The role of c*jun*-N-terminal kinase (JNK) the other kinase of MAPK, in osteoblastic differentiation has not been well elucidated. In this study, we first demonstrate that JNK1 is activated by BMP2 in C2C12 multipotent cell and MC3T3-E1 preosteoblastic cell lines. We then demonstrate that early and late osteoblastic differentiation, represented by ALP expression and mineralization, respectively, are significantly enhanced by JNK1 loss-of-function, such as treatment of JNK inhibitor, knockdown of JNK1 and exogenous overexpression of a dominant negative JNK1 (DN-JNK1). Consistently, BMP2-induced osteoblastic differentiation is reduced by JNK1 gain-of-function, such as enforced expression of a constitutively active JNK1 (CA-JNK1). Most importantly, we demonstrated that Runx2 is essential for JNK1-mediated inhibition of osteoblastic differentiation, and identified Ser104 of Runx2 is the site phosphorylated by JNK1 upon BMP2 stimulation. Finally, we found that overexpression of the Runx2 with Ser104 to Ala mutation stimulates osteoblastic differentiation of C2C12 and MC3T3-E1 cells to extent that was achieved by overexpression of wild-type Runx2 plus JNK inhibitor treatment. Taken together, these data indicate JNK1 negatively regulates BMP2-induced osteoblastic differentiation through phosphorylation of Runx2 at Ser104. In addition, unraveling these mechanisms may help to develop new strategies in enhancing osteoblastic differentiation and bone formation.

Keywords: Runx2; JNK; BMP2

A60

Spheroid Formation of Mesenchymal Stem Cells on Chitosan and Chitosan-Hyaluronan Membranes

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Stem cells can lose their primitive properties during in vitro culture. The culture substrate may affect the behavior of stem cells as a result of cell-substrate interaction. The maintenance of self-renewal for adult human mesenchymal stem cells (MSCs) by a biomaterial substrate, however, has not been reported in literature. In this study, MSCs isolated from human adipose (hADAS) and placenta (hPDMC) were cultured on chitosan membranes and those further modified by hyaluronan (chitosan-HA). It was observed that the MSCs of either origin formed three-dimensional spheroids that kept attached on the membranes. Spheroid formation was associated with the increased MMP-2 expression. Cells on chitosan-HA formed spheroids more quickly and the size of spheroids were larger than on chitosan alone. The expression of stemness marker genes (Oct4, Sox2, and Nanog) for MSCs on the materials was analyzed by the real-time RT-PCR. It was found that formation of spheroids on chitosan and chitosan-HA membranes helped to maintain the expression of stemness marker genes of MSCs compared to culturing cells on polystyrene dish. The maintenance of stemness marker gene expression was especially remarkable in hPDMC spheroids (vs. hADAS spheroids). Blocking CD44 by antibodies prevented the spheroid formation and decreased the stemness gene expression moderately; while treatment by Y-27632 compound inhibited the spheroid formation and significantly decreased the stemness gene expression. Upon chondrogenic induction, the MSC spheroids showed higher levels of Sox9, aggrecan, and collagen type II gene expression and were stained positive for glycosaminoglycan and collagen type II. hPDMC had better chondrogenic differentiation potential than hADAS upon induction. Our study suggested that the formation of adhered spheroids on chitosan and chitosan-HA membranes may sustain the expression of stemness marker genes of MSCs and increase their chondrogenic differentiation capacity. The Rho/Rho-associated kinase (ROCK) signaling pathway may be involved in spheroid formation.

Keywords: Adipose-derived adult stem cells, placenta derived multipotent cells, chitosan, Hyaluronan, Spheroid Formation.

A61

Regulation of Transcriptional Factor GATA4 by miR-200c in Human Embryonic Stem Cells

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Human embryonic stem cells (hESCs) can self-renew and differentiate into almost all types of cells of the body. *In vitro*, hESCs can differentiate into embryoid bodies (EBs) which mimic the process of gastrulation by producing three germ layers. MicroRNAs (miRNAs) are small non-coding RNAs that govern key cellular processes. The miR-200 family has been reported to attenuate mouse embryonic stem cell differentiation upon the removal of leukemia inhibitory factor. However, no studies have been performed to investigate the functional roles of human miR-200 family in hESC renewal and differentiation.

In this study, we found that miR-200c expression is most abundant in undifferentiated hESCs among the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429). Furthermore, miR-200c is enriched in undifferentiated hESCs and is downregulated in EBs. The knockdown of miR-200c in hESCs caused cell differentiation and induced GATA binding protein 4 (GATA4) expression. Interestingly, overexpression of miR-200c decreased the efficiency of EB formation and repressed the markers of all three lineages (ectoderm, endoderm, and mesoderm). We also found that knockdown of GATA4 in hESCs also decreased EB formation. miR-200c can directly and specifically target the 3'-untranslated regions of the GATA4 in a luciferase assay.

T aken together, miR-200c may play a pivotal role in inhibiting the differentiation of hESCs into EBs through the downregulation of GATA4 expression. During EB formation, the decreased expression of miR-200c is responsible for GATA4 expression and promotes the commitment to EBs. This finding uncovered a new mechanism of the undifferentiated and differentiated state of hESCs with a focus on the hESC to EB transition.

Key words: human embryonic stem cells, microRNAs, embryoid body, miR-200c, GATA4

A62

Fibirin Glue Mixed with Platelet-rich Fibirin as a Scaffold Seeded with Dental Bud Cells for Tooth Regeneration

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Odontogenesis is a complex process with a series of epithelial-mesenchymal interactions and odontogenic molecular cascades. In tissue engineering of teeth from stem cells, platelet-rich fibrin (PRF), which is rich in growth factors and cytokines, may improve regeneration. Accordingly, PRF was added into fibrin glue to enrich the microenvironment with growth factors. Unerupted second molar tooth buds were harvested from miniature swine and cultured *in vitro* for 3 weeks to obtain dental bud cells (DBCs). Whole blood was collected for the preparation of PRF and fibrin glue before surgery. DBCs were suspended in fibrin glue and then enclosed with PRF, and the DBCs–fibrin glue–PRF composite was autografted back into the original alveolar sockets. Radiographic and histological examination were used to identify the regenerated tooth structure 36 weeks after implantation. Immunohistochemical staining was used to detect proteins specific to tooth regeneration. One pig developed a complete tooth with crown, root, pulp, enamel, dentin, odontoblast, cementum, blood vessels, and periodontal ligaments in indiscriminate shape. Another animal had an unerupted tooth that expressed cytokeratin 14, dentin matrix protein-1, vascular endothelial growth factor, and osteopontin. This study demonstrated, with autogenic cell transplantation in a porcine model, that DBCs seeded into fibrin glue–PRF can regenerate a complete tooth.

Keywords: Odontogenesis; tooth regeneration; dental bud cell; fibrin glue; platelet-rich fibrin; scaffold

A63

Induction of Dendritic Cells from Serum-Free Expanded Hematopoietic Stem Cells

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Dendritic cells (DCs) are the most powerful antigen presenting cells (APCs) and play a pivotal role in initiating the immune response, which differentiated from $CD133^+$ hematopoietic stem cells (HSCs). Hence, we used the ex vivo expanded of hematopoietic stem cells as a source of DCs, and developed the optimal DCs induction medium.

In the previous study, we had developed a serum-free hematopoietic cells expansion system (SF-HSC medium), HSCs could expand in SF-HSC medium reaching 30-fold within one week.

According to the past researches, several cytokines, especially SCF, Flt-3 ligand, IL-1β, GM-CSF and TNF-α, have been identified as essential factors to induce and differentiate HSCs into DCs. Moreover, we tested the basal media (IMDM, RPMI-1640, Medium-199, α-MEM, H3000, DMEM/F12 and DMEM) combined with the various concentration of cytokines to finalize the optimal DC induction medium.

Finally, we confirmed the function and maturation of DCs by the assays of the mixed lymphocyte reaction (MLR), the ability of endocytosis, specific cytokines of secretion and the stimulation by lipopolysaccharides. When DCs become mature (mDCs), the specific surface markers of mDCs would change (CD1a, CD11c, CD14, CD80, CD83, CD86 and CD34), the ability of endocytosis would be more complete and the ability of stimulation would increase when co-cultured with $CD3^+$ T cells.

These results showed that DCs derived from the serum-free expanded $CD133^+$ HSCs exhibited both characteristics and functions of DCs. Therefore, we believed that combination of HSCs serum-free expansion medium and DCs induction medium would generate large amounts of functional DCs and would be a promising cell source for the basic research and translation media in the near future.

Keywords: dendritic cells、hematopoietic stem cells

A64

Tumorigenesis and Metastasis of EMT-phenotype CD133^{+} CSCs in Nude Mice

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CSCs are a minority of the cells found in malignant tumors and possess the capacity to self-renew and differentiate into heterogeneous lineages of cancer cells within tumor mass, known as cancer stem cells (CSCs) or tumor-initiating cells (TICs). Due to their stem-like cell properties, CSCs confer metastasis and drug resistance capacities in tumors. Recently, expanding evidence highlights the role of CD133 as a marker of CSCs in various human tumors. In addition, EMT (Epithelial-to-Mesenchymal Transition)-phenotype is a critical process for switching early stage in situ carcinomas into invasive malignancies that allow these cells to metastasize in distant sites thus enabling tumors to metastasize in multistage cancer progression, particularly with respect to metastasis. Nowadays, many studies participate in bring light to the connections between EMT-phenotype and CSCs metastasis. Evidence has shown that cells capable of EMT could be an enrich source for CSCs; this further supports the contention that CSCs, TICs and EMT-phenotype cells share some crucial properties. Therefore, researches drop a hint that EMT process is tightly linked with the characteristics and biology of CSCs.

In our result, we found preconditioned human SH-SY5Y cell line express higher ratio in CD133 cell surface marker (CD133^{+} cells) (91.48%) than control group (CD133^{-} cells) (0.11%) through flow cytometry (FCM) and immunocytochemistry (ICC). *In order to investigate the ability of CD133^{+} cells in tumorgenesis and metastasis,* these aliquot CD133^{+} cells were injected subcutaneously in nude mice (CD133^{-} cells as control group). We observed subcutaneous tumor formation and cancer metastasis in liver, spleen and ovary tissues (post injection 40 days) in *CD133^{+} cells treated group*. Among these subcutaneous tumors and metastasis organs, we detected human CD133 protein expression through immunohistochemistry (IHC) to prove these tumors formation was derived from human cell line. Moreover, we detected EMT-phenotype related markers in CD133^{+} cells treated group, Immunohistochemistry (IHC) results invent EMT-phenotype protein expression of mesenchymal markers (Vimentin, N-cadherin) but epithelial marker (E-cadherin) in CD133^{+} cells treated group, resulting in tumor aggressiveness, matastasis and drug resistance.In conclusion, we elucidate the molecular biology of CSCs that undergo EMT in the future; it will allow the development of novel therapies for combating cancers, which certainly improve the survival of patients diagnosed with cancers.

Keywords: Cancer stem cells, EMT-phenotype, CD133, Metastasis

A65

45RA$^-$CD90$^+$CD49f$^+$ Cells Present in CD34$^+$CD38$^-$ Rho$^-$ Cord Blood Cells Contain the Same Frequency of Cells with 12-week Lympho-myeloid Outputs on Engineered Stroma as in NSG Mice for 20 weeks

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Over the last 2 decades, the creation of various lines of increasingly immunodeficient mice has enabled parallel improvements in xeno-transplantation protocols to follow the engraftment, growth and differentiation of different types of human hematopoietic cells. As a result, however, the most efficient of these xeno-graft models requires at least 5 months of follow-up to infer a hematopoietic stem cell (HSC) origin from the regenerated human cells detected. In addition, such in vivo assays have limited power in dissecting events that regulate HSC functions. As a more tractable alternative, we have sought to develop a novel in vitro assay that may have parallel selectivity and efficiency for detecting human HSCs with long term repopulating activity. This assay involves co-culturing the test cells for an initial 6 weeks on immortal mouse stromal lines engineered to produce human Flt3-ligand, Steel factor and IL-3 with further addition for a second 6 weeks of the same stromal cells engineered to produce human G-CSF, IL-7 and IL-3 prior to phenotyping the cells at the end of the 12 weeks to detect the potential presence of CD7+CD14/15/33/66b- (pre-T), CD56+CD14/15/33/66b- (NK), CD19/20+CD14/15/33/66b- (B) and/or CD14/15/33/66b+ (GM) cells. Of 420 12 week-old co-cultures, each initiated with a single CD34+CD38- CD45RA-Rho- cord blood (CB) cell, 82 (20%) produced lineage-restricted hematopoietic clones; 4 of these clones (1% of the original cells) contained all 4 lineages assayed and 9 contained GM cells plus at least one lymphoid lineage (combined total = 3% of the original cells). These latter two frequencies are similar to the frequency of CD34+CD38-CD45RA-Rho- CB cells that we have found regenerate lymphoid and myeloid progeny in NSG mice at 20 weeks post-transplant (~1%). Recently, co-expression of CD90 and CD49f within the CD34+CD38-CD45RA-Rho- population was reported to further increase the purity of these cells to ~10%. Further tests of the activity of 44 such cells in our co-culture system showed the frequency of 12-week clones to be 25 (60% of initial cells) with 8 lympho-myeloid (20% of initial cells) and 2 (5% of initial cells) displaying all assayed lineage abilities. Our novel 12-week in vitro assay thus appears to offer the specificity for human CB HSCs with long term repopulating activity in vivo.

Keywords: hematopoieic stem cells, in vitro assay, in vivo assay, Rhodamine 123, CD49f, cord blood cells

A66

Human Orbital Fat-derived Stem Cells Promote Corneal Re-epithelialization and Attenuate Local Inflammation *in vivo*

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We have successfully established the platform of isolation of human orbital fat-derived stem cells (OFSCs) from minimal volume (0.5-1 ml) of orbital fatty tissue without destroying healthy ocular structure in our previous work. The characteristics of OFSCs are similar to human bone marrow-derived mesenchymal stem cells (BM-MSCs), and these cells possess corneal epithelial differentiation ability upon mix-cultured with human corneal epithelial cells *in vitro*. The aim of this study is to investigate the therapeutic effect and inflammation inhibition potential of OFSCs using alkali-induced corneal injury as an animal model. In this study, 70% or 100% of corneal epithelial wound on male BALB/c mouse cornea was created by covering a 0.5N NaOH containing filtering paper for 30 seconds. OFSCs (2×10^5) in 5 µl phosphate buffer saline (PBS) were topically administrated on right cornea twice a day, and 5 µl of PBS on left cornea served as control. OFSCs significantly decreased corneal opacity, promoted re-epithelialization and reduced acute alkali-induced corneal edema and stromal infiltration by *in vivo* fluorescein staining and histopathological examination. Using immunohistochemistry and immunofluorescence staining, human cells could be easily detected on mouse corneal epithelium, but only few of transplanted cells at limbal epithelium differentiated into cytokeratin 3 expressing cells within 3 days. However, western blot and its quantification analysis demonstrated that OFSCs did not alter neutrophil (Ly6G) level in cornea, but significantly reduced macrophage (CD68) infiltration after alkali injury. Among macrophage-releasing pro-inflammatory cytokines, although OFSCs had no effect on tissue necrosis factor-alpha, tissue growth factor-beta and vascular endothelial growth factor, these cells selectively inhibited inducible nitrous oxide synthetase (iNOS) production during the first 3 days after alkali injury. Taken together, OFSCs promoted corneal wound healing and possessed corneal epithelial differentiation potentiality *in vivo*. The therapeutic effect on acute alkali-induced corneal injury was achieved mainly through the anti-inflammation ability of OFSCs targeting on macrophage and iNOS production.

Key Words: orbital fat stem cells (OFSCs); alkali corneal injury; macrophage; inflammation

A67

Effect of Hypoxia on Dental Pulp Stem Cells (DPSCs) under a Serum-Free Culture System

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Human dental pulp from permanent tooth is a potentially promising source of progenitor cells. Sustaining and amplifying progenitor cell populations with serum-free culture system would greatly benefit for autograft transplantation and clinical cell therapy. Hypoxia is known to promote the undifferentiated cell state in various stem cell populations. However, the effect of hypoxia combined with serum-free culture media on dental pulp stem cells (DPSCs) has not been defined yet. In this aspect, dental pulps from adult orthodontic patients were cultivated and the DPSCs were selected by magnetic activated cell sorting system (MACS) for experiments. The DPSCs were cultivated in serum-free culture medium under normoxic (20% oxygen tension) and hypoxic (5% oxygen tension) culture conditions for cell proliferation assay. The WST assay showed a significant increased proliferation of hypoxic-DPSCs. Additionally, the hypoxic-DPSCs showed a better cell morphology, higher expression level of stem cell–specific markers (CD73 and CD29) and differentiation capacity (adipogenesis and osterogenesis) while comparing with the normoxic-DPSCs. Importantly, supplementing IGF-1 or insulin in medium greatly increased the stem cell properties of DPSCs. Furthermore, PPP (specific inhibitor of IGF-1R phosphorylation) effectively suppressed the stem properties of DPSCs, highlighting the important role of IGF-1R signaling in stemness maintenance of DPSCs. In summary, in this study, we found an important role of IGF-1R signaling and hypoxia in proliferation and stemness of DPSCs. Findings in this study would provide an effective culture condition for maintenance and expansion of adult DPSCs in-vitro which would facilitate the efficiency of individual human stem cell therapy.

Keywords: Dental pulp stem cells, hypoxia, serum-free media, proliferation, expansion

A68

HLA and ABO Genotypes of Eight Taiwanese Human ES cell lines

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The generation of human embryonic stem cells ignites a hope for patients who are incurable using current medical treatments. Human embryonic stem cells (hESCs) provide a potentially unlimited source for various cell therapies because they can propagate indefinitely in vitro and they are pluripotent, which means they can differentiate into all different kinds of tissues of a man. However, the first obstacle encountered in the human ES cell regenerative medicine applications is the histocompatibility problem. Detailed characterization of the blood-histotype of the human ES cells is thus vital to the construction of a human ES cell bank. Here we perform the high-resolution sequence based typing (SBT) of HLA-A,-B,-C,-DQ and –DR loci and acquire complete typing results of eight hESC lines in Taiwan. In our survey, there are several hESC lines that are predicted serologically homozygous at single or double HLA loci: one is homozygous for HLA-A; three are homozygous for HLA-C; and one is homozygous for both HLA-A and HLA-C loci. Three of these hESC lines are A blood type (A/A or A/O), three are type O and two are type B (B/B). These documented histotypes of human embryonic stem cell lines as a whole not only provide a detailed reference database for their use in cell transplantation, but also helps to identify their drug testing potentials of the banked human ES cells.

Keywords: Human ES cells, HLA, ABO

B01

Development of a Lab-on-a-chip Surface Plasmon Resonance Microscopy (SPRM) Technique for Real-time Imaging to Detect Osterogenic Differentiation of Mesenchymal Stem Cells

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【Introduction】 Surface plasmon resonance (SPR) biosensors have been recognized as a potentially powerful tool and widely used for dynamic analysis of molecular affinity and drug screening due to its high sensitivity to the change of the refractive index of tested objects. The selective binding and binding strength between molecules and drug/membranes can be obtained through the real-time monitoring of the shift of the surface plasmon resonance angle. Osteoblasts are the bone forming cells and are derived from mesenchymal stem cells (MSCs) present in the bone marrow stroma. The conventional methods to observe the extent of osteogenic differentiation includes methods such as RNA extraction, protein isolation and immunofluorescent stainings. However, these methods are invasive and require cell lysis or fixation which causes cell death. The purpose of this study is to investigate whether the SPR technique can be used as a non-invasive sensor to accurately define the different stages of osteogenic maturation with live cells.

【Materials and Methods】 MSCs were isolated from human bone marrow and were capable of being differentiated into osteoblasts, chondrocytes and adipocytes under culture induction. Parental MSCs, osteo-differentiated MSCs under osteogenic induction, and SaOS2 osteosarcoma cells as positive controls were individually mixed with PBS and injected onto a gold film pre-coated with OB-cadherin antibodies in which OB-cadherin is an osteoblast-specific transmembrane protein. The angle shift of captured cells by OB-cadherin antibodies was measured and recorded by the SPR system operated in an angular interrogation mode.

【Results】As MSCs matured into osteoblasts, OB-cadherin expression is upregulated with the highest expression levels in mature osteoblasts. The difference of the measured shift angle between MSCs and SaOS2 cells (θ_{SaOS2}-θ_{MSCs})≒0.13417338°. As the duration of osteogenic induction increases, the difference of the angle shift between MSCs under osteo-differentiation and SaOS2 cells gradually decreases during 15-day-experiment. The similarity in angle shifts between two cell types suggests that the cells are similar in surface OB-cadherin expression.

【Discussion】 In this study, we aimed to establish a non-invasive method to measure the osteo-differentiation process of MSCs. The results showed that the SPR system provides a precise, sensitive and specific way to observe the differentiation of cells by defining the angle shift of cells. This technology breakthrough for observation of

differentiation with live cells will facilitate further advances in a vast array of fields of scientific research and medical diagnosis.

Keywords: surface plasmon resonance; mesenchymal stem cell; osteogenic differentiation

B02

Hepatic Differentiation of Marrow Stromal Cells in Acellular Liver Scaffold

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Liver-related diseases often result in permanent damage to liver. The only effective treatment for severe hepatic failure is liver transplantation. However, lack of organ supply limits this approach. The cell source limitation can potentially be resolved by a number of alternative cells. Although previous studies offer significant efforts in identifying and utilizing alternative sources of cells that can be expanded easily in culture, and suitable growth factors subsequently manipulated to give rise to hepatocytes, there is a lack of an optimal three-dimensional scaffold to provide a conductive environment for generation of well-transplantable hepatic construct from marrow stromal cells (MSCs). In this study, we attempt to fabricate liver scaffold by decellularization, which is a promising technique for scaffold preparation in tissue engineering. The resulting material can potentially preserve the most extracellular matrix components and architecture of the original tissue, including the vascular network, which can be readily connected to the circulation, facilitating rapid oxygen and nutrient delivery. Through this approach, acellular liver scaffold (ALS) can provide a biomimetic three-dimensional environment for hepatic differentiation of MSCs characterized by heptic-relative genes expression and glycogen storage. It can found that hepatic differentiation in ALS superior than culturing in two-dimensional environment. ALS may therefore be useful for liver tissue engineering.

Key words: marrow stromal cells、acellular liver scaffold、hepatocytes

B03

Establishment of Immortalized Endothelial Progenitor Cell Line with Fluorescence Expression

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Function of Endothelial progenitor cells (EPCs) is associated with Angiogenesis. Besides, EPCs are not only responsible for development of blood vessel but also relative to tumor formation and cardiovascular disease. So, generation of large amounts of EPCs would be beneficial for basic research and cell therapy. In this study, firstly, we isolated primary EPCs ($CD31^+/34^+/133^+/144^+/309^+$ cells) from human cord blood. At the beginning, mononuclear cells derived from cord blood were seeded on fibronectin coated dish and cultured in EBM-2 medium supplemented with 20% FBS until EPC colonies observed. After that, primary EPCs and HUVECs were transfected with human telomerase reverse transcriptase (hTERT) and red fluorescence protein (RFP) genes by electroporation method respectively and the stable transfected EPCs were selected by using hygromycin and G418. Cells after transfection will be characterized by flow cytometry and tube formation assay. The preliminary result shows that the hTERT and RFP gene can be transfected to EPCs and HUVECs. Cell morphology and functions are very similar between transfected cells and primary cells. But these characteristics should be further proved for long period culture. In conclusion, immortalized EPCs were established in this study and these cells could be cultured to generate large amounts of cells as a promising cell source for above purposes.

Keywords: Endothelial progenitor cells, non-viral transfection, immortalized

B04

TW1 Human Embryonic Stem Cell Line Differentiates into Cardiomyocytes

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Human embryonic stem cells (hESCs) have pluripotency which can differentiate into three germ layers of embryo. With the pluripotent potential, hESCs are able to differentiation into cells in human body which includes cardiomyocyte. Previous studies have shown that hESC-derived cardiomyocytes which can form myocardium in heart may have potential to replace damaged cardiomyocytes. The purpose in this study is to differentiate TW1 human embryonic stem cells become cardiomyocytes and develop the application and physiology of hESC-derived cardiomyocytes.

TW1 human embryonic stem cell line was established by the research efforts of Biomedical Engineering Center of Industrial Technology Research Institute and Division of Infertility of Lee Women's Hospital. To start differentiation, TW1 hESCs were transfer into serum-free differentiation medium and supplemented with human recombinant bFGF, BMP4, ActivinA, Dkk-1 and VEGF. During differentiation, cells were collected at different time point to analyze the expression of mesoderm marker, Brachyury, and cardiac marker, Nkx2.5 and cardiac Troponin T (cTnT), by immunofluorescence.

During differentiation, the differentiated TW1 hESCs expressed mesoderm marker, Brachyury. On day 10, cardiac marker, Nkx2.5, can be detected from 10% of differentiated TW1 hESCs. After differentiation, some differentiated cells present contracting ability and express marker of cardiomyocyte, cTnT.

These data indicated that TW1 hESCs could differentiate into cardiomyocytes with this differentiation protocol. In regeneration medicine, TW1-derived cardiomyocytes may become a source to replace dead cardiomyocyte and repair damaged heart tissue. Moreover, the TW1-derived cardiomyocytes could be an in vitro system to discover the physiology of human cardiomyocyte.

Keywords: TW1, human embryonic stem cells, cardiac differentiation, cardiomyocyte.

B05

Highly Efficient Expansion of Human Mesenchymal Stem Cells in a Novel Serum-Free, Animal Components-Free and Chemically Define Medium

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Mesenchymal stem cells (MSCs) are adult multipotent cells which have been isolated from almost every type of connective tissue. They can self-renew and have the capacity to differentiate into adipogenic, chondrogenic and osteogenic lineages under appropriate *in vitro* conditions. Human mesenchymal stem cells (hMSCs) are promising candidates for stem cell therapy and regenerative medicine. However, despite the remarkable clinical advancements in this field, most applications still use traditional culture media containing fetal bovine serum. The highly variable nature of traditional culture media remains a challenge. Although there are several commercially available serum-free media, the cell expansion efficiency is low or required ECM coating. Here, we developed a serum-free, animal components-free and chemically define medium for efficient expansion of hMSCs without ECM coating. The medium is comprised of a chemical defined basal medium supplemented with human recombinant growth factors, growth hormones, lipids, amino acids, vitamins and antioxidants. Using this medium, hMSCs derived from bone marrow, adipose or Wharton's jelly were expanded 5 to 10 passages with low cell seeding density (0.325~1x103/cm2) on non-coated culture plates. These cells increased about 15~25, 40~50 and 35~50 folds in this serum-free medium for 5~7 days at each passage, respectively. The medium has higher cell expansion capacity compared to serum containing medium or commercially available medium. After several passages, the expanded cells maintained CD73+, CD90+, CD105+, CD34-, CD45- phenotype and exhibited the ability to differentiate into adipogenic, chondrogenic or osteogenic lineages. This serum-free, animal components-free and chemically define medium provides a substitute for serum-containing medium for hMSC expansion that has broad applicability for basic research and future clinical applications.

Keywords: mesenchymal stem cells, serum-free, cell expansion

B06

Molecular Cross-talk of Mouse Mesenchymal Stroma Cells with Different Kinds of Liver Cells

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Mesenchymal stroma cells (MSCs) are multipotent stem cells which are evidenced to participate in the process of inflammation, tissue regeneration and repair, and immuno-modulation by their paracrine effects. In our previous studies, systemic transplantation of MSCs successfully rescued acute liver injury in mice. However, there is little evidence showing the rescue of liver function was contributed by direct hepatocyte differentiation from transplanted MSCs. Thus, in this study, we hypothesize the rescue of liver injury by MSCs is due to the paracrine effect. The aim of this study is to elucidate the molecular cross-talk between MSCs and different types of liver cells in a mouse model. Mouse MSCs were isolated and co-cultured with hepatocytes, hepatic stellate cells, and kupffer cells in the in vitro transwell system. When co-cultured with mouse MSCs, increased expression of α-fetoprotein and onecut 2 and decreased expression of cytochrome P450 2b10 were noted in hepatocytes. Under the same condition, expression and secretion of IL-10 was increased in hepatic stellate cells, and TGF-beta was decreased in kupffer cells. In summary, MSCs may modulate the hepatocytes to an immature status and help liver regeneration through paracrine effect. Moreover, the increased secretion of interleukin-10 of the hepatic stellate cells and decreased secretion of TGF-beta of the kupffer cells may inhibit inflammation in the liver fibrosis. MSCs transplantation provides a new and potential therapeutic strategy for various liver diseases. This study offers more cues for the molecular interaction between MSCs and various kinds of liver cells.

Keywords: mesenchymal stroma cells; paracrine effect; hepatocytes; kupffer cells; hepatic stellate cells

B07

Development of Injectable Hyaluronan Modified Thermo-responsive Hydrogel for Adipose-derived Stem Cell Based Articular Cartilage Tissue Engineering

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The lesion of articular cartilage often results in progressive deterioration and eventual osteoarthritis. Current clinical used strategies meet difficulties to restore the native structure of cartilage. Tissue engineering has been suggested to provide more advantages over the strategies used clinically nowadays. It consists of three major components: cells, biomaterials and environmental factors. Adipose-derived stem cells (ADSCs) has been proposed as a better cell source because of ease of harvest, high proliferative property and possessing multilineage differentiation potential. Our previous study showed that hyaluronan (HA), as a micro-environmental factor, can both initiate and enhance chondrogenesis of ADSCs, and subsequently facilitate hyaline cartilaginous matrix formation. The thermoresponsive Poly(N-isopropylacrylamide) (PNIPAAM) hydrogel is an injectable biomaterial at room temperature and shifts to solid phase over 32℃, which may be a suitable cell carrier for tissue engineering. However, NIPAAM monomer has cytotoxicity and PNIPAAM has no chondroinductive property for ADSCs. Accordingly, we hypothesized that HA-modified PNIPAAM hydrogel (PNIPAAM-HA) may improve cell viability of ADSCs and enhance ADSCs' chondrogenesis for articular cartilage tissue engineering. The aim of this study is to develope the PNIPAAM-HA and to test the effect on chondrogenic differentiation of ADSCs. The effects of PNIPAAM and PNIPAAM-HA on cell viability, chondrogenic differentiation as well as cartilaginous matrix formation of rabbit ADSCs (rADSCs) in vitro or in vivo were tested. The rADSCs cultured PNIPAAM-HA showed significantly higher cell viability, cell aggregation and chondrogenesis than those in PNIPAAM in vitro. In vivo study, the rADSCs cultured PNIPAAM-HA exhibited better cartilaginous matrix formation than in PNIPAAM. These results indicate thermoresponsive PNIPAAM-HA demonstrated good cytocompatibility and chondrogenic differentiation property. We suggest that the copolymer may be used as cell vehicles for cartilage tissue engineering.

Keywords: Poly(N-isopropylacrylamide) (PNIPAAM); Adipose-derived stem cells (ADSCs); Hyaluronan (HA); Cartilage tissue engineering

B08

Gene Expression Analysis Reveals High Pluripotent Status and Metastatic Ability of CD133 Positive Cells in Primary Papillary Thyroid Cancer

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Papillary thyroid cancer (PTC) represents >70% of all thyroid cancers, and belongs to well-differentiated cancer. With appropriate surgery resection and ^{131}I ablation, PTCs are usually curable. However, local or regional recurrences occur in 5~15% of patients with PTC, with distant metastasis occurring in 5~10% of patients. We hypothesize a small portion of cells which escape from radiation ablation, contribute to recurrence and metastasis. CD133 is recently proposed as a marker of cancer stem cell in various cancers. Through quantitative PCR approach, we analyzed the expression of selected pluripotent and differentiated thyroid specific genes in both $CD133^{pos}$ and $CD133^{neg}$ cells derived from resected primary tumors. Papillary thyroid tumors resected from patients were subjected to primary culture. Cells expressing CD133 were isolated with magnetic microbeads and total RNA were isolated and reverse transcribed for subsequent real-time quantitative PCR using the TagMan Probe system. $CD133^{pos}$ and $CD133^{neg}$ cell proliferation was measured by MTS assay after cultured for different days. Invasion/migration experiment was performed on $CD133^{pos}$ and $CD133^{neg}$ cells using a dual chamber system. In radioresistance experiment, cells were irradiated with γ-ray then CD133 population was measured. Selected gene expression including regulators of ES cell self-renewal and pluripotency (Oct4, Sox2, nanog, Klf4, Lin28 and c-Myc), thyroid specific transcription factors (TTF-1 and Pax8) and thyroid specific genes(NIS, TPO, Tg, TSHR and Pendrin) were analyzed. $CD133^{pos}$ cells represent ~5% of the whole population and are enriched to >70% after magnetic microbeads isolation as analyzed by flow cytometry. ES cell regulators are expressed significantly higher in $CD133^{pos}$ cells than in $CD133^{neg}$ cells in all donors with variations in expression levels, including Oct4, nanog, Lin28 and Sox2. Thyroid specific gene expression was significantly higher in $CD133^{neg}$ than in $CD133^{pos}$ cells, including NIS, Tg, TPO and TSHR. $CD133^{pos}$ cells showed more radioresistant, more proliferative and also invasive than $CD133^{neg}$ cells. Conclusion: $CD133^{pos}$ cells within PTC cells have a great potential of contributing to the recurrence or distant metastasis of PTC.

Keywords: Papillary thyroid cancer, CD133, cancer stem cells

B09

Color-coded Imaging of the Interaction of Mesenchymal Stem Cells and Cancer Cells

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Mesenchymal stem cells (MSC) have been reported to promote tumor growth and metastasis. Through paracrine effects, cytokines such as TGFβ or CCL5 produced by MSC play a role in modulating cancer cell behavior. However, the interaction between MSCs and cancer cells is not only via cytokines but involves physical contact as well. In this study, using GFP mesenchymal stem cells and RFP murine mammary cancer cells, the interaction between these cells types was imaged in vivo and vitro. Murine MSCs were isolated from GFP transgenic mice and 4T1 murine mammary cancer cells expressed RFP. Cells were co-cultured in vitro or co-implanted into the mouse mammary fat pad. Cell movement and distribution were recorded with fluorescence video microscopy. 4T1 cells had an epithelial shape and moved toward each other to form clusters when cultured alone. In contrast, 4T1 cells showed a stretched, spindle like morphology when interacting with MSCs. In vivo imaging with the Olympus OV-100 or IV-100, clearly visualized GFP-MSC and RFP-4T1 cells within tumors. When co-injected with MSCs, as few as 5 4T1-RFP cells could form a tumor but could not form tumors at the low cell number without MSCs. The results demonstrate that MSCs have a powerful effect on breast cancer cells.

Keywords: mesenchymal stem cells, mammary cancer cell, molecular imaging

B10

Cycloxygenase-2 Contributes to Osteogenic-differentiation on Murine Bone Marrow Mesenchymal Stem Cells

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Cyclooxygenase (COX), including isoenzymes such as COX-1, COX-2and COX-3, is a central enzyme in converting arachidonic acid into prostaglandins (PGs). In several organs, COX-2 is expressed in a constitutive manner, playing a physiological role in controlling central nociceptive processes. Our previous study indicated that COX-2 is constitutively expressed in osteoblasts in the dynamic bone growth area, which facilitates osteoblast proliferation via PTEN/Akt/p27^{Kip1} signaling. COX-2 was also indicated to be important for mediating mechanical stimulation of bone. Therefore, COX-2 may play a role in promoting osteogenesis and bone formation. Besides, a recent report indicated that p27 ^{Kip1} and Akt contributes to promoting osteogenic cell differentiation. Accordingly, we hypothesize that COX-2 may also play an important role in controlling differentiation in osteogenic linage cell via Akt and p27 ^{Kip1} signaling. In this study, murine bone marrow mesenchymal stem cells are used to examine the function of COX-2 on osteogenic-differentiation. According to our data, the COX-2 has a dynamic change when D1 cells from proliferation stage (sub-confluence) to osteo-differentiation stage (confluence). When treated with NS398, the COX-2 specific inhibitor, the gene expressions of Runx-2 and osteocalcin significantly increase, but not in BMP-2. Besides, the p27 ^{Kip1} has a dynamics change that were suppressed in proliferation stage and increased in osteo-differentiation stage. It implies that the COX-2 may regulate the D1 cell osteo-differentiation via p27^{Kip1} to promote osteogenic gene expression. The results clarify the physiological roles and molecular mechanisms of the constitutively expressed COX-2 on osteo- differentiation. The finding provides new information for the basic medical science in bone physiology and provides knowledge for developing new drug that act via affecting COX-2 function in future.

Keywords: mesenchymal stem cells, COX-2, p27 ^{Kip1},Runx-2, osteoclacin

B11

The Role of the Eukaryote DNA End-Replication Problem

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Hermann Joseph Muller, 1930 Nobel Prize found the telomere structure in the end of chromosomes. James Watson, 1960 Nobel Prize, noted the length of DNA is decreased during replication, the end of the DNA 3' to 5' strand will lose 100 to 200 codes in each time of DNA replication and he mentioned it is an End-Replication Problem in DNA replication and the cells will be died when the telomere codes are used up.

There are even about 500,000 codes with a random like and not duplicated sequence in each telomere of the human chromosomes. Are there other roles of the codes of the telomere?

From other viewpoint, the end of the leading strand will have 100 to 200 nucleotides that are not paired with the lagging strand, and exposed in the nucleus. The exposed single strand DNA can be a template. There may be matched RNAs transcribed from the exposed single strand DNA and it may have 92 more or less transcribed RNAs produced from 46 chromosomes after each time of mitosis. The newly transcribed RNAs are new messages given to the daughter cells.

The zygote soon loses its totipotent differentiated entity after three times of mitosis. What are happening in the mitosis to lead the cell to lose its totipotent differentiated entity after the first three times of mitosis? The father cell and daughter cell are differentiated by the mitosis. The cells must produce some material to guide the differentiation during mitosis. The exposed DNAs of the End-Replication Problem can be a known DNA difference between father cell and daughter cell and may be playing the role in this event of differentiation. In other words, the codes of the telomere may play one of the roles to write the differentiated history from a zygote to the aged.

Keywords: DNA End-Replication Problem, Telomere, zygote, totipotent, differentiation, mitosis

B12

Cytokine Profiles in Various Graft-versus-Host Disease Target Organs Following Hematopoietic Stem Cell Transplantation

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Previous studies using genetic-deficient murine models suggest that different T helper subsets may contribute to different types of tissue damages in graft-versus-host disease (GvHD). However, there is limited information available on the distribution of T helper cytokines in the various GvHD target tissues. In the current study, an acute GvHD murine model was setup to directly assess the *in situ* cytokine profiles in various GvHD tissue lesions; in addition, we also studied GvHD tissues from patients who had undergone bone marrow transplantation procedures. We observed that interferon-gamma (IFN-γ was dominant in murine liver and gastrointestine tissue lesions, whereas IFN-γ and interleukin 17 (IL17) were abundant in murine skin lesions. Furthermore, in human GvHD tissues, interleukin 4 (IL4) and IFN-γ were predominant in liver lesions and colon lesions, respectively, while no specific cytokine was prevalent in human GvHD skin lesions. In addition, a low ratio of CD4+ T helper (Th) versus CD8+ T cytotoxic (Tc) cells in human GvHD tissue lesions, especially in the liver, was detected and this contrasts with the situation in murine GvHD tissues where CD4+ Th cells were predominant. Dual-staining for CD markers and cytokine expression showed that IFN-γ secreting T cells were enriched in all murine GvHD target tissue lesions and Tc1 and Tc2 cells were predominant in human GvHD colon and liver sections, respectively. However, IFN-γ+Th1, IL17+Th17, IFN-γ+Tc1 and IL17+Tc17 cells were slightly more frequent in human skin lesions compared to IL4+Th2 and IL4+Tc2 cells. To sum up, these results suggest that differences in cytokine imbalances may significantly contribute to tissue-specific pathogenesis in GvHD target organs and CD8+ Tc cells may play an important role in human GvHD induction.

Keywords: graft-versus-host disease (GvHD); bone marrow transplantation (BMT); T helper (Th) cell; cytotoxic T (Tc) cell

B13

Glucose Reduction Prevents Replicative Senescence and Increases Mitochondrial Respiration in Human Mesenchymal Stem Cells

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The unique self-renewal and multi-lineage differentiation potential of mesenchymal stem cells (MSCs) make them a promising candidate for cell therapy applications. However, during in vitro expansion of MSCs, replicative senescence may occur and will compromise the quality of the expanded cells. Since calorie restriction has been shown to effectively extend the lifespan of various organisms, the purpose of this study is to investigate the effect of glucose reduction on MSCs and the coordinated changes in energy utilization. It was found that the frequency of cycling cells was significantly increased, while senescence markers such as β-galactosidase activities and $p16^{INK4a}$ expression level were markedly reduced in MSCs under low glucose culture condition. Quantitative real-time PCR analysis demonstrated the preserved tri-lineage differentiation potentials of MSCs after low glucose treatment. Interestingly, the ability of osteogenic lineage commitment was improved, while the ability of adipogenic lineage commitment was delayed in MSCs after glucose reduction. In addition, we observed decreased lactate production, increased electron transport chain complexes expression, and increased oxygen consumption in MSCs after glucose reduction treatment. Increased antioxidant defensive responses were evidenced by increased antioxidant enzymes expression and decreased superoxide production after glucose reduction. Taken together, our findings suggest that MSCs utilize energy more efficiently under restricted glucose treatment and exhibit greater self-renewal and anti-senescence abilities, while their differentiation potentials remain unaffected.

Keywords: Glucose reduction, Mesenchymal stem cells, Replicative senescence, Mitochondria

B14

Developing a Safe Reprogramming Methods for Generating iPSC

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Stem cells can differentiate into most cell types while differentiated somatic cells have lost this pluripotency. Recent studies have shown that somatic cells can be reprogrammed by over-expression of defined transcription factors (Yamanaka factors, YFs) to become pluripotent stem cell (called as induced pluripotent stem cells, iPSC). Over-expression of YFs is usually mediated by retrovirus and whose integration into genome raises serious concern about the insertional mutagenesis. To develop safer iPSC, one reprogramming method has incubated reversibly permeable target cells in ES cell extracts and another has used recombinant cell–penetrating YFs proteins to generated iPSC. Although these two methods avoid insertional mutagenesis, unfortunately, they generate iPSC with only low efficiency. Therefore, we like to combine these two approaches by treating somatic cells with permeable system and cell-penetrating YFs proteins simultaneously to increase the reprogramming efficiency. Firstly, fibroblasts will be treated with streptolysin-O (SLO) to make their plasma membrane transiently permeable. Then, recombinant YFs proteins will be added to reprogram them to pluripotent state. The permeability of these cells will be determined by trypan blue uptake and the reprogramming efficiency will be examined by puromycin or GFP selection. Currently, we are using bacteria system to express recombinant YFs proteins and they are purified by His-Tag system. In the future, YFs proteins will be expressed by insect cells that allow post-translational modification of these factors. Once iPSC clones have been generated, their pluripotent state will be examined by ES like colony formation, gene expression profiling assays, and embryoid body formation.

Keywords: Pluripotency, stem cell, iPSC, cell-penetrating, streptolysin-O, Yamanaka factor

B15

Improvement of Behavioural Deficit by Transplantation of Human Amniotic Fluid Stem Cells in Parkinsonian Rats Model

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Parkinson's disease (PD) is the second most common chronic progressive neurodegenerative disorder and is characterized primarily by major loss of nigrostriatal dopaminergic neurons. Amniotic fluid stem cells (AFSCs) isolated from amniocentesis have been demonstrated to have the ability to self-renew and to give rise to not only mesenchyme-lineage but dopamine secretion cells in vitro. Here we investigated the feasibility of using AFSCs as therapeutic cell sources for PD rat model. The AFSCs expressed early developmental and neuronal markers. We also found that AFSCs samples can inhibit interferon (IFN)-γ–inducible major histocompatibility complex (MHC) class II expression in our culture condition. While the AFSCs were transplanted into the *striatum* of the PD rats with 6-hydroxydopamine lesion in the substantia nigra, the apomorphine-induced rotation was reduced by 75% in AFSCs grafted group. The histological results also showed that the implanted AFSCs keep viable over than 12 weeks and migrated into brain circuitry. The transplanted cells not only expressed neuronal markers including nestin and glial fibrillary acidic protein but could be detected the specific proteins of dopamine neurons, such as tyrosine hydroxlyase and dopamine transporter. AFSCs showed the powerful therapeutic effects in our report that about 80% PD rats got significant behavioral improvement. In this study, we firstly identified that AFSCs might have the specific immunomodulated function different from mesenchymal stem cells-derived from other sources, and we gave the direct evidence to improve the rotation syndrome of PD by AFSCs transplantation in vivo.

Keywords: amniotic fluid stem cells, Parkinson's Disease, dopamine neurons, cell therapy

B16

The Inflammatory Effects Increased Proliferation Rate and Maintained the Stemness of Pluripotent Germline Stem Cells

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One hypothesis of cancer stem cells (CSCs) formation is derived from its stem cell transformation. CSCs formation is constantly affected by the outside signaling from their niche, e.g. immune response (inflammation), endocrinal effects (growth factors from niche cells), and environmental factors (such as oxygen concentration). Accumulating evidence suggests that multiple CSC functions hinge on major receptor-mediated pathway, such as growth factor-mediated PI3K/Akt signaling, BMP signaling, Hedgehog regulation, and inflammatory signaling. Our previous results demonstrated an important role of insulin-like growth factor-1 receptor (IGF-1R) signaling in maintenance of germ cell pluripotency. However, signaling involved in transformation of stem cells into CSCs still remains largely unknown. To examine the effect of niche inflammatory stress on germline stem cells (GSCs), inflammatory conditioned medium(CM) were supplemented in culture medium for cultivation of neonatal mouse testis cells. The colony size and alkaline phosphatase activity of GSCs were examined. The AP activity was detected by Naphthol AS-BI phospate. While comparing with the control group0, optimal CM concentration (1/128 and 1/256) increased the colony size as well as the AP activity of GSCs significantly. The FACS result also showed the higher SSEA-1 marker expression in membrane by inflamatory CM addition. To examine the effect of IGF-1 regulation by inflammatory CM, IGF-1 expression level was detected. Inflammatory CM stimulation increased the IGF-1 expression level in GSC colonies. While comparing with the control group, the optimal CM concentration (1/128 and 1/256) increased the transcriptional level of pluripotent transcription factor Oct-4 by real-time PCR. A specific inhibitor of IGF-1R phosphorylation, PPP, was used to block the IGF-1R signaling in GSCs, and PPP significantly decreased the AP^{+}GSC colony formation. The result from PD98059 treatment showed the ERK pathway was not the pathway for the GSCs stemness. Together with these results suggest the important role of IGF-1/IGF-1R signaling in GSCs under niche inflammatory stress.

Keywords: Inflammation, germline stem cells, Oct4, IGF-1R

B17

Larger Wound in Porcine Full-Thickness Wound Healing

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It has been recognized that the treatment of cutaneous wound healing declines with larger burns and chronic wounds, yet the treatment approaches that affect the declines remain poorly understood. The purpose of this study was to establish standardized procedure on wound creation and understand re-epithelialization, contraction rate and scar formation in case of larger full-thickness wounds in swine. Multiple larger full-thickness excision wounds (10×10 cm) were created on the dorsum of 7-week-old (n=5) Landrace pigs. The extent of wound closure area and wound contraction were measured using clear plastic sheets from weeks 0 to 10. These transparent sheets were scanned and then ImageJ software was used to measure the area of each wound, and a contraction rate was calculated for each wound on each week using week 0 as "0 % contracted." Based on the gross appearance and biopsies taken from the center of the wound, we estimated the time to completed re-epithelialization for wound was 6±0.5 weeks (mean±SD). The mean wound closure area in each week was 8.0±2.2, 5.7±2.7, 4.2±2.3, 4.1±2.2, 4.0±2.2, 3.6±1.9, 3.8±1.8, 3.8±1.8, 3.9±1.8 and 3.9±1.8cm^2**.** The mean wound contraction rate was about 35±5, 67±8, 77±5, 83±5, 84±5, 87±4, 85±3, 85±3, 85±3 and 85±3%. Although wound contraction measurements from weeks 4 to 10 showed no statistical differences among the pigs, the wound contraction of full-thickness wounds was faster from the first week to third week ($P < 0.05$). These results suggest that this larger wound model can be used to test the effects of therapeutic approaches such as dermal and epidermal substitutes, efficacy, fine-tuning of required cell densities and safety aspects relating to immunoreactivity and biocompatibility intended to treat larger full-thickness wound.

Keywords: Landrace pig, skin, full-thickness, wound healing

B18

The Effects of Enterovirus 71 Infection on Mouse Neural Stem Cells

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Enterovirus 71(EV71), a member of *Picornaviridae* family, cause central nervous system (CNS) involvement and cardiopulmonary failure in children. Some of EV71 infected children are found associated with neurologic sequelae, delayed neurodevelopment, and reduced cognitive functioning. However, the mechanism that EV71 infection induced neurodevelopmental deficiency is still unclear. Previous study showed that coxsackievirus B3(CVB3), another member of *Picornaviridae* family, also cause CNS infection in neonatal, lead to serious neurodevelopmental defect. CVB3 infected neural progenitor cells lose the capacity of proliferation, but sill progress cell differentiation. Here we investigate that EV71 could infect mouse neural stem cell (mNSC), induced cell damage. These mouse brain-derived mNSC can differentiate into neuron and astrocyte had been characterized. After EV71 infection, the cytopathic effect (CPE) of mNSC would be the most apparent in 2 days post infection (p.i.). By using immunofluorence assay (IFA), we detected that EV71 protein expressed in around 3-5% of cells, and most of the infected cells had significant CPE. Moreover, we collected the protein extracted from different time points after EV71 infection and detect EV71 3D protein. We found that the highest expression of 3D was at 12hr post infection. Furthermore, the replication efficiency of EV71 in mNSC increased at 12hr post infection, not only in lysate but also in supernatant, showed that EV71 could finish its replication cycle and release from mNSC. We propose that EV71 can infect mNSC, finish a complete life cycle, and induce the cell damage.

Keywords: Enterovirus 71, neural stem cell

B19

Cell Lineage Tracing of Sox2 Expressing Cells in the Adult Mouse Brain

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Sox2 encodes a transcription factor which plays important roles in regulating neural development. It is also believed that *Sox2* is one of the neural stem cell markers in the adult brain. There are two major neurogenesis zones in the mouse brain, which include subgranular zone of hippocampal dentate gyrus and subventricular zone of lateral ventricle. Here we take a genetic approach to label Sox2 positive cells in the adult mice brain by crossing a *Sox2-CreERT2* transgenic mouse with an inducible organelle specific dual fluorescent protein reporter mouse (*R26RGR*). The dual fluorescent proteins are encoded by an inducible transgenic construct (H2B-EGFP-2A-mCherry-GPI) placed in the *Rosa*26 locus. The H2B-EGFP encodes a histone protein fusion with an enhance green fluorescent protein which allows us to observe chromatin in the nucleus, providing cell cycle information including mitosis, while mCherry-GPI encodes red fluorescent membrane anchored protein depicting cellular locations and morphologies. Under the induction of tamoxifen at the adult stage, we can label Sox2 positive cells, putative neural stem cells and trace their descendents *in vivo*.

Keywords: Sox2, Dual fluorescent, transgenic mouse

B20

Study of MRI Contrast Agent for Target Cell Isolation and Transplantation

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Cell based therapeutics face an important issue in monitoring cell localization to ensure cell homing at the desired region in vivo. This is important because the distribution of transplanted cells strongly correlates with the effectiveness and safety issues [1-2]. Due to this reason, we developed a contrast agent based on iron oxide materials with high relaxivity, and good biocompatibility. Our nanoparticle has PEG coating to decrease nonspecific interaction and to immobilize the CD34+ antibody onto the polymer to increase specific affinity. Thus, the contrast agent is intended to have specific CD34+ cell targeting ability which can be utilized to select specific cells in vitro from either cell solutions or cord blood by using a sorting device.

In this investigation, we conjugated either secondary antibody (Alexa594 IgG) or primary antibody (CD34+ antibody) onto the IOP individually. The photos of fluorescence electrophoresis of the contrast agent revealed the conjugation process was accomplished. The physical properties of the contrast agent were also measured: the core size is between 8~15nm and the hydrodynamic diameter is in the order of 50~150nm depending on the coating polymer types. Preliminary study show that over 95% KG1a CD34+ cell can bind to optimal IOP-antibody batch. Further cell isolation experiment indicated IOP-My10 can effectively bind to KG1a CD34+ cell in mixed PBMC cell solution, and more than $3*10^6$ of CD34+ cells can be purified by sorting tube with strong magnetic field. Moreover, MRI in animal model indicated that the nanoparticles remain in living organisms for at least 4 weeks after cell transplantation. The visualized MRI tracing reveal a good correlation with iron stain in rat brain indicating the IOP-antibody still bind to the target cell tightly and remain in the injection location.

The contrast agent has the ability for target CD34+ cells for the cell isolation, enrichment and as a labeling reagent for cell therapy as well as particular cell tracing in patients. The study revealed the potential of the contrast agent for further cell therapy applicatio**n.**

Keywords: MRI contrast agent, cell isolation, CD34 antibody, cell targeting & tracing, cell therapy

B21

A Serum-free Medium Containing TAT-HOXB4 to Support *in vitro* Expansion of HSCs

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Haematopoietic stem cells (HSCs) are commonly used in clinical transplantation protocols to treat a variety of diseases. However, efficient transplantation requires a substantial amount of HSCs from different sources and may require expansion. Effective expansion of HSCs remains therefore a technical hurdle blocking the development of advanced cell therapies. The product of the human homeobox B4 (HOXB4) gene was recently demonstrated to effectively expand HSCs with a animal serum containing medium. In this study, we purified TAT-HOXB4 proteins and demonstrated their ability to expand umbilical cord blood (UCB) and peripheral blood (PB) progenitor cells by a serum free system. The results showed that the number of total MNCs increased approximately four fold and $CD34^+$ cells increased approximately eight fold. The results from a semisolid cloning assay, a human long-term culture-initiating cells (LTC-ICs) assay and a nonobese diabetic-severe combined immunodeficiency (NOD-SCID) mice repopulating assay all showed that this serum free medium expanded hematopoietic progenitor cells while retaining their repopulating capacity and multipotency. The results demonstrated the feasibility of using TAT-HOXB4 to expand HSCs, which are readily for clinical usage.

Keywords: HOXB4 protein, Expansion, Haematopoietic stem cells (HSCs).

B22

Manual Isolation of Differentiated Oct4-EGFP-positive Human Embryonic Stem Cells Enriches the Potential Germ Cells Expressing VASA and GDF9

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Recent evidences in mice and human have suggested the great potential of germ cell development from human embryonic stem (hES) cells. In addition to be an effective experimental model, germ cell development from hES cells may become clinically relevant in overcoming a number of human diseases related to the quality and quantity of germ cells. In this study we differentiated two hES cell lines, NTU1 and H9 Oct4-eGFP cells, *in vitro* using a number of niche environments, including laminin-coated dishes, human granulosa cell co-culture or conditioned medium, mouse ovarian stromal cell co-culture or conditioned medium, and addition of growth factors (retinoid acid, SCF and BMP4). We showed that granulosa cells co-culture and its conditioned medium increased the percentages of cells expressing early germ cell marker SSEA1 (SSEA1+) (up to 40%) and/or Oct4-eGFP (Oct4-eGFP+) compared to laminin alone (18%). Manual collected Oct4-eGFP+ cells on day 14 of differentiation significantly expressed higher levels of specific germ cell marker gene *VASA* and late marker *GDF9*, although meiosis markers SCP1 and SCP3 and mature germ cell marker ZP3 were not up-regulated. These Oct4-eGFP cells developed to ovarian follicle-like structures after extended culture for 28 days. In addition, supplement of retinoid acid in the medium also enhanced VASA and GDF9 expression in the differentiated hES cells, whereas SCF and BMP4 did not. It is concluded that granulosa cells co-culture and conditioned medium may increase the percentages of cells showing germ cell potential. However SSEA1 alone may not be a good marker for isolating primordial germ cells from differentiated hES cells, except perhaps at a very early stage of differentiation. Whereas GDF9 can become a useful marker for selection at later stage (after day 21). We propose that the protocol of differentiating hES cells in granulosa cell co-culture/conditioned medium and/or retinoid acid supplement, followed by early manual selection of Oct4-eGFP+ cells, and finally by selection with VASA and/or GDF9 (especially) markers can become an effective strategy to enrich the cells with greatest potential for germ cell formation.

Keywords: differentiation/ embryonic stem cells/ germ cell/ granulosa cells/ co-culture

2011 International Symposium on Recent Advances in Pluripotent Stem Cells & 7th Annual Meeting of Taiwan Society for Stem Cell Research

About TSSCR

臺灣幹細胞學會章程

第一章　總　　則

第　一　條　本會名稱為臺灣 幹細胞學會(以下簡稱本會)。

第　二　條　本會為依法設立、非以營利為目的之社會團體。宗旨如下：促進幹細胞學之發展及配合政府推行相關幹細胞醫療之政策及方案。

第　三　條　本會以全國行政區域為組織區域。

第　四　條　本會會址設於主管機關所在地區，並得報經主管機關核准設分支機構。

前項分支機構組織簡則由理事會擬訂，報請主管機關核准後行之。

會址及分支機構之地址於設置及變更時應函報主管機關核備。

第　五　條　本會之任務如左：

一、提倡幹細胞學之研究、教育與應用。

二、舉辦幹細胞相關演講及研討會。

三、促進國內外相關學會之密切聯繫與發展。

四、協助政府與民間審議制定幹細胞醫療之政策及方案。

五、得發行會誌及有關幹細胞科技書刊，及倫理規範資料，以供社會參考。

六、聯繫、促進國內外幹細胞事業之發展，供產、官、學界參考，促進幹細胞學術與相關產業之合作。

七、其他相關之事項與活動。

第　六　條　本會之主管機關為內政部，本會之主要目的事業主管機關為行政院衛生署。本會之目的事業應受各該事業主管機關之指導、監督。

第二章　會　員

第　七　條　本會會員申請資格如左：

一、個人會員：凡贊同本會宗旨、年滿二十歲、具有實際從事有關幹細胞學工作之經驗，且具相關生命科學專業學歷背景者，並由會員二人推薦，經理監事會審查通過，得為本會個人會員。

二、團體會員：凡贊同本會宗旨之公私立學術或研究機構或團體。

三、贊助會員：凡贊同本會宗旨之工商團體或個人。

四、準會員：凡贊同本會宗旨的學生、博士後研究員、住院醫師、研究助理或等同資格者等，由會員二人推薦，經理監事會審查通過，得為本會準會員。

申請時應填具入會申請書，經理事會通過，並繳納會費。團體會員應推代表一人，以行使會員權利。

第　八　條　個人會員(團體會員代表)有表決權、選舉權、被選舉權與罷免權。

每一會員(會員代表)為一權。

贊助會員無前項權利，贊助會員所享有權利，可由理監事會再行討論定奪。

第　九　條　會員有遵守本會章程、決議及繳納會費之義務。未繳納會費者，不得享有會員權利；連續二年未繳納會費者，視為自動退會。會員經出會、退會或停權處分，如欲申請復會或復權時，應繳清前所積欠之會費即可復會，或以新加入會員重新申請重新，並由理事會審核方可復會。

第 十 條 會員(會員代表)有違反法令，章程或不遵守會員大會決議時，得經理事會決議，予以警告或停權處分，其危害團體情節重大者，得經會員(會員代表)大會決議予以除名。

第 十一 條 會員喪失會員資格或經會員大會決議除名者，即為出會。

第 十二 條 會員得以書面敘明理由向本會聲明退會。

第三章 組織及職權

第 十三 條 本會以會員大會為最高權力機構。

會員人數超過三百人以上時得分區比例選出會員代表，再召開會員代表大會，行使會員大會職權。會員代表任期 2 年，其名額及選舉辦法由理事會擬訂，報請主管機關核備後行之。

第 十四 條 會員大會之職權如左：

一、訂定與變更章程。

二、選舉及罷免理事、監事。

三、議決入會費、常年會費、事業費及會員捐款之數額及方式。

四、議決年度工作計畫、報告及預算、決算。

五、議決會員(會員代表)之除名處分。

六、議決財產之處分。

七、議決本會之解散。

八、議決與會員權利義務有關之其他重大事項。

前項第八款重大事項之範圍由理事會定之。

第 十五 條 本會置理事 15 人、監事 5 人，由會員(會員代表)選舉之，分別成立理事會、監事會。

選舉前項理事、監事時，依計票情形得同時選出候補理事 2 人，候補監事 1 人，遇理事、監事出缺時，分別依序遞補之。

本屆理事會得提出下屆理事、監事候選人參考名單。

理事、監事得採用通訊選舉，但不得連續辦理。通訊選舉辦法由理事會通過，報請主管機關核備後行之。

第 十六 條　理事會之職權如左：

一、審定會員(會員代表)之資格。

二、選舉及罷免常務理事、理事長。

三、議決理事、常務理事及理事長之辭職。

四、聘免工作人員。

五、擬訂年度工作計畫、報告及預算、決算。

六、其他應執行事項。

第 十七 條　理事會置常務理事 5 人，由理事互選之，並由理事就常務理事中選舉一人為理事長。

理事長對內綜理督導會務，對外代表本會，並擔任會員大會、理事會主席。　理事長因事不能執行職務時，應指定常務理事一人代理之，未指定或不能指定時，由常務理事互推一人代理之。理事長、常務理事出缺時，應於一個月內補選之。

第 十八 條　監事會之職權如左：

一、監察理事會工作之執行。
二、審核年度決算。
三、選舉及罷免常務監事。
四、議決監事及常務監事之辭職。
五、其他應監察事項。

第 十九 條　監事會置常務監事 1 人，由監事互選之，監察日常會務，並擔任監事會主席。

常務監事因事不能執行職務時，應指定監事一人代理之，未指定或不能指定時，由監事互推一人代理之。監事會主席(常務監事)出缺時，應於一個月內補選之。

第 二十 條　理事、監事均為無給職，任期 2 年，連選得連任。理事長之連任，以一次為限。每次改選至少三分之一需為新任理監事。

第二十一條　理事、監事有左列情事之一者，應即解任：
一、喪失會員(會員代表)資格者。
二、因故辭職經理事會或監事會決議通過者。
三、被罷免或撤免者。
四、受停權處分期間逾任期二分之一者。

第二十二條　本會置秘書長一人，承理事長之命處理本會事務，其他工作人員若干人，由理事長提名經理事會通過聘免之，並報主管機關備查。但秘書長之解聘應先報主管機關核備。
前項工作人員不得由選任之職員擔任。
工作人員權責及分層負責事項由理事會另定之。

第二十三條　本會得設各種委員會、小組或其他內部作業組織，其組織簡則經理事會通過後施行，變更時亦同。

第二十四條　本會得由理事會聘請名譽理事長一人，名譽理事、顧問各 若干人，其聘期與理事、監事之任期同。

第四章　會　　議

第二十五條　會員大會分定期會議與臨時會議二種，由理事長召集，召集時除緊急事故之臨時會議外應於十五日前以書面通知之。

定期會議每年召開一次，臨時會議於理事會認為必要，或經會員(會員代表)五分之一以上之請求，或監事會函請召集時召開之。

本會辦理法人登記後，臨時會議經會員（會員代表）十分之一以上之請求召開之。

第二十六條　會員(會員代表)不能親自出席會員大會時，得以書面委託其他會員(會員代表)代理，每一會員(會員代表)以代理一人為限。

第二十七條　會員(會員代表)大會之決議，以會員(會員代表)過半數之出席，出席人數較多數之同意行之。但章程之訂定與變更、會員(會員代表)之除名、理事及監事之罷免、財產之處分、本會之解散及其他與會員權利義務有關之重大事項應有出席人數三分之二以上同意。

本會辦理法人登記後，章程之變更以出席人數四分之三以上之同意或全體會員三分之二以上書面之同意行之。本會之解散，得隨時以全體會員三分之二以上之可決解散之。

第二十八條　理事會、監事會至少每六個月各舉行會議一次，必要時得召開 聯席會議或臨時會議。

前項會議召集時除臨時會議外，應於七日前以書面通知，會議之決議，各以理事、監事過半數之出席，出席人數較多數之同意行之。

第二十九條　理事應出席理事會議，監事應出席監事會議，不得委託出席;理事、監事連續二次無故缺席理事會、監事會者，視同辭職。

第五章　經費及會計

第 三十 條　本會經費來源如左：

一、入會費：個人會員新台幣 1000 元，團體會員新台幣 10000 元，準會員新台幣 500 元，於會員入會時繳納。準會員之入會費得依當年入會時參與學術活動海報之張貼，予以減免。

二、常年會費：個人會員新台幣 1000 元，團體會員新台幣 3000 元。準會員新台幣 500 元。

三、事業費。

四、會員捐款。

五、委託收益。

六、基金及其孳息。

七、其他收入。

第三十一條　本會會計年度以曆年為準，自每年一月一日起至十二月三十一日止。

第三十二條　本會每年於會計年度開始前二個月由理事會編造年度工作計畫、收支預算表、員工待遇表，提會員大會通過(會員大會因故未能如期召開者，先提理監事聯席會議通過)，於會計年度開始前報主管機關核備。並於會計年度終了後二個月內由理事會編造年度工作報告、收支決算表、現金出納表、資產負債表、財產目錄及基金收支表，送監事會審核後，造具審核意見書送還理事會，提會員大會通過，於三月底前報主管機關核備(會員大會未能如期召開者，先報主　　管機關。)

第三十三條　本會解散後，剩餘財產歸屬所在地之地方自治團體或主管機關指定之機關團體所有。

第六章　附　　則

第三十四條　本章程未規定事項，悉依有關法令規定辦理。

第三十五條　本章程經會員(會員代表)大會通過，報經主管機關核備後施行，變更時亦同。

第三十六條　本章程經本會94年4月16日第一屆第一次會員大會通過。報經內政部94年6月20日台內社字第0940073166號函准予立案。95年9月2日第一屆第二次會員大會第一次修正通過。99年10月9日第三屆第六次會員大會第二次修正通過。

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