Conference Honorary Chairman:

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Tao Cheng, Center for Stem Cell Medicine and Institute of Hematology, Chinese Academy of Medical Sciences and Peking Union Medical College, China
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Yong-Guang Yang, Columbia University, USA
Daohong Zhou, University of Arkansas for Medical Sciences, USA
Weiping Yuan, Institute of Hematology, Chinese Academy of Medical Sciences and Peking Union Medical College, China

Conference Speakers:

Irving L. Weissman, Professor and Director, Institute for Stem Cell Biology and Regenerative Medicine at Stanford University. Director, the Stanford Ludwig Center for Stem Cell Research, USA

Edward F. Srour, Professor, Departments of Medicine and Pediatrics, Herman B Wells Center for Pediatric Research, Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN, USA

Gerald de Haan, Professor and Chairman, Department of Stem Cell Biology, University of Groningen, Netherlands

Armand Keating, Professor of Medicine, Director of Division of Hematology, University of Toronto, Canada

Sheng Ding, Associate Professor, the Scripps Research Institute, USA

John Yu, Distinguished Research Fellow & Director, Stem Cell Program, Genomics Research Center and Institute of Cellular and Organismic Biology, Academia Sinica, Taiwan

Shaorong Gao, Investigator, National Institute of Biological Sciences; Center for Stem Cell Medicine, Chinese Academy of Medical Sciences, China

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Zhongchao Han, Professor, Institute of Hematology & Hospital of Blood Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Academician of French Academy of Sciences, China

Lingsong Li, Professor and Director, Peking University Stem Cell Research Center, China

Yael Porat, CEO, BioGenCell, Israel

Jung-Woog Shin，Professor，Dept. of Biomedical Engineering, College of Bioscience and Bioengineering. Director，National Research Lab(NRL) of Cellular and Biomechanical Engineering, Inje University, Korea

Xiaomei Zhai, Professor and Director, Centre for Bioethics, Ethics Committee on Stem Cell Research, Chinese Academy of Medical Sciences and Peking Union Medical College，China

Shi-Jiang Lu, Senior Scientist and Scientific Director, Stem Cell & Regenerative Medicine International and Advanced Cell Technology, USA

Feng Ma, Assistant Professor, Institute of Medical Science, University of Tokyo, Japan; Adjunct Professor, Institute of Transfuction Medicine, Chinese Academy of Medical Sciences and Peking Union Medical College, China

Xiao Hu, Investigator, State Key Laboratory of Experimental Hematology, Institute of Hematology & Hospital of Blood Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, China

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Lugui Qiu, Professor, State Key Laboratory of Experimental Hematology, Institute of Hematology & Hospital of Blood Diseases, Chinese Academy of Medical Sciences & Peking Union Medical College, China

Zack Wang, Principle Investigator, Maine Medical Center; University of Maine, USA

Charles Wang, Research Associate Professor and Director, Functional Genomics Core, City of Hope and Beckman Research Institute, USA

Yue Ma, Investigator, Institute of Biophysics, Chinese Academy of Sciences, China

Yufang Shi, Professor and Director, Chinese Academy of Sciences and Shanghai Jiao Tong University School of Medicine, China

Yong-Guang Yang, Professor, Columbia Center for Translational Immunology, Columbia University College of Physicians and Surgeons, USA

Daohong Zhou, Professor, Division of Radiation Health, Department of Pharmaceutical Sciences, University of Arkansas for Medical Sciences, USA

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Nadia Carlesso, Associate Professor, Wells Center for Pediatric Research, Department of Pediatrics, Department of Medical and Molecular Genetics, Indiana University School of Medicine, USA

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26) Direct Differentiations of Atrial and Ventricular Myocytes from Human Embryonic Stem Cells (Yue Ma)
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28) Macrophages prevent human red blood cell reconstitution in immunodeficient mice (Yong-Guang Yang)
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41) Characterization and Suppression of Pten Null Leukemia Stem Cells (Wei Guo)

42) Hematopoiesis suppression in the aGVHD condition: intrinsic or extrinsic? (Jianmin Wang)
43) Marrow microenvironment and pathogenesis of hematopoietic defects in murine model of Fanconi Anemia (Feng-Chun Yang)
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46) Novel molecular pathways regulating the function of leukemia stem cells (Shaoguang Li)
47) Balancing Self-Renewal and Differentiation in Stem Cells (Ting Xie)
48) Generation and Regeneration of Pancreatic Beta Cells (Hongkui Deng)
49) Human iPS and blood cells: gene targeting and disease modeling (Linzhao Cheng)
50) Stem Cell Tourism: Hope without Reason? (Douglas Sipp)

Poster Presentations (in alphabetic order of the name of first author)

1. Manufacture of Bone Marrow Stromal Cells (BMSCs, aka Mesenchymal Stem Cells) using a novel closed system bioreactor preserves “stemness”
2. Comparison of Cellular Characterization of Mesenchymal Stem & Haemetopoetic Stem Cell Isolated from Bone Marrow & Cord Blood
3. Wnt signaling regulates porcine pancreatic stem cell proliferation
4. A Mathematical Model Reveals the Bistability and All-or none Characters of Extraembryonic–endoderm Differentiation
5. Functional alteration of normal hematopoietic stem and progenitor cells in MLL-AF9 induced acute myeloid leukemia
6. The effects of telomerase activity and telomere length on leukemogenesis
7. Peripheral blood derived stem cell implantation for patients with critical limb ischemia
8. Systematic Expression, Refolding and Purification of a Human 11R tag Transcription Factor Protein for PiPS and in vitro Cell Differentiation Studies
9. Nanoparticles labeled Stem Cells: A Novel Therapeutic Vehicle
10. Directing mesenchymal stem cells to chondrocytes via specific surface chemistry
11. Molecular Cloning of Nanog Gene Promoter from Dairy Goat and Construction of Reporter System
12. New Insight in Cell Therapy for Trinucleotide Repeat Expansion Diseases
13. Derivation of Human Induced Pluripotent Stem Cells from Patients with Parkinson’s Disease
14. An effective serum- and xeno-free chemically defined cryopreservation with simple procedure for human ES and iPS cells
15. CCR5-delta32 iPS cells as a potential anti-HIV therapy for AIDS patients

16. Characterization of female germ cells derived from mouse embryonic stem cells through expression of GFP under the control of Figla
17. Isolation and differentiation of germline stem cells from the postnatal mouse ovary
18. Standardization study of G-CSF mobilized peripheral blood stem cells for the treatment of patients with lower limb ischiaemia
19. A new method of type 1 diabetes treatment：T lymphocytic series clearance and autologous hematopoietic stem cell transplantation（with 2 cases analysis）
20. Improving reconstitution of human-blood lineage cells in humanized mice by ex vivo culture of hematopoietic stem cells with mesenchymal stem cells expressing angiopoietin-like-5 and in vivo expression of human cytokines
21. Protection of mice against irradiation injuries by the post-irradiation combined administration of p38 inhibitor and G-CSF
22. Targeting on the hematopoietic stem cell niche to protect stem cell from chemotherapy and G-CSF
23. Stem cell transplantation in ataxia patients
24. Long-term Survival of Embryonic Stem Cells in Adult Bone Marrow
25. An Oct4/Sox2 Co-Expression Vector Can Activate Endogenous Nanog Expression In Human Embryonic Kidney 293 Cells
26. Amyloid beta peptide induces genetic programming in mesenchymal stem cells and neuronal PC-12 cells
27. Inhibition of mTORC1 hyperactivity promoted self-renewal capacity of mouse hematopoietic stem cells during ex vivo expansion
28. A novel approach to promote germ cell differentiation from mouse embryonic stem cells (mESCs)
29. Human umbilical cord mesenchymal stem cells accelerate recovery of cisplatin-induced kidney injury and attenuate renal interstitial fibrosis in rats
30. The Use of Bone Marrow and Fat Stem Cells in an Office Setting for the Treatment of Musculoskeletal Conditions
31. Phosphorylation of ERK Contributes to Differentiation from Human Umbilical Cord Mesenchymal Stem Cells into Cardiomyocyte-like Cells Induced by 5-azacytidine
32. Competition between leukemic cells and normal hematopoietic cells in non-irradiated recipient mice
33. Vam3 stimulates phenotypic and functional maturation of murine bone marrow-derived dendritic cells
34. Human umbilical cord mesenchymal stem cells expand CD4+CD25+ forkhead boxp3(FOXP3)+regulatory T cells to improve survival and resolve experimental lung injury in mice
35. Impact of notch1-induced murine T cell leukemic environment on normal hematopoietic stem cells

36. Inducing Pluripotency in Somatic Cells from the Snow Leopard (Panthera uncia), an Endangered Felid
37. Comparing liver differentiation potential of different source stem cells
38. Functionalization of poly (epsilon-caprolactone) surface with bioresponsive molecular components for accelerating in situ endothelialization
39. The increased stability of p16 mRNA in murine hematopoietic stem cell after irradiation was mediated by p38 signal pathway
40. Trancriptome analysis of undifferentiated human ES cells and primary erythroid cells at different developmental stages
41. Differentiation of Lymphatic Endothelial Cells From Bone Marrow Mesenchymal Stem Cells with VEGF
42. Analysis the advantage of autologous mobilized peripheral blood mononuclear cells transplantation on lower limbs ischemia disease
43. Regulation of erythroid differentiation by skeletal and membrane proteins
44. Stem Cells Transplantation Clinical Therapy for Children Cerebral Palsy （Attach 40 patients report）
45. Analysis of erythroid-specific enhancers in KLFS genomic regions
46. Umbilical Cord Derived Mesenchymal Stem Cells Isolated by a Novel Explantation Technique Can Differentiate Into Functional Endothelial Cells and Promote Revascularization
47. Chemotaxis of AGM Mesenchymal Stem Cells Induced by Mouse Embryonic Circulation
48. ERK 1/2 signaling in hepatic differentiation of human umbilical cord-derived mesenchymal stem cells
49. From stem cells to platelets: the role of cytokines, serotonin and herbal medicines
50. Safety evaluation of allogeneic umbilical cord blood mononuclear cell therapy for degenerative conditions
51. Induction of human bone marrow mesenchymal stem cells differentiation into neural-like cells using cerebrospinal fluid
52. Human umbilical cord Mesenchymal stem cells affect the immunomodulation in autoimmunemyasthenia gravis rats
53. Pancreatic Carcinoma Treated Using Dendritic Cells Pulsed by Cancer Cells with α-Gal Epitopesand DC Activated Naïve T cells
54. Resveratrol and its analogues - isorhapontigeni, heyneanol-A, protect mouse hematopoietic cells from ionizing radiation damage
55. Regulation of erythroid differentiation by KLF3
56. Derivation and characterization of ovine embryonic stem-like cell lines in defined conditions
57. Isolation and Characterization of Cervical Cancer Stem Cells through Stem Cell-Specific Marker Sox2
58. Construction of RNAi lentiviral vector targeting mouse Islet-1 gene
59. A Combinatorial Approach for Development of Synthetic Surfaces for Stem Cell Culture

60. A novel mesenchymal stem cells can be obtained from pubertal goat testis

致谢（Acknowledgements）

Program of 2010 International Forum on Stem Cells
(2010 国际干细胞论坛日程)
November 11-13 (11 月 11 日-13 日)
Tianjin Saixiang Hotel (天津赛象酒店)

Date (日期)	Title (报告题目)
11/11/2010, Thursday (周四) **Conference Registration, Exhibition Arrangement, Hotel Check-in, and Poster Set-up.** **(会议注册，展览安排，酒店入住和海报设置)**	
11/12/2010, Friday (周五)	
Morning (上午)	**Plenary Session (大会报告)**
8:30-9:00	**Welcome Ceremony and Opening Remarks (欢迎仪式及开幕致辞)**
9:00-9:50 Keynote speech (特邀报告) Chairperson (主持人)： Tao Cheng (程涛)	Title：Normal and Neoplastic Stem Cell Keynote Speaker：Irving Weissman
9:50-10:10	**Coffee Break and Group Photo (照相与茶歇)**
10:10-11:50	**Plenary Session (大会报告)**
Plenary Session I **(大会报告 一)** Chairpersons (主持人): Ed Srour Zhongchao Han(韩忠朝) (Each talk 25 mins, including 5 mins discussion)	1、Title：Modulation of Stem Cell Function by Cellular Components of the Hematopoietic Niche Speaker： Edward Srour
	2、Title： Aging of Hematopoietic Stem Cells: A Program or Noise? Speaker：Gerald de Haan
	3、Title： Overcoming Challenges to the Enhancement of Translational and Clinical Research in Cellular Therapy Speaker： Armand Keating
	4、Title： A Chemical Approach to Controlling Cell Fate Speaker： Sheng Ding (丁盛)
12:00	**Lunch (午餐)**
Afternoon (下午)	**Concurrent Sessions (专题报告)**
13:30-15:10 **Concurrent Session I** **(专题报告一)：** **Stem Cell Pluripotency & Development** Chairpersons(主持人)： John Yu	1、Title：Aberrant Gene Expressions and Fgf3-Fgfr2 Signaling in Parthenogenetic Preimplantation Embryos Speaker：John Yu
	2、Title：Pluripotency of Induced Pluripotent Stem Cells Speaker：Shaorong Gao (高绍荣)
	3、Title：Functional Role of Myostatin-induced Gene X in Regulating Muscle Stem Cell Activation and Muscle Regeneration in Mice Speaker：Dahai Zhu (朱大海)

Shaorong Gao (高绍荣) （Each talk 20 mins, including 5 mins discussion)	4、Title：New Insights into the Epigenetic Regulation of Stem Cell Pluripotency by Polycomb Proteins Speaker：Xiaohua Shen (沈晓骅)
	5、Title：Interaction of Mouse Embryonic Stem Cells with Feeder Cells at the Ultrastructural Level Speaker：Ping Xia
13:30-15:10 **Concurrent Session II (专题报告二): Stem Cell Therapeutics** Chairpersons(主持人)： Linsong Li(李凌松) Daohong Zhou(周道洪) (Each talk 20 mins, including 5 mins discussion)	1、Title：Therapeutic Uses of Different Sources of Stem Cells in Patients with Critical Limb Ischemia: Mechanisms of Action Speaker：Zhongchao Han (韩忠朝)
	2、Title：Inhibition of S1P Signaling Blocks Migration of Mouse Mesenchymal Stem Cells and Improves the Condition in Chemically Induced Fibrotic Liver Speaker：Lingsong Li (李凌松)
	3、Title：Development of BC1 - A Therapeutic Cellular Product for the Treatment of Microvascular Complications of Diabetes Speaker：Yael Porat
	4、Title ：The Roles of Biomechanics Based on Bio-inspired Environments for the Stem Cell Research Speaker：Jung-Woog Shin
	5、Title：Ethical and Governance Issues in Stem Cell Research and Clinical Application in China Speaker：Xiaomei Zhai(翟晓梅)
15:10-15:30	**Coffee Break and Poster Viewing (茶歇和海报)**
15:30-17:10 **Concurrent Session III (专题报告三): Blood Cell Development** Chairpersons(主持人)： Xuetao Pei(裴雪涛) Zack Wang (王征宇) (Each talk 20 mins, including 5 mins discussion)	1、Title：Translating Human Pluripotent Stem Cell Research into Clinical Products Speaker：Shijiang Lu (卢世江)
	2、Title：Generation of Functionally Mature Blood Cells from Human Pluripotent Stem Cells Speaker：Feng Ma (马峰)
	3、Title：Regulatory Roles of miRNAs in Erythroid Differentiation Speaker：Xiao Hu (胡晓)
	4、Title：FEV, a Novel ETS Transcription Factor, is Required for Hematopoietic Stem Cell Development Speaker：Feng Liu (刘峰)
	5、Title：Hemangioblast Populations Derived from Baboon Pluripotent Stem Cells Speaker：Qiang Shi
15:30-17:10 **Concurrent Session IV**	1、Title：TBD Speaker：Yilin Cao (曹谊林)

(专题报告四): **Stem Cell Tissue Engineering** Chairpersons(主持人): Yilin Cao(曹谊林) Qi-Qun Tang(汤其群) (Each talk 20 mins, including 5 mins discussion)	2、Title：Construction of Implantable Stem Cell-Derived Hepatocytes/Liver Tissue Speaker：Yunfang Wang (王韫芳)
	3、Title：Study of Transgenic Rhesus Monkeys Speaker：Weizhi Ji (季维智)
	4、Title：Commitment of Pluripotent Stem Cells to Adipocyte Lineage Speaker：Qi-Qun Tang (汤其群)
	5、Title：The Current Status of Cord Blood Stem Cell Banking and Transplanation Speaker：Lugui Qiu (邱录贵)
17:15-18:15	**Poster Session With Presenter (海报交流)**
18:30-20:00	**Welcome Reception (欢迎宴会)**

11/13/2010, Saturday (周六)	
Morning (上午)	**Concurrent Sessions (专题报告)**
8:30-10:10 **Concurrent Session V (专题报告五): Cardiovascular Differentiation & Immune Regulation of Stem Cells** Chairpersons(主持人): Yong-Guang Yang(杨永广) Yufang Shi(时玉舫) (Each talk 20 mins, including 5 mins discussion)	1、Title：Regulation of ES Derived-Cardiomyocytes by Endothelial Cells Speaker：Zack Wang (王征宇)
	2、Title： Dedifferentiation and Cell Cycle Reprogramming of Mouse Cardiomyocytes into Cardiac Progenitor Cells Speaker： Charles Wang
	3、Title：Direct Differentiations of Atrial and Ventricular Myocytes from Human Embryonic Stem Cells Speaker：Yue Ma (马跃)
	4、Title： Mesenchymal Stem Cells: A Double-edged Sword in Regulating Immune Responses Speaker： Yufang Shi (时玉舫)
	5、Title：Macrophages Prevent Human Red Blood Cell Reconstitution in Immunodeficient Mice Speaker：Yong-Guang Yang (杨永广)
8:30-10:10 **Concurrent Session VI (专题报告六): Hematopoietic Stem Cells** Chairpersons(主持人): Gerald de Haan Zhenyu Ju(鞠振宇)	1、Title：An Innate Immune Stimulation with Polyinosinic: Polycytidylic Acid Attenuates Total Body Radiation-Induced Hematopoietic Genetic Instability Speaker：Daohong Zhou (周道洪)
	2、Title：Slug Regulates Hematopoietic Stem Cell Regeneration under Stress Conditions Speaker：Wen-Shu Wu (吴文书)
	3、Title：Puma Dependent Apoptosis Checkpoints in Telomere Dysfunctional Mice Speaker：Zhenyu Ju (鞠振宇)

(Each talk 20 mins, including 5 mins discussion)	4、Title：Identification of Secreted Factors Inducing Hematopoietic Stem Cell Self-Renewal Speaker：Eric Deneault
	5、Title：Definitive Gene Expression Profiling of Hematopoietic System by Gene Expression Commons Platform Speaker：Jun Seita
10:10-10:30	**Coffee Break (茶歇)**
10:30-12:10 **Concurrent Session VII (专题报告七): Neural & Skin Differentiation from Stem Cells** Chairpersons(主持人)： Kursad Turksen Qin Shen(沈沁) (Each talk 20 mins, including 5 mins discussion)	1、Title：Use of Embryonic Stem Cells(ESC) to Delineate the Epidermal Lineage Speaker：Kursad Turksen
	2、Title：Region-specific Neurons Differentiated from Human iPS Cells Speaker：Renhe Xu (徐仁和)
	3、Title：Tracking of Neural Stem Cells and iPS Cells in Human and Monkey CNS Speaker：Jianhong Zhu (朱剑虹)
	4、Title：Neural Stem Cells Ensconced in the SVZ niche Speaker：Qin Shen (沈沁)
	5、Title：High Efficiency Induction of Neural Conversion in hESCs and hiPSCs with a Single Chemical Inhibitor of TGF- Superfamily Receptors Speaker：Jiaxi Zhou (周家喜)
10:30-12:10 **Concurrent Session VIII (专题报告八): Stem Cells in Diseases** Chairperson(主持人)： Nadia Carlesso Cheng-Kui Qu(瞿成奎) (Each talk 20 mins, including 5 mins discussion)	1、Title：Abnormal Regulation Of Hematopoietic Stem Cell Differentiation And Self-Renewal During Acute Inflammatory Stress Speaker：Nadia Carlesso
	2、Title：Non-Lineage Restricted Effects of a Gain-of-Function Mutations in Tyrosine Phosphatase Ptpn11(Shp2) on Leukemia Stem Cell Development Speaker： Cheng-Kui Qu (瞿成奎)
	3、Title：Characterization and Suppression of Pten Null Leukemia Stem Cells Speaker：Wei Guo (郭伟)
	4、Title：Hematopoiesis Suppression in the aGVHD Condition: Intrinsic or Extrinsic? Speaker：Jianmin Wang (王健民)
	5、Title：Marrow Microenvironment and Pathogenesis of Hematopoietic Defects in Murine Model of Fanconi Anemia Speaker：Feng-Chun Yang (杨逢春)
12:15	**Lunch (午餐)**
Afternoon (下午)	**Plenary Session (大会报告)**
13:30-15:10 **Plenary Session II (大会报告二)** Chairpersons(主持人)：	1、 Title：Hematopoietic Stem Cells in the Hypoxic Niche Speaker：Toshio Suda
	2、 Title：Targeting Dormant Drug-Resistant Cancer Stem Cells Speaker：Linheng Li (李凌衡)

Yu Zhang (张愚) Linzhao Cheng(程临钊) (Each talk 25 mins, including 5 mins discussion)	3、Title：Novel Molecular Pathways Regulating the Function of Leukemia Stem Cells Speaker：Shaoguang Li (李少光)
	4、Title：Balancing Self-Renewal and Differentiation in Stem Cells Speaker：Ting Xie (谢亭)
15:10-15:30	**Coffee Break (茶歇)**
15:30-17:10 **Plenary Session III (大会报告三)** Chairpersons(主持人)： Toshio Suda Armand Keating (Each talk 25 mins, including 5 mins discussion)	1、Title：TBD Speaker：Qi Zhou (周琪)
	2. Title：Generation and Regeneration of Pancreatic Beta Cells Speaker：Hongkui Deng (邓宏魁)
	3、Title：Human iPS And Blood Cells: Gene Targeting and Disease Modeling Speaker：Linzhao Cheng (程临钊)
	4、Title：Stem Cell Tourism: Hope Without Reason? Speaker：Douglas Sipp
17:10-17:25	**Closing Remarks (闭幕式)**
17:30-18:30	**Roundtable Discussions on International Collaborations on Stem Cell Medicine**
18:30-20:00	**Dinner (自助晚餐)**

Keynote Presentation

A Brief Introduction of Irving L. Weissman, M.D.

Irving L. Weissman, M.D., is the Director of the Institute for Stem Cell Biology and Regenerative Medicine at Stanford and Director of the Stanford Ludwig Center for Stem Cell Research. Dr. Weissman has many first discoveries to his credit that opened fields in which he has participated. His most important achievement is developing the general method to identify and to isolate stem and progenitor cells. Using that method he and his lab and collaborators were first to isolate prospectively any stem cell from any tissue in any species. That was accomplished first by isolation of mouse blood-forming (hematopoietic) stem cells (HSC), followed by the human HSC. Dr. Weissman is a member of the National Academy of Sciences, the Institute of Medicine at the National Academy, the American Association of Arts and Sciences and the American Academy of Microbiology. He has published numerous papers that impacted the whole stem cell research field. Throughout his career, He has received numerous awards including the Outstanding Investigator Award from the National Institutes of Health, the Pasarow Award in Cancer Research, the Irvington Institute Immunologist of the Year Award, the California Scientist of the Year Award, the J. Allyn Taylor International Prize in Medicine etc.

Plenary session, Keynote speech: Friday November 12, 2010, 9.00-9:50

Normal and Neoplastic Stem Cell

Irving L. Weissman, M.D.

Director，Institute for Stem Cell Biology and Regenerative Medicine at Stanford University. Director, the Stanford Ludwig Center for Stem Cell Research

Following embryonic development, most of our tissues and organs are continuously regenerated from tissue/organ specific stem cells. The principal property that distinguishes such stem cells from their daughter cells is self-renewal; when stem cells divide they give rise to stem cells (by self-renewal) and progenitors (by differentiation). In most tissues only the primitive stem cells self-renew. Stem cell isolation and transplantation is the basis for regenerative medicine. Self-renewal is dangerous, and therefore strictly regulated. Poorly regulated self-renewal can lead to the genesis of cancer stem cells, the only self-renewing cells in the cancerous tumor.

Oral Presentations

Plenary session I: Friday November 12, 2010, 10:10-11:50

Modulation of Stem Cell Function by Cellular Components of The Hematopoietic Niche

Edward F. Srour[1,2,3] , Brahmananda R. Chitteti[1], Ying-Hua Cheng[4], Sonia Rodriguez-Rodriguez[3], Nadia Carlesso[3], and Melissa A. Kacena[4,5]

Departments of Medicine[1], Pediatrics, Herman B Wells Center for Pediatric Research [2], Microbiology and Immunology[3], Orthopaedic Surgery[4], and the Department of Anatomy and Cell Biology[5], Indiana University School of Medicine, Indianapolis, IN

Hematopoietic stem (HSC) and progenitor (HPC) cell fate is governed by intrinsic and extrinsic parameters. Most of the extrinsic parameters are encountered by HSC in the hematopoietic niche in the form of interactions with other cellular components of the niche or with soluble factors. Osteoblasts (OB) play a critical role in HSC function and self-renewal. At present, it is widely believed that HSC associated with the endosteal region have high proliferative and repopulating capacities. We examined the impact of hematopoietic niche elements on HSC and HPC function by analyzing the combined effect of OB and stromal cells (SC) on Lineage-Sca-1-CD117- (LSK) cells. CFU expansion and marrow repopulating potential of cultured LSK cells were significantly higher in OB compared with SC cultures, thus corroborating the importance of OB in the competence of the hematopoietic niche. OB-mediated enhancement of HSC and HPC function was reduced in co-cultures of OB and SC, suggesting that SC suppressed the OB-mediated hematopoiesis-enhancing activity. Expression of *Notch 2*, *Jagged* 1 and 2, *Delta* 1 and 4, *Hes* 1 and 5, and *Deltex* was increased in OB cultures and suppressed in SC and OB/SC cultures suggesting that stromal cells antagonize OB-mediated enhancement of hematopoiesis by negatively modulating Notch signaling. Further analysis of the suppressive effects of SC revealed that this activity is mediated by adipocytes through the upregulation of Neuropilin-1, a co-receptor to a tyrosine kinase receptor. In order to phenotypically identify cells of the osteoblastic lineage mediating this hematopoiesis enhancing activity (HEA), we fractionated OB based on the expression of lineage markers (CD45, CD31, and Ter119), Sca1, ALCAM (CD166), osteopontin (OPN), CD90, and CD44. Isolated cells were examined by classical OB functional assays (Ca deposition

and Alkaline Phosphatase (ALP) activity) and by QRT-PCR quantification of OB-specific lineage markers (Runx-2, osteocalcin, and type I collagen) and were assessed for their HEA in co-cultures with marrow-derived LSK cells. While we were able to identify Lin-Sca1-OPN+ALCAM+ as less mature OB in contrast to the more mature Lin-Sca1-OPN+ALCAM- cells, these fractionations did not compartmentalize the HEA and both groups of cells had comparable OB functional properties and expressed similar levels of Runx-2 and osteocalcin. These populations were then further separated based on CD44 and CD90 expression. Using this strategy we found that Lin-Sca1-OPN+ALCAM+CD44+ CD90+ cells exhibit a >5-fold increase in Runx2 expression compared to their fractionated counterparts that did not express ALCAM, with a concomitant >4.6-fold increase in the sustained proliferation and production of primitive hematopoietic cells. The selective expression of Runx2 by ALCAM+ cells and the fact that this marker was lost in culture over time, strongly suggest that ALCAM+ cells are more immature elements of the osteoblastic lineage and illustrate that these markers are excellent tools to identify and characterize classes of mature and immature OB. These data illustrate that OB promote the in vitro maintenance of hematopoietic functions, including in vivo repopulating potential by up-regulating Notch-mediated signaling between HSC and OB and that stromal cells, especially adipocytes can antagonize the supporting activity of OB. Furthermore, these studies begin to define the hierarchical organization of osteoblastic cells and identify cells with high Runx2 activity as the class of OB mediating the highest level of hematopoiesis enhancing activities.

Aging of hematopoietic stem cells: a program or noise?

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Hematopoietic stem cells are capable to sustain the production of multiple distinct blood cells throughout the lifetime of an organism. At the same time, their self-renewal potential allows maintenance of stem cell numbers. Yet, numerous studies have indicated that changes in both stem cell pool size and functional activity are evident during normal aging. Our previous studies have indicated that these age-dependent phenotypes are mouse-strain dependent, and suggest the presence of a genetic component to stem cell aging.

Efforts to identify a “HSC aging signature” by comparing gene expression profiles of HSCs purified from pooled groups of young and old mice have resulted in surprisingly little overlap. While this may be in part due to technical variables, it is also possible that a unique aged stem cell profile does not exist. Rather, age-related changes may arise as a result of stochastic changes, in combination with cellular (darwinian) selection of stem cell clones over time. One approach to resolve this issue is to determine whether HSC aging occurs similarly from mouse to mouse, and from strain to strain. Therefore, we measured a variety of physiological and HSC-specific parameters in 20 individual young and 40 individual old C57Bl/6 or DBA/2 mice. “LT-HSCs” (defined as CD34-Lin-Sca+cKit+ CD48- CD150+EPCR+) and “progenitors” (defined as CD34+Lin-Sca+cKit+ CD48+CD150-) were purified from each mouse As expected, there was little variation in the frequency of phenotypically defined LT-HSCs in young mice. In contrast, marked variation was observed in individual old mice, ranging from no change to a 20-fold increase. To measure functional frequency, we performed multiple in vitro and in vivo stem cell assays. “Progenitors” purified from old or young mice had a similar functional frequency, while “LT-HSCs” from old mice had a consistently lower functional frequency than “LT-HSCs” from young mice. Combining the phenotypic and functional measurements from each mouse revealed that the frequency of functionally defined HSCs increased with age an average of 3-fold. However, this coincided with an extensive mouse-to-mouse variability, ranging from a 2-fold decrease to a 10-fold increase. Global gene expression analysis was performed on LT-HSCs and progenitors from the individual C57Bl/6 mice, and analysis of this data is ongoing. We expect this to

reveal genes that correlate with the functional parameters tested. Most importantly, our data should allow to determine whether a specific stem cell-aging signature actually exist, or whether transcriptional infidelity (generating random noise), is a hallmark of stem cell aging and could contribute to demise of stem cell function.

Overcoming Challenges to the Enhancement of Translational and Clinical Research in Cellular Therapy

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Despite considerable international activity in the area of regenerative medicine, delays continue at each step, from discovery research to the final implementation of early phase clinical trials. Challenges include: the lack of appropriate sources of funding for non-discovery research, especially for pre-clinical, scale-up and validation studies; the need for extended periods of funding to ensure progression of studies from pre-clinical models to the submission of biologics license applications (BLAs) to government agencies for regulatory approval prior to initiation of clinical trials; selection of the most appropriate animal models for particular indications, including small versus large animals; the choice of allogeneic versus autologous sources and of stem versus progenitor versus differentiated cell types; the identification of potency assays for the cells administered; the tracking of introduced cells *in vivo* in real time; the development of more appropriate clinical trials platforms than those based on the classical anti-cancer drug model; monitoring for long-term adverse effects; and the relative lack of involvement of industry in supporting translational and clinical research in cellular therapy. These issues appear to be common to many jurisdictions, including north America and Europe, and have been the subject of workshops conducted under the auspices of the US National Institutes of Health and the American Society of Hematology. This presentation will focus on potential solutions to overcoming the challenges outlined and offers an approach to the enhancement of translational and clinical research in cellular therapy.

A Chemical Approach to Controlling Cell Fate

Sheng Ding

The Scripps Research Institute

Recent advances in stem cell biology may make possible new approaches for the treatment of a number of diseases. A better understanding of molecular mechanisms that control stem cell fate as well as an improved ability to manipulate them are required. Toward these goals, we have developed and implemented high throughput cell-based phenotypic screens of arrayed chemical and gene libraries to identify and further characterize small molecules and genes that can control stem cell fate in various systems. This talk will provide latest examples of discovery efforts in my lab that have advanced our ability and understanding toward controlling stem cell fate, including self-renewal, survival, differentiation and reprogramming of pluripotent stem cells.

Session I：Stem Cell Pluripotency & Development: Friday November 12, 2010, 13:30-15:10

Aberrant gene expressions and Fgf3-Fgfr2 signaling in parthenogenetic preimplantation embryos

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Parthenogenetic mammalian embryos (parthenotes) are reported to have abnormal development of both embryonic and extraembryonic lineages. We found that parthenote morulae and blastocysts contained decreased numbers of cells expressing the epiblast-specific transcription factors *Sox2* and *Nanog*, but increased numbers of cells expressing the primitive endoderm-specific transcription factor *Gata4*. These results indicate a diminished epiblast population and an expanded primitive endoderm population in the parthenote inner cell mass (ICM), consistent with the tendency of parthenote ICM cells to differentiate into primitive endoderm derivatives. In order to account for the marked increase of Gata4 expression in parthenotes, we demonstrate for the first time that this may be explained by the strong up-regulation of Fgf3 expression and Fgfr2 phosphorylation. In agreement with decreased total cell numbers in parthenogenetic blastocysts, up-regulated *Fgf3* expression in parthenotes was predominant in the nucleoli, where Fgf3 inhibits cell proliferation. Interestingly, inhibition of Fgfr2 phosphorylation by SU5402 restored normal *Nanog* and *Gata4* expressions without affecting *Fgf3* expression, indicating that *Gata4* expression was up-regulated by Fgfr2 signaling instead of Fgf3 per se. In parthenote trophectoderm, we detected normal *Cdx2* expression but ectopic *Gata4* and reduced *Tbr2*(*Eomes*) expressions, as indicated by significantly decreased numbers of $Tbr2^+$ cells. Interestingly, the trophectoderm defects of parthenote embryos were similar to those of *Tbr2-/-* mice, suggesting that reduced *Tbr2* expression contributes to the trophectoderm defects. Taken together, our work provides insight into the molecular mechanisms of the developmental defects of parthenogenetic embryos in both the trophectoderm and ICM, which had not been clarified before.

Pluripotency of induced pluripotent stem (iPS) cells

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Ectopic expression of four transcription factors including Oct4, Sox2, Klf4 and c-Myc in differentiated fibroblast cells could reset the cell fate of fibroblast cells to pluripotent state. Subsequently, fully pluripotency of these so-called induced pluripotent stem cells (iPSCs) has been demonstrated as viable mice could be generated autonomously from iPS cells through tetraploid blastocyst complementation. Moreover, the generation of human and patient-specific iPS cells has raised the possibility of utilizing iPS cells clinically. However, the utilization of c-Myc in iPS cells induction greatly increased the incidence of tumorigenecity in the iPS-chimeric mice and also might hinder the clinical application of human iPS cells in the future. Fortunately, c-Myc has been recently found dispensable for iPS induction even though the iPS induction efficiency is greatly reduced in the absence of c-Myc. However, it remains unknown if these three factors-induced iPS cells are fully pluripotent. In the present study, we have successfully demonstrated that 3-factor iPS cells could also be fully pluripotent as viable mice could be generated from 3-factor iPS cells autonomously via tetraploid complementation and moreover, our data indicated that the pluripotency regulatory mechanism in 3-factor iPS cells might be distinct from 4-factor iPS cells.

New Insights into the Epigenetic Regulation of Stem Cell Pluripotency by Polycomb Proteins

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Epigenetic mechanisms control stem cell differentiation and organogenesis by influencing chromatin structure and gene expression programs. Epigenetic deregulation contributes to disease. Polycomb group (PcG) proteins are central players in mammalian development, and are often linked to human cancer. An initial step in PcG regulation of gene expression is the targeting of a protein complex named Polycomb repressive complex 2 (PRC2) to specific regions of chromatin to establish the repressive trimethylation mark on histone H3 lysine 27 (H3K27me3). H3K27me3 and PRC2 have been implicated in maintaining the pluripotent state of embryonic stem (ES) cells. To study the function and regulation of PRC2 in the epigenetic regulation of stem cell pluripotency, we constructed a core PRC2 interaction network in ES cells. We identified EZH1 as an alternative H3K27 methyltransferase, and JMJ (JUMONJI or JARID2) and MTF2 (or PCL2) as novel regulators of PRC2. We demonstrated critical roles for PRC2 and its associated proteins in execution of stem cell pluripotency. Our work highlights the importance of chromatin dynamics in cellular differentiation, and suggests greater complexity in the composition of the Polycomb repressive complex 2 (PRC2), which may render epigenetic specificity during cell-fate transitions.

Interaction of Mouse Embryonic Stem Cells with Feeder Cells at the Ultrastructural Level

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Mouse embryonic stem (ES) cells grow better with feeder cells in culture compared to feeder free culture systems. Details on how ES cells interact with feeder cells at an ultrastructural level have never been reported due to technical difficulties. In this study, we revealed the ultrastructure of mouse ES cells and adjacent fibroblast feeder cells using transmission electron microscopy. Mouse ES cells were derived from inner cell mass of 3.5 days old blastocysts of C57BL6/129svj F1 hybrid strain, passage 8, and were cultured with feeder cells which were irradiated mouse embryonic fibroblast cells. After 2-3 days in culture, the ES cells and feeder cells were fixed and processed for observation of the ultrastructure. The results showed that mouse ES cells in the center of the colony appeared to be heterogeneous in morphology. In contrast, ES cells attaching to feeder cells are more homogeneous. The protrusion of the feeder cells forms tight junctions and desmosomes with the ES cells. The feeder cells contained far more mitochondria and rough sarcoplasmic reticulum than those in the ES cells. The morphology of mitochondria was different in feeder cells compared to those in the ES cells. Our study, for the first time, provided morphological evidence at an ultrastructural level that feeder cells play an important role in maintaining the pluripotent status of ES cells in culture.

Session II: Stem Cell Therapeutics: Friday November 12, 2010, 13:30-15:10

Therapeutic uses of different sources of stem cells in patients with critical limb ischemia: mechanisms of action

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It has been shown that the critical limb ischemia (CLI) could be improved by transplantation of autologous bone marrow mononuclear cells (BM-MNC) G-CSF-mobilized peripheral blood mononuclear cells (M-PBMNC) or allogeneic bone marrow mesenchymal stem cells. However, little information is available to assess which is the better option. This prospective study presents the results of intramuscular implantation of autologous G-SCF-mobilized peripheral blood mononuclear cells and dual intramuscular and intravenous transplantation of allogeneic placenta-derived mesenchymal stem cells for the treatment of patients with CLI in whom amputation was considered the only viable treatment option. Success was defined as meeting the following four criteria: improvement in ABI measurements; relief of rest pain; ulcer healing, if applicable; and absence of major limb amputations. Patients not undergoing major limb amputations continued to be monitored for subsequent procedures. One hundred fifty patients with CLI were randomized to three groups: group A (76 cases implanted with M-PBMNC), group B (74 cases implanted with BM-MNC) and group C (6 cases treated with allogeneic placenta derived MSC) followed up for 12 weeks. Clinical outcomes indicate that all three treatments were safety and efficacy based on analysis per protocol. Significant improvement of the main clinical index was observed in all groups after transplantation. No transplantation-related complication was observed in these groups. Comparative analysis revealed that at 12 weeks after cell implantation, improvement of ABI (difference 0.06 +/- 0.01; $p < 0.0001$), skin temperature (difference 0.55 +/- 0.25; $p = 0.028$), and rest pain (difference -0.57 +/- -0.15; $p< 0.0001$) was significantly better in group A patients than group B patients. However, there was no significant difference among three groups for pain-free

walking distance, transcutaneous oxygen pressure, ulcers, and rate of lower limb amputation. These data indicate the use of stem cells either from bone marrow, G-CSF-mobilized PBMNC or allogeneic placenta as a means of limb salvage therapy for patients with CLI shows promise in avoiding amputation in a patient population currently presented with few alternatives to amputation. The mechanisms of action of stem cells in treating CLI were also addressed.

Inhibition of S1P signaling blocks migration of mouse mesenchymal stem cells and improves the condition in chemically induced fibrotic liver

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We have previously reported that sphingosine 1-phosphate (S1P) regulates the migration of mesenchymal stem cells (MSCs) from the mouse bone-marrow into the damaged liver. Using green-color MSCs isolated from GFP-transgenic mice, here we show that inhibition of S1P signaling by FTP720, a functional inhibitor for S1P receptors, S1P1 and S1P3, significantly blocks migration of mouse MSCs and improves conditions in chemically induced fibrotic liver. Our study will bring cautious for the safety of MSC transplantation, and provide S1P as a new target for potentially treating liver fibrosis.

Development of BC1 - a therapeutic cellular product for the treatment of microvascular complications of diabetes

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Both types of diabetes result in devastating morbidity and mortality due to vascular blockage leading to gradual loss of affected organs, such as kidney failure, coronary vascular diseases engendering myocardial infarctions and strokes, critical limb Ischemia (CLI) and amputations, neuropathy and retinopathy. There is no fully effective treatment against these complications.
An innovative approach for treatment of these and other vasculopathies is stem/progenitor cells obtained from patients' peripheral blood. This method avoids the risks and discomfort associated with existing sources of cells such as bone-marrow, fat or G-CSF-mobilized blood.
Applying specific purification and a phased direction of activation/ differentiation of stem/progenitor cells in culture, a cell population named BC1 was produced. BC1 contains a significant number (0.3+/-0.04x10^6/ml blood) of highly viable cells (97+/-0.3%) composed of a mixture of 33.5+/-5.3% endothelial-progenitor-cells and 19.8+/-4.3% multipotent-adult-stem/ progenitor-cells. BC1 was found safe and effective in animal models of hind limb ischemia and retinopathy. Recovery of blood supply to ischemic mouse leg caused by double ligations and excision of the thigh artery demonstrated using laser Doppler flow measurement and of visual function in RCS model using ERG.
BC1, designed as a treatment of vascular insufficiency in diabetic patients, is being developed for a phase I/II study in CLI patients.

The roles of biomechanics based on bio-inspired environments for the stem cell researches

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Sophisticated methodologies and outcomes have introduced to the areas of stem cell researches during past decades. However, most methodologies and related outcomes are based on the pure biology and biochemistry, especially focused on the biochemical reagents. Recently various potential methodologies are being introduced in these areas in relation to bio-inspired environments. Among them the roles of biomechanics and substrate materials properties are currently being focused. In this lecture, various recent methodologies based on biomechanics are to be presented for the control of stem cell differentiations. Specifically the potential methodologies in the control of mesenchymal stem cell differentiation utilizing various biomechanics-based bioreactors and their outcomes will be presented. In addition non-invasive techniques in the analysis for the evaluation of stem cell differentiation shall be introduced. Also, potential research target areas to bridge biology and biomechanics to explain mechano-biology for the application to stem cell researches.

Ethical and Governance Issues in Stem Cell Research and Clinical Application in China

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Stem cell research has been developed in China in a cultural context different from western countries. Among the differences whether human embryo enjoys personal status is primary. With huge investment from the government and conscientious work by scientists, great progress has been made in stem cell research. However, with the scientific and technological breakthroughs, there are new emerging issues both in ethics and governance which need to be dealt with , such as hybrids and chimeras, parthenogenesis, genetic modification, iPS cells, artificial gametes from adult cells, PGD and pre-implantation tissue typing, designer babies, translation of stem cell science from bench to bedside, stem cell tourism, and oocyte trading over Internet. On the other hand there are regulatory gaps both in the regulations on stem cell research and in their implementation. So the Ethical Guiding Principles for Human Embryonic Stem Cell Research which promulgated by MOST/MOH in 2003 should be updated. Now more urgent ethical and governance issue in China is that there are a great number of health institutions independently or in collaboration with biotech companies are conducting unproven and unregulated adult stem cell therapies. Many of them did not conduct pre-clinical studies and clinical trials, did not provide adequate and complete information to patients, did not do follow-up, but charge too much costs to patients. Although in December 2009 MOH in Regulation on Clinical Application of Medical Technologies classifies stem cell therapy as the III type of medical technologies which needs to be approved by MOH, and its implementation met a lot of challenges. Ethics Committee of MOH recently drafted the Ethical Guidelines on Clinical Trials and Clinical Application of Human Adult Stem Cells. In these Guidelines, any clinical application of human adult stem cells should not made until positive results of clinical trials on safety and efficacy, and before clinical trials positive results of the pre-clinical studies should be obtained. Both clinical trials and clinical application have to be

approved by provincial or municipal health care administration. Under certain special conditions human adult stem cell therapy can be used as innovative therapy or experimental treatment in response to patient’s insistent request.

Session III: Blood Cell Development: Friday November 12, 2010, 15:30-17:10

Translating human pluripotent stem cell research into clinical products

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Human embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSC) represent a new source of stem cells that can be propagated and expanded in vitro indefinitely, providing a potentially inexhaustible and donorless source of cells for human therapy. For hESCs, the ability to create banks of cell lines with matched or reduced incompatibility could potentially decrease or eliminate the need for immunosuppressive drugs. Inasmuch as hiPSCs could potentially be produced from a patient's own tissues, cells derived from such hiPSC lines would perforce be histocompatible with the patient. Retinal pigment epithelial cells (RPE) derived from hESCs under GMP have been shown to significantly improve the vision of RCS rats. Histological examination demonstrated extensive photoreceptor rescue 5-7 cells deep in outer nuclear layer. ACT has filed an IND for the treatment of Stargardt's Macular Degeneration using hESC-RPE cells last year. We have developed a robust method to generate functional erythroid cells and megakaryocytes (MKs) from hESCs on a clinical relevant scale under feeder and serum-free conditions. Erythroid cells derived from hESCs possessed oxygen-transporting capacity comparable to normal red blood cells (RBC) and responded to changes in pH and 2,3-DPG. These cells underwent a progressive decrease in size, chromatin condensation and extrusion of the pycnotic nucleus to form enucleated erythrocytes, and over 15% of them expressed the adult β-globin chain. MKs derived from hESCs expressed CD41a, CD42a and CD42b, and underwent endomitosis and formed mature polyploid MKs. Upon further maturation these cells generated platelets that can be activated by thrombin stimulation and able to spread on fibrinogen and vWF surfaces. The platelets also formed micro-aggregates, and facilitated clot formation and retraction comparable to normal human platelets. Our results show hESC-derived RBCs and platelets are functional and represent an important step towards generating an unlimited supply of blood substitutes for human transfusion.

Generation of functionally mature blood cells from human pluripotent stem cells

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The clinical utilization of human embryonic stem (hES) and induced pluripotent stem (iPS) cells is based on whether they can generate terminally mature progenies with normal function. We recently developed an efficient method to produce hematopoietic progenitors from hESCs by coculture with mouse fetal hematopoietic niche-derived stromal cells (Fetal liver & AGM region). The large-scale production of hESCs-derived erythroid progenitors with high purity enabled us to analyze the development of hESC-derived erythrocytes and their function. The mature erythrocytes showed high proportion of β-globin expression and could function as oxygen carrier, providing a novel potential source for therapeutic transfusion. More recently, we achieved large production of mature mast cells (MCs) and eosinophils from hESC/iPSCs, both cell types playing central roles in allergic diseases and multiple immuno-responses. The hESC/iPSC-derived MCs are of a tryptase+chymase+ phenotype from an early stage. They perform mature function in response to IgE receptor, substance P and compound 48/80 to release histamine, identical to human MC-TCs. On the other hand, hESC/iPSC-derived mature eosinophils not only showed a mature phenotype, but also could exert function through stimulation of secretory IgA to release EDN. Since the early development of both human MCs and eosinophils remains largely blank, our study may highlight a new understanding for MC and eosinophil development and finally benefit novel therapies for related disorders.

Regulatory roles of miRNAs in erythroid differentiation

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MicroRNAs (miRNAs) are a class of small, non-coding RNA molecules that regulate gene expression through binding to their targeted mRNAs. MiRNAs play key roles in many cellular pathways including hematopoiesis. Aberrant expression of miRNAs has been found associated with many diseases, including various hematological malignancies. We have focused on studying the regulatory roles of miRNAs in erythropoiesis, the processes that involve red cell production, with an *in vitro* erythroid culture system. Based on a microarray technique, we have found that expression of miRNA in erythroid cells is temporally regulated which reflects the developmental stage and cellular properties of corresponding erythroid cells. Our functional studies have identified miRNAs that can increase fetal gamma-globin gene expression in adult CD34+ hematopoietic stem / progenitor cells (HSPC) derived erythroid cells and adult beta-globin gene expression in fetal CD34+HSPCs derived erythroid cells, respectively. miRNA target gene searching has identified cell cycle regulatory genes (CDKs) and hematopoietic regulatory genes (GATA-1) as targets. Future studies will be aimed at fine delineating the targeted genes and genetic pathways associated with these miRNAs. In parallel, we will investigate the applications of those miRNA in the diagnosis and treatment of erythroid disorders, such as the sickle cell anemia and polycythemia vera, and in directing adult erythroid differentiation of human embryonic stem cells and iPS cells.

FEV, a Novel ETS Transcription Factor, is Required for Hematopoietic Stem Cell Development

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Summary: The gene *fev* (Fifth Ewing Variant) is a novel ETS family member, which acts as a transcription repressor and is expressed in serotonergic neurons in mammals. However, its role in hematopoiesis is unknown. We have characterized the expression pattern of *f*ev in zebrafish and found it is expressed in hematopoietic and vascular endothelial cells during early development. Loss-of-function assay using antisense morpholinos (MO) showed that all the HSC markers were decreased and no T cells were found in the thymus in the *fe*v-deficient embryos. Further analysis demonstrated that artery-vein differentiation was disrupted as well in the *fev*-deficient embryos. TUNEL and BrdU essays showed that the HSC defects were not caused by iregular apoptosis and cell proliferation. Finally, by combining genetic analysis and chemical treatment, we have showed that Fev regulates HSC development through VEGF-Foxc1-Notch pathway. Taken together, our data reasoned that the ETS transcription factor, Fev, is an important regulator in HSC development.

Hemangioblast Populations Derived from Baboon Pluripotent Stem Cells

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Hemangioblast is a common precursor that commitment to the hematopoietic and endothelial lineages. Hemangioblast can be extracted from embryonic stem cell cultures and manipulated by a combinational cocktail of cytokines to differentiate along either hematopoietic or endothelial line. To generate hemangioblast cells from baboon pluripotent stem cells, we established a protocol in our laboratory. Briefly, entire colonies were loosely detached from feeder cells and transferred into feeder-free, AggreWell™ plates. 5,000–10,000 cells were expected per embryoid body, and the cells were cultured for 5–7 days. To induce hemangioblast differentiation, ESCs were cultured without feeder cells in hemangioblast differentiation medium containing 5% Knockout - serum replacement, supplemented with a cocktail of 0.5 ng/mL BMP-4, 5 ng/mL bFGF, 10 ng/mL VEGF, 5 ng/mL stem cell factor, 5 ng/mL Tpo, 10 ng/mL Fit3 ligand in DMEM. ESC-derived hemangioblast cells present cobblestone morphology under the microscope (100X) from day 3 to day 9. If ESC-derived hemangioblast cells at day 3 are cultured in EGM-2 medium, they form tubular and branching networks in vitro. The table indicated the characteristics of phenotypic expressions of several hemangioblast markers in pluripotent ESCs, in ESC-derived cells after 3 days differentiation, in ESC-derived cells after 9 days differentiation. Our results suggested that there exist many putative hemangioblst populations using this protocol. Based on phenotypic characteristics by flow cytometry, we postulated the following populations to be putative hemangioblast cells and will use them in this research. (1) $CD31^+$ at day 3; (2) $CD34^+$ at day 9; (3) $CD31^+/CD34^+$ at day 9; (4) $CD117^+/CD34^+$ at day 3; (5) $VEGFR3^+$ at day 3; (6) $CXCR4^+/UEA\text{-}1^+$ at day

3; (7) $CD117^{+}$ at day 3. Further investigations are needed to demonstrate their differentiation progenies and characteristics.

Session IV: Stem Cell Tissue Engineering: Friday November 12, 2010, 15:30-17:10

Construction of implantable stem cell-derived hepatocytes/liver tissue

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Liver transplantation has been used as the only way to rescue some of the patients suffering from late-stage liver failure. The shortage of donor livers for transplantation is an ongoing issue and ways to alleviate the shortage are being sought. Alternatives include hepatocyte and/or tissue engineered liver transplantation, but the efficacy of the approach is currently limited by cell source, low engraftment efficiency, the integration of the scaffold that mimics the organ's microarchitecture and its extracellular matrix composition. During the past several years, we worked on the stem cell hepatic-lineage restriction, material selection and three dimensional tissue-engineered liver tissue construction. The potential of human hepatic stem cells and/or other stem/progenitors for cell-based therapies and tissue engineering relies on being able to isolate them, propagate them in culture and differentiate them to a functional mature cell fate(s). Paracrine signals from mesenchymal cell populations govern the expansion and differentiation of hepatic stem cells have been investigated and human fetal liver derived stem cells, embryonic stem cells, and adult extra-hepatic biliary tree stem cells have been used for the lineage restriction to adult liver fates. The other critical issue is the matrix and scaffold. Synthetic materials such as PLGA or PGA have been used widely and proofed helpful for the engineered tissue construction and implantation. We figured out a new strategy to prepare tissue-specific

biomatrix scaffolds to efficiently differentiate human hepatic stem cells to mature fates and to maintain mature parenchymal cells as fully functional for long periods of time. This technique offers considerable opportunities for academic, industrial, and clinical programs enabling the use of well-differentiated cell types for analytical study, and, excitingly, enabling an improved way to generate implantable, revascularized tissues or organs that might be used for basic research and clinical programs.

Study of transgenic rhesus monkeys

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Transgenic mice are widely used to model human disease, but some diseases, such as neurological disorders, are difficult to study in mice. Non-human primates are genetically and physiologically similar to humans, but the limited success of strategies to produce transgenic animals has limited their use in research. Creating valuable animal models of human physiology so that the etiology of diseases can be studied and potential therapies for their amelioration may be developed. Depended on our works on monkey superovulation, oocytes maturation, embryo development in vitro and embryo transfer, we have developed an improved methodology for the production of transgenic rhesus monkeys, making use of a simian immunodeficiency virus (SIV)-based vector that encodes EGFP and a protocol for infection of early-cleavage–stage embryos. We show that infection does not alter embryo development. Moreover, the timing of infection, either before or during embryonic genome activation, has no observable effect on the level and stability of transgene expression. Our results demonstrate the usefulness of SIV-based lentiviral vectors for the generation of transgenic monkeys and improve the efficiency of transgenic technology in nonhuman primates.

Commitment of Pluripotent Stem Cells to Adipocyte Lineage

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The developmental pathway that gives rise to mature adipocytes involves two distinct stages: commitment and terminal differentiation. Although the important proteins/factors contributing to terminal adipocyte differentiation have been well defined, the proteins/factors in commitment of mesenchymal stem cells (MSCs) to the adipocyte lineage cells have not. The pluripotent stem cells have the potential to undergo commitment and then differentiate into adipocytes, as well as myocytes, osteocytes, and chondrocytes. Results from our research indicate that both bone morphogenetic protein (BMP)2 and BMP4 can induce commitment of C3H10T1/2 pluripotent stem cells into adipocytes. We applied proteomic analysis profiling to characterize differences between uncommitted C3H10T1/2 pluripotent stem cells and those which have been committed to adipocyte lineage by BMP4 or BMP2 with the goal to identify such proteins/factors and to understand the molecular mechanisms that govern the earliest stages of adipocyte lineage commitment. 8 proteins were found to be up-regulated by BMP2 and 27 proteins were up-regulated by BMP4, while a total of 5 unique proteins were up-regulated at least 10-fold by both BMP2/4, including 3 cytoskeleton-associated proteins (i.e. lysyl oxidase (Lox), translationally-controlled tumor protein 1 (Tpt1) and alphaB crystallin). Western blotting further confirmed the induction of the expression of these cytoskeleton-associated proteins in the committed C3H10T1/2 induced by BMP2/4. Importantly knock-down of Lox expression totally prevented the commitment, while knock-down of Tpt1 and alphaB crystallin expression partially inhibit the commitment. Several published reports suggest that cell shape can influence the differentiation of partially committed precursors of adipocytes, osteoblasts, and chondrocytes, we observed a dramatic change of cell shape during the commitment process, and we showed that knockdown of these cytoskeleton-associated proteins prevented the cell shape change and restored F-actin organization into stress fiber and inhibited the commitment to adipocyte lineage. Our studies indicate that these differentially expressed cytoskeleton-associate proteins might determine the fate of MSCs to commit to adipocyte lineage through cell shape regulation.

Session V: Cardiovascular Differentiation & Immune Regulation of Stem Cells: Saturday November 13, 2010, 8:30-10:10

Regulation of ES derived-cardiomyocytes by endothelial cells

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Cardiovascular disease, including hypertension, coronary heart disease, stroke, and congestive heart failure, remains the number one killer in the developed world. The damage to cardiomyocytes resulting from ischemic injury is irreversible and leads to progressive heart failure. Restoring damaged heart function may result from implantation of cardiomyocytes to the damaged myocardial tissue. The signals that direct ES cell differentiation into cardiomyocytes are largely unknown. We explored the mechanisms by which endothelial cells provide signals to induce cardiomyocyte generation from ES cells. A strong contender involved in the mechanism is EphB4, a receptor tyrosine kinase that is expressed in endothelial cells underneath the cardiomyocytes in beating EBs. Inactivation of EphB4 results in decrease of beating EBs and decrease of cardiac gene expression. The cardiac defect in EphB4-deficient ES cells is rescued by coculture of endothelial cells with EphB4-deficient ES cells, suggesting that EphB4 is a critical component of endothelial niche to induce cardiomyocyte generation from ES cells.

Dedifferentiation and Cell Cycle Reprogramming of Mouse Cardiomyocytes into Cardiac Progenitor Cells

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It has been believed that mammalian cardiomyocytes are terminally differentiated and are unable to proliferate. Recently, using a bi-transgenic mouse model and an in vitro culture system, we found out that adult mouse cardiomyoctyes can dedifferentiate into cardiac progenitor cells (CPCs) which can further differentiate into adult cardiomyocytes (ACMs). To understand the molecular mechanism of dedifferentiation of ACMs: (1) the mature cardiomyocytes, isolated from adult MerCreMer/ZEG bi-transgenic mice expressing GFP following 4-OH tamoxifen induction, were cultured continuously using a modified cardiac explant culture technique, until the cardiomyocytes dedifferentiate into CPCs that expressed cKit, but lost the expression of mature myocyte filament protein a-MHC or cTnT; (2) single CPCs isolated using a microfluidic chip were subject to transcriptomic profiling using a cDNA amplification technique coupled with Affymetrix MG 430 2.0 arrays. It was found that 3845 probe sets (genes) are significant changed (2430 down vs. 1415 up, 2-fold plus P=0.05) in CPCs as compared to AMCs. As expected, genes in cytoskeleton and myosin complex, and those for cardiac ion channel function, contractile regulation are remarkably down-regulated (up to 192 folds) in CPCs, while cell cycle genes are co-regulated, signifying a process in dedifferentiation and proliferation. Pathway analysis reveals the regulated genes involved in p53, EGF, insulin and PI3K/Akt signaling pathway, cyclin and cell cycle, and proliferation regulation, mitochondrial function, telomeres and telomerase activity. Moreover, genes pertaining to stem cell phenotype and function, such as Klf4,

Nanog, Tbxs and stem cell factor (kit ligand) are upregulated in CPCs. In conclusion, ACMs retain substantial cellular plasticity and they are able to dedifferentiate into CPCs orchestrated by cell cycle reprogramming.

Direct differentiations of atrial and ventricular myocytes from human embryonic stem cells

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Although cell transplantation studies have suggested promising therapeutic potentials, the incapability to obtain relatively homogeneous ventricular myocytes is one major obstacle to the development of clinical therapies for myocardial infarction. Human embryonic stem cells (hESC) are a promising source of cardiomycoytes. Here we report that relatively homogeneous embryonic ventricular and atril myocyte populations can be efficiently derived from human hESCs by specifically influencing a signaling switch during human ES cell differentiation.

Mesenchymal Stem Cells: A Double-edged Sword in Regulating Immune Responses

Yufang Shi

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Mesenchymal stem cells (MSCs) have been employed successfully to treat various immune disorders in animal models and clinical settings. We have reported that MSCs can become highly immunosuppressive upon stimulation by inflammatory cytokines, an effect exerted through the concerted action of chemokines and nitric oxide (NO). We show here that MSCs can also enhance immune responses. This immune-promoting effect occurred when proinflammatory cytokines were inadequate to elicit sufficient nitric oxide production. When iNOS production was inhibited or genetically ablated, MSCs strongly enhanced T cell proliferation in vitro and DTH response in vivo. Furthermore, iNOS-/- MSCs could significantly inhibit melanoma growth. Interestingly, iNOS-/- MSCs have reduced immune-promoting effect in CCR5-/-CXCR3-/- mice, suggesting that it is dependent on chemokines. Thus, NO acts as a switch in MSC-mediated immunomodulation. In the absence of NO, chemokines mediate immune upregulation by inflammatory cytokine-activated MSCs. This study provides novel information for better clinical application of MSCs.

Macrophages prevent human red blood cell reconstitution in immunodeficient mice

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An animal model supporting human erythropoiesis will be highly valuable for assessing the biological function of human RBCs under physiological and disease settings, and for evaluating protocols of *in vitro* RBC differentiation from human embryonic stem cells. Although immunodeficient mice on the NOD background have been widely used to study human hematopoietic stem cell function in vivo, the successful use of these mice in the study of human erythropoiesis and RBC function has not been reported. Cotransplantation of human fetal thymic tissue and $CD34^+$ fetal liver cells in NOD/SCID or NOD/SCID/$\gamma c^{-/-}$ mice resulted in the development of multilineage human hematopoietic cells. However, human RBCs were undetectable in blood of these mice despite the presence of a large number of human immature nucleated normoblasts in the bone marrow. Recipient mouse macrophage-mediated rejection was found to be the major factor causing the lack of human RBCs in these mice. Furthermore, human RBC rejection by macrophages in humanized mice was predominately induced by CD47-SIRPα pathway-independent mechanisms. Thus, strategies of preventing human RBCs from rejection by macrophages are required for using immunodeficient mice as an *in vivo* model to study human erythropoiesis and RBC function.

Session VI：Hematopoietic Stem Cells: Saturday November 13, 2010, 8:30-10:10

An innate immune stimulation with polyinosinic: polycytidylic acid attenuates total body radiation-induced hematopoietic genetic instability

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Exposure to ionizing radiation (IR) induces genomic instability, which can lead to hematopoietic stem cell (HSC) transformation and leukemia. In this study, we show that the induction is likely attributable to HSC quiescence, because quiescent HSCs cannot efficiently repair IR-induced DNA double-strand breaks (DSBs) and accrue more DNA damage than cycling HSCs and their progeny after IR. In addition, we demonstrate that activation of HSCs to enter cell cycle is prerequisite for quiescent HSCs to effectively repair DSBs, which can be achieved by an innate immune stimulation with polyinosinic:polycytidylic acid (pIC). More importantly, we found that administration of pIC after total body irradiation (TBI) can significantly inhibit IR-induced hematopoietic genetic instability. These findings reveal a novel impact of immune reaction on HSCs and raise the possibility that activation of HSCs with an immune stimulus may represent a novel approach for the prevention of genotoxic stress-induced genomic instability and hematological malignancies.

Slug regulates hematopoietic stem cell regeneration under stress conditions

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Both extrinsic and intrinsic mechanisms tightly govern HSC decisions of self-renewal and differentiation. However, transcription factors that can selectively regulate HSC self-renewal division after stress remain to be identified. Slug is an evolutionarily-conserved zinc-finger transcription factor that is highly expressed in primitive hematopoietic cells and is critical for the radioprotection of these key cells. We studied the effect of Slug in the regulation of HSCs in *Slug*-deficient mice under normal and stress conditions by using serial functional assays. We show that *Slug* deficiency does not disturb hematopoiesis or alter HSC homeostasis and differentiation in bone marrow, but increases the numbers of primitive hematopoietic cells in the extramedullary spleen site. Deletion of *Slug* enhances HSC repopulating potential but not its homing and differentiation ability. Furthermore, *Slug* deficiency increases HSC proliferation and repopulating potential in vivo after myelosuppression and accelerates HSC expansion during in vitro culture. Therefore, we propose that Slug is essential for controlling the transition of HSCs from relative quiescence under steady-state condition to rapid proliferation under stress conditions. Our data suggest that inhibition of Slug in HSCs may present a novel strategy for accelerating hematopoietic recovery, thus providing therapeutic benefits for patients after clinical myelosuppressive treatment.

Benefit Points:

- A novel negative regulator of HSC regeneration under stress condition is demonstrated

The potential application of Slug inhibitor will be discussed.

Puma dependent apoptosis checkpoints in telomere dysfunctional mice

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Apoptosis and cell cycle arrest may contribute to aging associated tissue atrophy, especially in the context of DNA damage. In telomere dysfunctional mice, abrogation of p21-dependent cell cycle arrest improved maintenance of somatic tissues, whereas the combined inactivation cell cycle arrest and apoptosis in response to p53 deletion resulted in an accelerated atrophy of somatic tissues associated with chromosomal instability at stem cell level. The specific contribution of p53-dependent apoptosis to telomere dysfunction induced aging has not been delineated. Here we show that abrogation of Puma-dependent apoptosis improves progenitor cell function, organ maintenance, and lifespan of telomere dysfunctional mice. Chromosome breakage cycles and DNA damage increased in response to Puma deletion, but increasing expression of p21 associated with the prevention of chromosomal imbalances at stem cell level and with a limitation in the rescue period. These results provide the first experimental evidence that apoptosis and cell cycle arrest act in parallel but compensatory pathways limiting tissue maintenance in the context of telomere dysfunction. A selective inhibition of apoptosis can result in temporary improvements in organ maintenance without increasing stem cell instability or cancer risk.

Identification of Secreted Factors Inducing Hematopoietic Stem Cell Self-Renewal

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Even a modest expansion of cord blood or adult hematopoietic stem cells (HSCs) would have a profound impact on the number of patients that would become eligible for a transplant therapy. Toward this goal, our group has recently reported a novel gain-of-function screen, which identified a series of nuclear factors inducing high levels of murine HSC activity similar to those previously achieved with our positive control Hoxb4 (Deneault et al., Cell 2009). In total, 18 new determinants have emerged, several of which showed a non-cell autonomous influence on HSC activity, i.e., *Fos, Tcfec, Hmgb1* and *Sfpi1*. Evidences are now provided that seven additional factors, i.e., *Smarcc1*, *Vps72*, *Sox4*, *Klf10*, *Ski*, *Prdm16* and *Erdr1* significantly enhance unmanipulated HSC activity non-cell autonomously. Two of these factors, i.e., *Fos* and *Klf10*, induce the expansion of unmanipulated human cord blood HSCs transplanted into immunodeficient mice. This expansion does not depend on the physical contact with feeder cells overexpressing *Fos*, *Sox4*, *Klf10* or *Ski*, confirming that some factors are secreted by engineered feeder cells and diffuse in the culture media to promote HSC expansion. These results reveal a set of secreted molecules that functionally behave as bona fide HSC growth factors.

Definitive Gene Expression Profiling of the Hematopoietic System by Gene Expression Commons Platform

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Hematopoietic stem cells continuously produce more than 10 distinct types of functional cells by step-wise differentiation through progenitor and precursor cells. To understand the global picture of molecular mechanisms regulating this lineage commitment process, it is essential to investigate normal and abnormal hematopoiesis. However, the current methodology of genome-wide gene expression analysis is limited to identifying differentially regulated genes based on their relative expression difference, and not the definitive (or absolute) profiling of global gene expression. Further, each probeset in the gene expression microarray has a different dynamic range because of fundamental variations in hybridization. Therefore, the results of such studies are very sensitive to the samples used for comparison. Based on this limitation, some biologically important information, e.g. genes that are highly expressed in all samples or significant up-regulation of genes detected by probesets with narrow dynamic-range, is often missed. Hence, using current techniques it is very difficult to determine global gene expression with absolute expression levels.

To address this issue, we created a database of dynamic range for each probesets using over 10,000 publicly available datasets from Gene Expression Omnibus. We computed the dynamic range of each probeset by normalizing all the arrays together using RMA algorithm. For each probeset, a threshold separating “high” and “low” expression values was computed using our StepMiner algorithm. The definitive gene expression profiling of a sample of interest is achieved by comparing against this dynamic-range database.

Together with microarray datasets of 40 distinct, highly-purified hematopoietic stem/progenitor/precursor/differentiated cells, we built an intuitive web-based

definitive gene expression profiling platform called “Gene Expression Commons”. Gene expression datasets of any biological model with diverse microarray platforms can be easily incorporated on our highly flexible Gene Expression Commons platform.

Session VII：Neural & Skin Differentiation from Stem Cells: Saturday November 13, 2010, 10:30-12:10

Use of Embryonic Stem Cells (ESC) to Delineate the Epidermal Lineage

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The epidermis is a stratified squamous epithelium, which is in a constant state of proliferation, commitment, differentiation, and elimination so that the functional integrity of the tissue is maintained. The intact epidermis has the ability to respond to diverse environmental stimuli by continuous turnover to maintain its normal homeostasis throughout an organism's life. This is achieved by a tightly regulated balance between stem cell self-renewal and the generation of a population of committed progenitor cells that undergo a limited number of divisions before giving rise to nonproliferative, terminally differentiating cells. This process makes it an excellent model system to study lineage, commitment, and differentiation, although neither the unambiguous identity of epidermal stem cells nor all the regulatory steps and mediators that lead to mature epidermal cells have yet been determined. Furthermore, the identities of genes that initiate epidermal progenitor commitment to the epidermal lineage, from putative epidermal stem cells, are unknown. This is mainly due to the lack of an in vitro model system, as well as the lack of specific reagents, to study the early events in epidermal lineage. Our recent development of a differentiating embryonic stem cell model for the epidermal and hair follicle lineages offers the opportunity to refine and analyze the factors that regulate the epidermal lineage. We will discuss current understanding of the field, and the assays to evaluate stem and progenitor cell potential. Our ongoing studies promise to provide new insights into epidermal stem cells and

their future utility in regenerative medicine. [Our studies are supported by the Canadian Institutes of Health Research]

Region-specific Neurons Differentiated from Human iPS Cells

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My laboratory is interested in understanding external and internal mechanisms that regulate pluripotency of human pluripotent stem cells and their lineage-specific differentiation for realization of their therapeutic values. Directed differentiation of human induced pluripotent stem cells (hiPSC) into functional region-specific neural cells is a key step to applying these therapeutically promising cells to treatment of various neural disorders. Here, I present the generation of region-specific neurons from hiPSC lines derived with either 4 or 6 reprogramming factors. Although we observed heterogeneity in neural differentiation efficiency between different hiPSC lines, the neural differentiation process resembles that from human embryonic stem cells (hESC) in morphology, timing, and gene expression. Moreover, the neural differentiation from hiPSC also requires an active FGF signaling. The differentiated neural epithelial cells possess a default rostral phenotype and can be caudalized to form the midbrain or spinal progenitors. Upon further differentiation, these rostrocaudal neural progenitors mature, respectively, to develop forebrain glutamatergic projection neurons, midbrain dopaminergic neurons, and spinal motor neurons. Typical ion channels and action potentials were recorded in the hiPSC-derived neurons, suggesting their functionality. The efficiency of caudalization is comparable to that of similar neurons from hESC. These results support the highly expected value of hiPSC for study and treatment of patient-specific neural disorders. The variations of the neural induction efficiency between different hiPSC lines also suggest a need to evaluate cell lines and to explore the mechanisms underlying the heterogeneity of hiPSC.

Tracking of Neural Stem Cells and iPS Cells in Human and Monkey CNS

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Investigations of stem cell therapy have required non-invasive analysis of the fate and migration of implanted stem cells. We demonstrate the feasibility of labeling human iPS cells with nanoparticle and functional tracking of iPS cells-induced neural progenitor cells in monkey and human central nervous system (CNS). The day before implantation, we incubated the cells with SPIO nanoparticle. They were then autologously implanted stereotactically around the damage region of the brain or spinal cord. Imaging was obtained by gradient reflection echo with an MR imager. Pronounced hypointense signals were initially detected at the cell injection sites, and were later found to progressively extend to lesion regions, demonstrating that iPS cells-derived neural progenitor and neural stem cells could migrate to lesion sites. The therapeutic efficacy of stem cells was concomitantly tested through functional recovery tests of injury model animals. This data demonstrates the possibility of successful functional tracking of stem cells in CNS and collectively provide necessary foundation for future application of neural stem cells and iPS cells in CNS.

Neural Stem Cells Ensconced in the SVZ niche

Qin Shen, PhD

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Neural stem cells (NSCs) in the mature brain continue to give rise to new neurons only in specialized microenvironment or niches, mainly the subventricular zone (SVZ) of the lateral ventricle and the hippocampal dentate gyrus. The neural stem cell lineage in the SVZ includes type B cells, which express GFAP and contain the slow-dividing neural stem cells, Type C cells that are transit-amplifying cells, and type A neuroblasts which migrate anteriorly along the rostral migratory stream into the olfactory bulb. Maintenance and lineage progression of NSCs are regulated by interplays between the NSC intrinsic properties and the stem cell niche factors. We previously showed that secreted factors from endothelial cells stimulate the self-renewal of neural stem cells and enhance their neurogenic potential in vitro. Using confocal imaging analysis, we characterized the 3-D cellular architecture of the SVZ niche, showing that adult SVZ NSCs and their progeny are bordered by the ependymal cells, which line the lateral ventricles, and blood vessels that form a planar plexus in the SVZ. NSCs and progenitor cells are closely apposed to the laminin-containing extracellular matrix surrounding vascular endothelial cells. Our work has identified a vascular niche in the SVZ and revealed an interaction between laminin and its receptor alpha6beta1 integrin which plays a functional role in binding SVZ stem cells within the vascular niche. Interestingly, apical GFAP-expressing cells are admixed in the ependymal layer and have contact with both the lateral ventricle and blood vessels, occupying a unique position. We have further investigated molecules and signaling pathways regulating the placement and movement of NSCs in the SVZ niche, which will help understand how stem cell niche factors affect NSC activities and adult neurogenesis.

High-Efficiency Induction of Neural Conversion in hESCs and hiPSCs with a Single Chemical Inhibitor of TGF-βSuperfamily Receptors

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Chemical compounds have emerged as powerful tools for modulating embryonic stem cell (ESC) functions and deriving induced pluripotent stem cells (iPSCs), but documentation of compound-induced efficient directed differentiation in human ESC (hESCs) and human iPSC (hiPSCs) is limited. By screening a collection of chemical compounds, we identified compound C (also denoted as dorsomorphin), a protein kinase inhibitor, as a potent regulator of hESC and hiPSC fate decisions. Compound C suppresses mesoderm, endoderm and trophoectoderm differentiation and induces rapid and high-efficiency neural conversion in both hESCs and hiPSCs (88.7% and 70.4%, respectively). Interestingly, compound C is ineffective in inducing neural conversion in mouse ESCs (mESCs). Large-scale kinase assay revealed that compound C targets at least seven TGF-beta superfamily receptors, including both type I and type II receptors, and thereby blocks both the Activin and BMP signaling pathways in hESCs. Dual inhibition of Activin and BMP signaling accounts for the effects of compound C on hESC differentiation and neural conversion. We also identified muscle segment homeobox gene 2 (MSX2) as a downstream target gene of compound C and a key signaling intermediate of the BMP pathway in hESCs. Our findings provide a single-step cost-effective method for efficient derivation of

neural progenitor cells in adherent culture from human pluripotent stem cells. Therefore, it will be uniquely suitable for the production of neural progenitor cells in large scale and should facilitate the use of stem cells in drug screening and regenerative medicine and study of early human neural development.

Key words: human embryonic stem cells, neural conversion, compound C, TGF- superfamily receptors, induce pluripotent stem cells

Session VIII: Stem Cells in Diseases: Saturday November 13, 2010, 10:30-12:10

Abnormal Regulation of Hematopoietic Stem Cell Differentiation and Self-Renewal during Acute Inflammatory Stress

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While progress has been made in identifying key regulators of hematopoietic stem cells (HSC), little is known on the regulation of HSC during the host response to severe infection. Using an in vivo model of sepsis induced by burn and inoculation of Pseudomonas aeruginosa, we defined the bone marrow (BM) response to acute bacterial infection. Analysis of septic BM at different time points from challenge demonstrated a dramatic increase in the absolute number of HSC (5 to 10-fold). 'Septic' HSC were characterized by a more quiescent status and by decreased ability to egress into the pool of more differentiated subsets, resulting in a significant decline in common myeloid progenitor (CMPs) and granulocyte-monocyte progenitors (GMPs). The reduction in myeloid progenitors led ultimately to profound neutropenia and decreased host defenses. Although immunophenotypically indistinguishable from healthy control HSC, 'septic' HSC displayed diminished stem cell activity in transplantation assays, combined to a defective ability to differentiate in vitro and in vivo. Molecular characterization of "septic" HSC show that they have a distinct transcriptional and microRNA signature. By using an LPS defective strain of P. aeruginosa we identified LPS as the key bacterial component, and TLR4 as the critical upstream signaling pathway necessary to induce the alterations observed in the 'septic' bone marrow. Interestingly, dysfunctional regulation of HSC during sepsis was accompanied by changes in the bone marrow niche. Specifically, the BM endothelium showed increased expression of the Notch ligand Jagged2 and of Notch signaling. Further studies are ongoing to define how alterations of the BM microenvironment may converge with cell autonomous signals in the HSC to induce a defective BM response to infection. Interestingly, changes similar to those occurring during sepsis can be found in BM disorders such as bone marrow failure, myelodysplasia, and preleukemic states. Exploring the mechanisms involved in maintaining BM

homeostasis during conditions of inflammatory stress may contribute to identifying molecular mechanisms relevant not only to “normal stem cell biology” but also to the understanding of the process of leukemogenesis and BM failures.

Non-lineage restricted effects of a gain-of-function mutation in tyrosine phosphatase *Ptpn11* (Shp2) on leukemia stem cell development

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Ptpn11 (Shp2), a *Src* homology 2 (SH2) domain-containing protein tyrosine phosphatase (PTP) implicated in multiple intracellular signaling processes, plays a crucial role in normal and leukemic hematopoiesis. We previously showed that Shp2 was required for myeloid and lymphoid cell development. Recently, genetic mutations in *Ptpn11* that cause Shp2 to be hyperactive have been identified in various childhood leukemias, such as juvenile myelomonocytic leukemia (JMML) (35%), myelodysplastic syndromes (MDS, also called pre-AML) (10%), B cell acute lymphoblastic leukemia (B-ALL) (7%), and acute myeloid leukemia (AML) (4%). The molecular and cellular mechanisms by which *Ptpn11* mutations are associated with these hematopoietic malignancies, however, are poorly understood. Although previous studies including ours have demonstrated that single *Ptpn11* activating mutations are sufficient to induce JMML-like myeloproliferative disorder (MPD) in mice, it remains unclear whether *Ptpn11* mutations play a causal role in childhood malignant leukemias, and if so, the cell origin of transformed leukemia-initiating/stem cells (LSCs) remains undefined. We have created conditional knock-in mice with the most common and most active *Ptpn11* activating mutation (E76K) found in childhood leukemias. Global $Ptpn11^{E76K/+}$ mutation results in early embryonic lethality. Induced knock-in of this mutation leads to MPD followed by malignant evolution into T cell ALL (T-ALL), AML, and B-ALL. $Ptpn11^{E76K/+}$ mutation aberrantly activates both hematopoietic stem cells (HSCs) and lineage progenitors prior to leukemia evolution by enhancing growth factor/cytokine signaling. Remarkably, $Ptpn11^{E76K/+}$ mutation also promotes genomic instability in these cells by targeting centrosomes, and this mutation induces LSC development not only in stem cells but also in lineage committed progenitors. These findings reveal non-lineage specific effects of $Ptpn11^{E76K}$ mutation on LSC development and suggest a causal role for this mutation in childhood acute leukemias.

Characterization and Suppression of Pten Null Leukemia Stem Cells

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Cancer stem cells (CSCs), which share many common properties and regulatory machineries with normal stem cells, have been recently proposed to be responsible for cancer initiation and to contribute to cancer resistance. It is very important and challenging to identify CSCs and selectively target them. We have recently established a *Pten*-null acute T lymphoblastic leukemia (T-ALL) model by crossing *Pten*$^{loxP/loxP}$ mice with *VE-Cadherin-Cre* transgenic mice. PTEN loss in hematopoietic stem cells (HSCs) leads to a transient myeloproliferative disorder, followed by acute T lymphoblastic leukemia (T-ALL) with 100% penetrance. Besides *Pten* deletion, at least two subsequent spontaneous alterations, namely β-catenin activation and a *Tcrα/δ-c-myc* translocation, have been identified, which lead to the transformation of T progenitor cells to self-renewable leukemia stem cells (LSCs) enriched in the c-Kit^{mid}CD3^{+}Lin^{-} subpopulation. Interestingly, the NOTCH1 pathway is not altered in the *Pten*-deficient T-ALL model, which mimics the genetics of a subset of pediatric T-ALL patients with only *PTEN* mutations. With identifying mTOR as a novel regulator of β-selection, we reveal that rapamycin, an mTOR specific inhibitor, alters nutrient sensing and blocks T cell differentiation from CD4^{-}CD8^{-} to CD4^{+}CD8^{+}, the stage where the *Tcrα/δ-c-myc* translocation occurs. Long-term rapamycin treatment of pre-leukemic *Pten*-null mice prevents the *Tcrα/δ-c-myc* translocation, LSC formation and halts T-ALL development. However, rapamycin alone fails to inhibit mTOR signaling in LSCs

and eliminate them. Our study indicates that multiple genetic or molecular alterations cooperatively contribute to LSC transformation and provides *in vivo* evidence that preventing LSC formation and selectively targeting LSCs are promising approaches for anti-leukemia therapies.

Hematopoiesis suppression in the aGVHD condition: intrinsic or extrinsic?

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Acute Graft-versus-host disease (aGVHD) remains a major obstacle for successful allogeneic bone marrow transplantation (BMT). The principal organs affected by aGVHD are the skin, liver, gastrointestinal tract and lung. However, bone marrow (BM) suppression (bicytopenia, pancytopenia) without infection is often observed in patients undergoing allogeneic BMT as GVHD symptoms appear, suggesting that BM could be a target of GVHD. Complications considered to be resulted from BM dysfunction, such as infections after delayed immune reconstitution or bleeding, account for ~30% of deaths after allo-BMT. Although BM is a likely target for GVHD and its dysfunction could have a serious negative impact on clinical outcomes, however, how GVHD impairs bone marrow hematopoiesis is not well understood. Recent studies have highlighted important roles for dysfunctional osteoblast niche in reconstituting immunity in aGVHD hosts. Since the hematopoiesis is donor-derived in aGVHD host, we hypothesized the dysfunctional hematopoiesis is extrinsic, not intrinsic. In MHC haplo-identical murine models of GVHD, we have demonstrated the reduced frequency of hematopoietic stem cell (HSC)-enriched population (CD150+CD48-Lin-) in the aGVHD hosts, while their repopulation and multiple differentiation potential were well preserved. Since T cells is the principle alloreactive immune cells in aGVHD milieu, we observed the CD3+CD4+ subset was significantly expanded and the levels of cytokine IFN-γ and TNF-α were dramatically increased in the aGVHD hosts, which was parallel to the severity of hematopoiesis dysfunction. These data suggested that the biased polarization of T cells may entail a severe aGVHD reaction and be responsible for the defective regeneration of hematopoiesis. In summary, our current work provides definitive evidence for the inhibition of normal hematopoietic regeneration by aGVHD and its plausible underlying mechanisms, thereby having important implications for clinical management of aGVHD.

Marrow microenvironment and pathogenesis of hematopoietic defects in murine model of Fanconi Anemia

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Hematopoiesis is a dynamic and highly regulated process orchestrated by self-renewing hematopoietic stem/progenitor cells (HSPCs) and their supporting microenvironment. The hematopoietic microenvironment is a complex network of cells including osteoblasts, endothelial cells, and a common progenitor of mesenchymal origin, which give rise to osteoblasts, chondrocytes and adipocytes. It is generally accepted that the osteoblast and endothelial niches play critical roles in modulating HSPC functions and cellular fates. However, despite the fact that mesenchymal stem/progenitor cells (MSPCs) are stem cells for osteoblasts, the role of MSPCs in HSPC supportive activity by the BM microenvironment and the impact of MSPCs on the pathogenesis of hematopoietic diseases is incompletely understood.

Fanconi Anemia (FA) is a complex heterogeneous genetic disorder. It is well documented that FA has defects in the HSC compartment; however, the impact of the loss of *FA* in other stem cell compartments has received limited attention. Our recent study show that loss of the murine homologue of *FANCG* (*Fancg*) results in a defect in MSPC proliferation and in their ability to support the adhesion and engraftment of murine syngeneic HSPCs *in vitro* or *in vivo*. Transplantation of wildtype (WT) but not *Fancg-/-* MSPCs into the tibiae of *Fancg-/-* recipient mice enhances the HSPC engraftment kinetics, the BM cellularity and the number of progenitors per tibia of WT HSPCs injected into lethally irradiated *Fancg-/-* recipients. These studies provide for the first time quantitative evidence that syngeneic WT MSPCs enhance the engraftment of WT HSCs in *Fancg-/-* mice. Collectively, these data demonstrate that FA proteins are required in the BM microenvironment to maintain normal hematopoiesis and provide genetic and quantitative evidence that adoptive transfer of WT MSPCs enhances hematopoietic stem cell engraftment.

Plenary Session II: Saturday November 13, 2010, 13:30-15:10

Hematopoietic Stem Cells in the Hypoxic Niche

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Hematopoietic stem cells (HSCs) are sustained in a specific microenvironment known as the stem cell niche. Adult HSCs are kept quiescent during the cell cycle in the endosteal niche of the bone marrow (BM). The quiescent state is thought to be a characteristic property for the maintenance of HSCs. Normal HSCs maintain intracellular hypoxia, stabilize the hypoxia-inducible factor-1α (HIF-1α) protein and generate ATP by anaerobic metabolism. In HIF-1α-deficiency, HSCs became metabolically aerobic, lost cell cycle quiescence, and finally exhausted. An increased dose of HIF-1α protein in VHL mutated HSCs and their progenitors induced cell cycle quiescence and accumulation of HSCs in the BM. Restored glycolysis by pyruvate dehydrogenase kinases ameliorated cell cycle quiescence and stem cell capacity. Taken together, HSCs directly utilize the hypoxic microenvironment to maintain their cell cycle by HIF-1 -dependent metabolism. I would like to discuss the similarities of stem cell niche to cancer cell niche.

Targeting dormant drug-resistant cancer stem cells

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Akin to tissue stem cells (TSCs), cancer stem cells (CSCs) may have adopted a dual active-dormant mechanism that enables them to sustain rapid tumor growth and simultaneously avoid depletion from environmental stress, such as chemotherapy. We observed in our studies of mice bone marrow, intestine, and leukemia tissues that administering 5-fluorouracil (5FU) initially depleted active stem/progenitor cells; in response to this stressor, dormant stem/progenitor cells became activated for a narrow window of time; then, some of the forcibly activated stem/progenitor cells subsequently reverted to dormancy.

To effectively target this duality mechanism in cancer (active-dormant CSCs), we propose a novel two-step strategy and have named this protocol ADAPT - activate from dormancy and prime for targeting. Step I applies chemotherapy and/or radiation to deplete active drug-sensitive proliferating CSCs. This also subsequently activates the dormant drug-resistant CSCs. Step II then applies a "stemness" trait inhibitor within the narrow timeframe when the forcibly activated CSCs are primed to be sensitive to drug treatment before reverting to their dormant drug resistant state. Both steps are repeated to fully exhaust remaining dormant CSCs.

Retrospectively, the application of an ADAPT-like protocol in clinic treatment of patients with advanced colorectal cancer (CRC) resulted in much longer survival rate.

Novel molecular pathways regulating the function of leukemia stem cells

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We have previously shown that the arachidonate 5-lipoxygenase gene (*Alox5*) functions as a critical regulator of leukemia stem cells (LSCs) in BCR-ABL-induced chronic myeloid leukemia (CML) in mice. The Alox5 pathway appears to represents a major molecular network in LSCs. We took advantage of our DNA microarray analysis for the identification of critical genes regulated by BCR-ABL in LSCs. We identified a small group of candidate genes that likely play tumor suppressor roles in these stem cells, and among them, a gene called Scd1 was shown to have a strong inhibitory effect on survival of LSCs in CML mice. BCR-ABL transduced bone marrow cells from Scd1-/- mice induced CML much faster in recipient mice than BCR-ABL transduced wild type bone marrow cells, and overexpression of Scd1 delayed CML development. A drug that enhances Scd1 expression had an inhibitory effect on CML development and on the survival of human leukemia cells. Scd1 appears to link to the Pten pathway, at least partially explaining its inhibitory effect on LSCs. Together, our results indicate a novel negative regulatory pathway in LSCs, and provide a rationale for suppressing LSCs by enhancing Scd1 expression. It will be important to fully study this Scd1 pathway and its connection with the Alox5 pathway for developing curative therapeutic strategies for CML.

Balancing Self-Renewal and Differentiation in Stem Cells

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Stem cells in adult tissues have the capacity to self-renew and generate differentiated progeny that replenish lost or damaged cells. The *Drosophila* ovary is an effective system for studying relationships between the niche and stem cells because the stem cells and their associated niches are well defined and versatile genetic tools for dissecting gene functions are readily available. My lab has been using two types of stem cells in the *Drosophila* ovary, germline stem cells (GSCs) and follicular stem cells (FSCs), to study stem cell-niche relationships, niche formation, stem cell quality control and aging. Using a combination of genetic and cell biological approaches, we have studied multiple signaling transduction pathways for their roles in controlling stem cell self-renewal and differentiation, such as BMP, Wnt and JAK-STAT. In addition, we have revealed essential roles of cadherin-mediated cell adhesion in anchoring GSCs and FSCs to their niches, and have defined essential functions of ATP-dependent chromatin remodeling factors and the microRNA pathway in controlling GSC and FSC self-renewal. Recently, we have shown that Notch signaling is required for GSC niche formation during early development, and is also used by GSCs to signal back to maintain niche integrity in the adult ovary, indicating that stem cells and their niche are mutually dependent. We have also shown that stem cell competition serves as a quality control mechanism and that stem cell aging is controlled intrinsically and extrinsically. Finally, we have demonstrated that the balance between stem cell self-renewal and differentiation is controlled by concerted actions of extrinsic signals and intrinsic factors. In my presentation, I will summary our current understanding of stem cell regulation in the *Drosophila* ovary.

Plenary Session III: Saturday November 13, 2010, 15:30-17:10

Human iPS and blood cells: gene targeting and disease modeling

Linzhao Cheng, PhD

Johns Hopkins University School of Medicine

It is highly desirable to reprogram blood cells that are easily accessible and less exposed to environmental mutagens. For example, umbilical cord blood (CB) cells that are collected and stored in various cell banks could be used as a source of iPS cell lines. Last year we reported a protocol to derive human iPS cells from CB, adult bone marrow and peripheral blood (PB) immature hematopoietic cells that express the CD34 surface marker by using the standard 4 retroviral vectors (Ye, 2009). To generate high-quality iPS cells by a virus-free method that would not alter the genome of reprogrammed postnatal somatic cells, we constructed novel episomal vectors and attempted to reprogram un-fractionated mononuclear cells (MNCs) isolated from CB or adult PB. With several key improvements, we can now generate 900 and 14 iPS-like colonies per 2 million transfected CB and PB MNCs, respectively, 14 days after one-time transfection of two plasmids of MNCs cultured for 8-9 days. A single plasmid (pEB-C5) express 5 reprogramming is sufficient to derive iPS cells from CB and PB MNCs although the efficiency is lower, especially for adult MNCs, than the 2 plasmid combination. The blood-derived iPSCs are free of vector DNA after 10-12 passages and lack the V(D)J DNA rearrangements, therefore having a native genome as compared to the previous iPS cell lines derived from human T cells with or without genome-integrating vectors. This facile method of generating integration-free human iPSCs from blood MNCs will accelerate their use both in research and future clinical applications. In addition, we have derived human iPS cell lines from sickle cell disease, X-CGD, polycythemia, other forms of myeloprolifereative disease and aplastic anemia. We are also conducting targeted gene correction in some of these disease-specific human iPS cells by

the homologous recombination method we previously described (Zou, 2009). This would allow us to create an isogenic pair of diseased and corrected iPS cell lines for research, and to develop a novel combined strategy for cell and gene therapy of genetic diseases from patient-specific iPS cell lines.

Stem cell tourism: Hope without reason?

Douglas Sipp

Riken Center for Developmental Biology, Japan

The past decade has seen remarkable advances in the study of stem cells, driven both by their fundamental biological importance and their promise for uses in regenerative medicine. The scientific interest in this field has been accompanied by a high level of governmental support in many nations, great excitement in media reports, and generally high expectations from all sectors. Clinical trials have already been launched for a number of potential stem cell-based treatments, and many others are under consideration. However, companies and clinics around the world have begun to preempt responsible science and bypass established methods for the validation of clinical innovations by advertising unproven and unregulated stem cell treatments directly to patients via the internet. There are now hundreds of companies with English-language websites claiming to provide therapeutic applications that either contain stem cells of some sort, or to stimulate stem cell function, in the treatment of conditions including spinal cord injury, ALS, congestive heart failure, diabetes, multiple sclerosis and autism. I will provide an overview of this growing industry, and outline the regulatory and ethical issues at stake, with special reference to stem cell R&D in Asia.

Poster Presentations

Manufacture of Bone Marrow Stromal Cells (BMSCs, aka Mesenchymal Stem Cells) using a novel closed system bioreactor preserves “stemness”

Delbert Antweiler, Arun Balakumaran, Harvey Klein, Sergei Kuznetsov, Rebecca Peters, Jiaqiang Ren, Pamela Robey, David Stroncek, Brain Sworder, Marianna Sabatino.

National Institutes of Health (NIH)

Ex-vivo expanded bone marrow stromal cells (BMSCs, aka mesenchymal stem cells) are being translated into the clinic in patients with auto-immune disorders, acute graft versus host disease, tissue injury, and for bone formation. The clinical scale-up of BMSC manufacture has been hampered by expansion in an open process with its inherent risks of contamination. Developing a closed system has been difficult for adherent cells such as BMSCs due to the constant shear stresses caused by flow of media, and because these cells do not naturally bind to the surface materials in many closed system. CaridianBCT has developed a hollow fiber bioreactor that promotes adhesion of cells. We wanted to determine whether culture of BMSCs in this bioreactor maintains the “stemness” of cells within the BMSC population *in vivo*. Primary BMSCs were plated in both flasks and two prototype bioreactors, one requires no coating and the other requires fibronectin coating. Assays performed included a panel of surface antigen markers- CD73, CD 105, CD 90 and CD146as positive markers, and CD 34, CD 45 and HLA-DR as negative markers, gene expression profiling, and the “gold standard” of evaluating bone and myelosupportive stroma formation *in vivo* in immune compromised mice. Culture in either of the two bioreactors resulted in cells that were equivalent to those grown in flasks. Comparable abundant bone with adjoining host hematopoietic cells were seen in cohorts of mice that were implanted subcutaneously with BMSCs cultured in flasks or in bioreactors. This study confirms that growth in the novel closed system bioreactors does not alter the differentiation potential of the subset of stem cells within the BMSC population.

Comparision of Cellular Characterization of Mescenchymal Stem & Haemetopoetic Stem Cell Isolated from Bone Marrow & Cord Blood

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The comparative study of isolation and characterization of mononuclear cells from bone marrow and Cord blood is performed and the population of HSC & MSC is characterized using CD 34, CD 90 & CD 105 by running the samples in FACS. The CD34+, CD90- cells, CD34+ CD90+ cells & the CD cells represent the stem and progenitor cell populations in the sample. The percentage of both CD34+, CD90 cells, CD34+, CD105+cells & CD105 CD34 cells are analyzed using different gating strategy in FACS with fluorescent tags APC for CD105, PE for CD34 cells and PerCP, CD 90. The CD34 & CD 90 highly expressed by stem cells than the non-stem cell population hence they are used as the criteria for characterizations. CD34 is the well known hematopoietic stem cell marker. Mononuclear cell number yielded after Ficoll density gradient separation were 3.6 X 10^7 in cord blood and 8.9 X 10^6 in bone marrow respectively.

Cord blood found to contain 59.3% of CD34- CD90- (progenitor) cells as well as 51.8% of CD34- CD105- and 48.7% CD90- CD105- bone marrow found to contain 53.3% of CD34- CD90- (progenitor) cells as well as 24.1% of CD 34- CD105- and 21.8% of CD90- CD105- cells. This shows the high percentage of mesenchymal cell in cord blood cell. Cord blood found to contain 9.7% of CD34+ CD 90- (progenitor) cells as well as 7.1% of CD34+ CD105- and bone marrow found to contain 4.7% of CD34+ CD90- (progenitor) cells as well as 1.5% of CD 34+ CD105- cells. This shows the high percentage of heamatopoIetic cell in cord blood cell. From our study, cord blood proved to be the better option when looking for more HSC/ MSC yield compared with bone marrow blood.

Methods & Results: Aspiration by ficoll method, Total cell count, cell viability count, enumeration by FACS.

Conclusion: From our study, cord blood proved to be the better option when looking for more HSC/ MSC yield compared with bone marrow.

Bone marrow sample aspiration is little painful and complicated comparing to

obtaining cord blood. Patient needs to be admitted and under observation for bone marrow aspiration but for cord blood HSC isolation admission not needed and observation not required.

Cord blood sample provides more number of HSCs, and MSCs in respect of bone marrow which has the self renewal ability. So it maintains the homeostasis in the tissue. Hence most clinical trial study utilizes cord blood stem cell due to their higher rates of proliferation, immunological immaturity, and reduced exposure to viruses and aging.

Wnt signaling regulates porcine pancreatic stem cell proliferation

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With the increasing morbidity of diabetes mellitus, the diabetes mellitus has now become the third highest incidence following cardiovascular disease and tumour which are chronic diseases heavily threatening people's common health. Transplantation of donor islet β-cells has been shown to successfully restore precise fine tuning of insulin release, but is limited by the scarcity of donor islets. However, pancreatic stem cell might provide unlimited resources by the capacity to differentiate into islet-like cells for beta cell regenerative therapies. So there is a widespread interest in specific factors and mechanisms that stimulate proliferation of pancreatic stem cells. Wnt signaling is an important regulator of cell growth and fates. However, the precise role of Wnt signaling for pancreatic stem cell growth and survival is incompletely understood at present. To determine whether a molecule that can maintain stem cell properties can also participate in controlling the proliferative capability of stem cell, we examine the effect of genes encoding Wnt signaling factors (Wnt3a, DKK1 and BIO) in porcine pancreatic stem cell derived from fetal porcine pancreas. In this study, we established a porcine pancreatic stem cell line derived from 2 months fetal porcine pancreases，named pPSCs. Further using potent and the specific Wnt signaling factors, we found that Wnt3a and BIO can robustly stimulate pPSCs proliferation and formation mass analyzed by morphology (significantly smaller and more compact), QRT-PCR, western blotting, 5-Bromo-2-deoxyuridine (BrdU) immunostaining assay and cell cycle analysis, and DKK1 not. β-catenin is activated when pPSCs were cultured in BIO medium. However, we did not find the related role of BIO on

beta cell differentiation by immunostaining and QRT-PCR assay. These results suggested that Wnt signaling plays a key role in the regulation of pPSCs, cell mass proliferation, and maintaining the undifferentiated status.

A Mathematical Model Reveals the Bistability and All-or none Characters of Extraembryonic–endoderm Differentiation

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Self renewal and differentiation of embryonic stem cells (ESCs) are controlled by signaling network. The Erk/MAPK pathway is a key player in ECSs differentiation, whereas transcription Oct4 is known to be significant for maintaining undifferentiated states of ESCs.

According to the recent data, extraembryonic–endoderm (ExEn) differentiation in mouse ESCs is related to activating Erk/MAPK pathway. The serine/threonine kinase 40 (Stk40), whose expression can be suppressed by Oct4, activate Ras-Erk/MAPK pathway through Rcn2. Besides, Rcn2 is functional dependent on Ras, which makes a positive feedback loop between Rcn2 and Ras.

A mathematical model is constructed according to the signaling network, ordinary differential equations (ODEs) are used to describe the reactions of the network. The ODEs can be solved numerically using MATLAB, with results properly fit into two experimental observations: (1)it can well recapitulate the bistability and all-or-none characters of extraembryonic–endoderm (ExEn) differentiation; (2) Over-expression of Oct4 could induce the endoderm cells back to ESCs.

Functional alteration of normal hematopoietic stem and progenitor cells in MLL-AF9 induced acute myeloid leukemia

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Background:

Hematopoietic stem cells (HSCs) are the most primitive cells in the hematopoietic hierarchy, which can produce different classes of progenitors (HPCs). Although the relationship between HSCs and HPCs has been extensively investigated under homeostasis, the kinetics and functional alteration of the hematopoietic compartment in leukemic environment remains largely unknown. Our recent study demonstrated that the HSCs/HPCs were reversibly suppressed in Notch 1 induced acute T lymphoblastic leukemia (T-ALL) condition (Hu et al., 2009). To further explore the paradigm in other types of leukemias, we have examined the kinetics of normal HSCs/HPCs in acute myeloid leukemia (AML).

Methods:

We employed the MLL-AF9 induced AML mouse model (Stubbs et al., 2008) with some modifications. Briefly, the standard hematopoietic assays, involving colony-forming cell (CFC) assay and competitive bone marrow transplantation (cBMT) in conjunction with the phenotypic analysis by flow cytometry at different time points were used to quantify the changes of numbers and functions of normal HSCs/HPCs. In addition, microarray was used to explore the potential molecular mechanisms.

Results:

AML was induced with 100% penetrance and three-week median survival in non-irradiated recipients. Normal hematopoiesis was progressively suppressed during leukemia development. The leukemic environment imposed different effects on HSCs and HPCs, thereby resulting in different outcomes. The frequency of normal LKS^+ or $LKS^+CD150^+CD48^-$ cells in the $CD45.1^+$ population in the leukemia hosts were progressively increased from day 7 to day 21 compared to the controls. In contrast, the normal HPCs in

leukemic mice demonstrated accelerated proliferation and exhaustion during leukemia development. However, the absolute numbers of HSCs and HPCs in the bone marrow were both decreased during the development of leukemia. In accordance with the increased frequency of primitive cells, the normal $CD45.1^+$ BMNCs from leukemic mice had a significantly higher colony-forming *[(46.00±0.52) vs (36.50±1.17) CFCs/ 10^4 BMNCs, p<0.001,n=6]* and long-term engraftment capacity *[5×10^5 $CD45.1^+$ BMNCs were sorted and transplanted via i.v. into lethally irradiated (950 cGy) C57BL/6J ($CD45.2^+$) recipient mice in competition with 5×10^5 $CD45.2^+$ BMNCs. (48.25±5.86)% vs (85.65±0.83)%, p<0.001]* than that from control mice at day 14. Finally, to define the molecular mechanisms, we sorted the $CD45.1^+LKS^+$ population from control or leukemia mice at different time points (day 7, day 10 and day 14) and then performed microarray analysis. The preliminary results showed 351, 34 and 1914 genes were respectively up- or down-regulated at these time points.

Conclusion:

Our current study provides definitive evidence and a molecular signature for the reversible hematopoietic suppression in a AML environment. Interestingly, our findings are similar to the results with the T-ALL model (Hu et al., 2009), thereby suggesting a similar paradigm of HSC alteration under different types of leukemias.

Key words: AML, HSCs/HPCs, MLL-AF9, kinetics, molecular signature

The effects of telomerase activity and telomere length on leukemogenesis

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Background:

Most cancer cells maintain telomere length via telomerase. However, in some cancers telomeres were maintained by alternative lengthening of telomeres (ALT) instead of telomerase. What's more, telomerase activity appears to be reduced in some types of bone marrow diseases, such as CML, AML and myeloproliferative neoplasms (MPN), leading to shortened telomeres which correlate with leukemogenesis. Therefore, our current study is to investigate the role of telomere and telomerase in leukemogenesis. In addition, we also studied the influence of aging micro-environment on the leukemia development.

Methods:

The 3rd generation of Terc-/- KO mice (G3) and heterozygous control mice (F1) were used in the experiments. Bone marrow cells were transduced via retrovirous over-expressing MLL-AF9 fusion gene (AML). The same number of transduced cells were transplanted together with 5×105 CD45.2+ BM cells into lethal-irradiated C57BL/6J (CD45.2) recipients. In addition, one year old F1 and G3 recipients were irradiated, followed by injecting 2,000 leukemia cells together with 5×105 CD45.1+ BM cells as radioprotection, or directly injected 10,000 leukemia cells into non-irradiated F1 and G3 mice via tail-veins.

Results:

Our data showed that leukemia developed from MLL-AF9 transduced bone marrow cells of different genotypes are all acute myeloid leukemia, but latency was different. Transduced TREC-/- (G1, G2, G3 and iG4) bone marrow cells need longer time to developed leukemia. Interestingly, 50% and 30% of mice

that transplanted with G3 and iG4 transduced cells did not get leukemia. It is possible that telomere shortening could prevent the cells from becoming leukemia cells. Moreover, when we injected 10,000 leukemia cells into non-irradiated TERC+/- and TERC-/- mice, the median survival of TERC-/- group was significant shorter than that of the TERC+/- group.

Conclusion:

Our preliminary results suggested that leukemia could be induced in the absence of telomerase activity, but short telomeres could prevent leukemogenesis. Telomere dysfunction induced aging micro-environment could accelerate the development of leukemia.

Key words: telomerase, telomere, leukemogenesis

Peripheral blood derived stem cell implantation for patients with critical limb ischemia

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Angiogenic stem/progenitor cells are a novel, promising therapeutic method for patients with critical limb ischemia (CLI). We performed a controlled Phase I/II study to evaluate the safety and efficacy of peripheral blood-derived, cultured autologous angiogenic cell progenitors (VesCell™) in end-stage, 'no option' CLI patients (n=20).

Autologous cells were administrated in a single session of 30 intramuscular injections into the gastrocnemius muscle of the affected leg. Our results show that the method is safe, and efficacious in preventing major amputations during a 3 months follow-up. Major lower extremity amputations were performed in 6 out of 10 patients in the control group while only 2 minor amputations in the 10 patients treated with autologous angiogenic progenitor cells. The main effect was accompanied by improvement in ankle brachial index (ABI) and transcutaneous oxygen index (TcO_2). Preliminary results from ongoing 24 months follow-up show long-lasting effect of the treatment.

These findings suggest that implantation of angiogenic progenitor cells is safe and effective method in patients with critical limb ischemia. Further tests of alternative cell preparates as well as administration routes are needed in order to find the most promising treatment for CLI patients.

Systematic Expression, Refolding and Purification of a Human 11R tag Transcription Factor Protein for PiPS and in vitro Cell Differentiation Studies

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The ability of a transcription factor (TF) to regulate its targets is modulated by a variety of genetic and epigenetic mechanisms, resulting in highly context-dependent regulatory networks. Various cell type specific differentiation are tightly controlled by step-wise changes of TF regulatory networks. Recent data have demonstrated that by delivering cell type specific master TFs, trans_differentiation of fibroblast cell to neuronal cells, cardiomyocytes, or generating iPS cells can be achieved in vitro. As cell penetration peptide (CPP) technology, such as poly_arginine (11R) tag fusion system now be widely utilized in recombinant protein intracellular delivery, successful engineering human TF protein with 11R tag will benefit for in vitro controlling cell differentiation by simply incubating TF-11R protein in culture medium. Aim in our project is to develop a unique/high throughput *E.coli* based TF_11R manufacture technology for stem cell and cell differentiation applications. In this presentation, we will describe *E.coli* derived TF-11R protein expression, refolding, purification and function assay. Protein derived iPS cell (PiPS) generation using human Oct4_11R, Sox2_11R, KLF4_11R and cMyc_11R proteins will be highlighted. Additional discussion about *E.coli* inclusion body based Matrix_Buffer_Temperature controlled protein refolding system will focus on an example of key transcription factors for hematopoietic stem cell development in vitro.

Nanoparticles labeled Stem Cells: A Novel Therapeutic Vehicle

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Nanotechnology has been described as a general purpose technology. It has already generated a range of inventions and innovations. Development of nanotechnology will provide clinical medicine new prospective in different areas such as medical imaging, medical diagnosis, drug delivery, cancer detection and management. Nanoparticles such as manganese, polystyrene, silica, titanium oxide, gold, silver, carbon, quantum dots and iron oxide have received enormous attention in the creation of new types of analytical tools for biotechnology and life sciences. Labeling of stem cells with nanoparticles overcame the problems in homing and fixing stem cells to desired site and guiding extension of stem cells to specific direction. Although the biological effects of some nanoparticles have already been assessed, information on toxicity and possible mechanisms of various particle types are insufficient. The aim of this review is to give an overview on the mechanisms of internalization and distribution of nanoparticles inside the stem cells, and the influence of different nanoparticles on the stem cell viability, proliferation, differentiation and cytotoxicity, and to assess the role of nanoparticles in tracking the fate of stem cells employed in tissue regeneration.

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Directing mesenchymal stem cells to chondrocytes via specific surface chemistry

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Mesenchymal stem cells (MSCs) can give rise to chondrocytes, thereby having the potential to treat cartilage injuries, including osteoarthritis, by replacing damaged cartilage. However, the mechanisms behind chondrogenesis induction are complex, necessitating micromass culture and the addition of expensive growth factors.

The aim of this study was to design novel culture substrates capable of directing MSC chondrogenesis in 2D culture, in order to

i. investigate the molecular mechanisms behind the initial stages of chondrogenesis
ii. circumvent the need for expensive growth factors
iii. improve scale-up

Substrates were designed with Biomer Technology Limited (BTL), which has developed novel synthetic accelerateTM polymeric coatings for use as biomaterials, and were modelled on the functional composition and distribution found at integrin binding sites of ECM proteins. These coatings present a combination of amine, carboxylic acid and hydroxyl groups at controllable density and proportion. These functionalities are known to influence MSC behaviour and differentiation; however their combined influence is less studied.

Murine MSCs (mMSCs) and human MSCs (hMSCs) were cultured on BTL substrates and after several time points analysed for expression of chondrocyte-specific markers. Interestingly, on substrate ESP01004 mMSCs began to aggregate following seeding, and after 14 days QPCR analysis showed expression levels of collagen II and aggrecan had increased significantly compared with controls. Confocal microscopy demonstrated Collagen II expression in the centre of aggregates, reminiscent of cell condensation in limb-bud formation. Furthermore, on substrate ESP01007 hMSCs aggregated

and expression of chondrogenesis markers was significantly increased following 20 day culture. These results suggest that biomaterials designed to mimic ECM molecules have the potential to direct MSC chondrogenesis without need of additional stimuli.

Molecular Cloning of Nanog Gene Promoter from Dairy Goat and Construction of Reporter System

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Abstract: Nanog is a divergent homeodomain protein that plays an important role in maintaining self-renewal and pluripotency in embryonic stem cells. Nanog gene expresses specifically in embryonic stem cells, embryonic carcinoma cells and embryonic germ cells, but is downregulated in differentiated ES cells, suggesting that Nanog is one of markers suitable for recognizing the undifferentiated state of stem cells in the mouse and human. However, it does not well understand the molecular feature and transcription regulation of Nanog gene in domestic animal. In this study, we, for the first time, have cloned, sequenced, and characterized Naong promoter of Guanzhong dairy goat. The cloned promoter represents the tissue specificity, which activates in ES and EC cells, but is quiescent in fibroblasts and myoblastes. The xxx bp length goat Nanog promoter and regulation region was subcloned into the pEGFP-1 vector to construct the EGFP reporter system, which could sensitively determine the expression level of Nanog through EGFP, and indicate the differentiation status of ES cells or the reprogram status of somatic cells. This reporter system was used to generate a stable transfected Nanog-EGFP reporter goat embryonic fibroblast cell line that is very useful in the study of induced pluripotent stem cells (iPS) of goat.

Keywords: Nanog promoter, Goat, reporter system, iPS

New Insight in Cell Therapy for Trinucleotide Repeat Expansion Diseases

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Many known neurodegenerative and muscular diseases such as Huntington, fragile X, spinocerebellar ataxia, Friedreich's ataxia, spinobulbar muscular atrophy or Kennedy disease, and myotonic dystrophy are caused by instability in DNA microsatellite repeats. These repeats has trinucleotide tandem array. Instability in the number of repeats is established during DNA replication, repair, recombination and transcription. Expansion of these repeats produces abnormal protein that impair cell function .Introduce normally expanded DNA repeat cells may result recovery of the disturbed cell function. For this purpose, application of autologous stem cells that cultured and differentiated into the related functional cells those the number of repeats has been reduced is a therapeutic intervention.

Derivation of Human Induced Pluripotent Stem Cells from Patients with Parkinson's Disease

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Human induced pluripopotent stem cells (iPS cells) have been recently reported using the combinations of OCT4, SOX2, c-MYC and KLF4 or OCT4, SOX2, NANOG and LIN 28 to reprogram the somatic cells including the fibroblasts, adipose cells and blood cells. Establishing of mutation-specific iPS cells from patients with inherited disorders will allow researchers to study the molecular pathological mechanisms of the specific mutations in the human cells, screen for drugs using human cells and explore the autologous cell therapies for inherited diseases. Recently several studies have shown the generation of the disease-specific iPS cells from different genetic diseases including muscular dystrophy, Parkinson disease (PD) and Huntington disease (HD) and amyotrophic lateral sclerosis (ALS), but no pathological changes were reported in these studies. Here, we have generated mutation-specific iPS cells using 4 transcription factors of OCT4, SOX2, c-MYC and KLF4 to infect the adult fibroblasts from PD patients with LRRK2 and alpha-synuclein (SCNA) gene mutations. We have generated 5 iPS cell lines from 3 patient fibroblasts. Of these 5 iPS lines, 2 iPS lines are generated from patient with G2019S mutation in the LRRK2 gene, 2 iPS lines are generated from patient with R1441C mutation in the LRRK2 gene and one iPS cell line from patient with alpha-synuclein (SCNA) triplication. These iPS cell lines have been characterized by morphological observation, immunostaining, RT-PCR analysis and teratoma study. We continue to characterize these iPS cell lines by neuronal differentiation and study the molecular pathological mechanisms of the different mutations in the LRRK2 and SCNA genes.

An effective serum- and xeno-free chemically defined cryopreservation with simple procedure for human ES and iPS cells

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Introduction:
Both human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) possess a great potential in regenerative medicine. In addition to optimized clinical grade culture conditions, efficient and chemically defined cryopreservation methods for these cells are needed. The earlier established slow freezing protocols have, even after recent improvements, resulted in low viability and thawed cells had a high tendency to differentiate. Obtaining good survival after thawing has been problematic.
Methods:
We describe a simple and effective, chemically defined xeno-free cryopreservation system for cryo-banking of hESCs and iPSCs. The medium used is a completely serum and animal substance free product containing DMSO, dextrose and a polymer as cryoprotectants. Cells were directly frozen at -70^{o}C, without a programmed freezer.
Results:
The number of frozen colonies versus the number of surviving colonies differed significantly for both HS293 and HS306. After thawing, the cells had a high viability (90–96%) without any impact on proliferation and differentiation, compared with the standard freezing procedure where viability was much lower (49%). The frozen–thawed hESCs and iPSCs had normal karyotype and maintained properties of pluripotent cells with corresponding morphological characteristics, and expressed pluripotency markers after 10 passages in culture.

They formed teratomas containing tissue components of the three germ layers.

Conclusion:

The defined freezing–thawing system described here offers an excellent simple option for banking of hESCs and iPSCs.

CCR5-delta32 iPS cells as a potential anti-HIV therapy for AIDS patients

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CCR5B is a critical cell membrane co-receptor for the HIV virus to enter and infect lymphocytes and macrophages. CCR5-delta32, a normal CCR5 variant with a 32bp deletion in the CCR5B gene, is widely dispersed among Northern Europeans and their descendents (5-14%). Individuals homozygous for the CCR5-delta32 allele are healthy, suggesting that “normal” CCR5B is largely dispensable. Interestingly, homozygous delta32 alleles provide strong protection against HIV infection (Cell, 1996;86:367). Transplantation of bone marrow from a homozygous CCR5-delta32 trait donor cured AIDS in a HIV-infected leukemia patient (New Engl J Med 2009;360;692). Thus, CCR5B provides an ideal gene target for AIDS therapy without the concern for the mutability of the HIV virus as is often encountered in HIV vaccine development.

To generate induced pluripotent stem (iPS) cells that are resistant to HIV virus, we used a protein evolution approach to develop a DNA eliminase HIVase. The enzyme specifically recognizes two sequences flanking the delta 32bp and eliminates the delta 32bp in the CCR5B gene. After treatment with HIVase, skin fibroblasts derived from AIDS patients were selected for homozygousity of the CCR5-delta32 allele, and were used to generate iPS cells with the Oct4-Sox2-ID1-Nanog polycistronic retrovirus. The resulting iPS will be useful for generating HIV-resistant hematopoietic stem cells, aiming to cure AIDS.

Characterization of female germ cells derived from mouse embryonic stem cells through expression of GFP under the control of *Figla*

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Abstract: Previous studies have demonstrated the germ cells can be derived from mouse embryonic stem cells (ESCs). However, there is still no efficient system, which can visualize the stage of germ cell formation *in vitro*, and further to identify and enrich germ cells derived from ESCs. *Figla* (factor in the germline, alpha) gene encodes a germ cell specific transcription factor that coordinates the expression of the oocyte-specific zona pellucida (ZP) genes and is essential for folliculogenesis in the mouse. Here, we first constructed an Figla-pEGFP-1 recombinant plasmid that expressed green fluorescent protein (GFP) under the control of *Figla* promoter, and generated and characterized an ESC line stably carrying this Figla-pEGFP-1 reporter construct. Then we induced the ESCs to differentiate into female germ cells by culturing adherent embryoid bodies (EBs) in retinoic acid (RA) differentiation medium or with ovarian cells for 7 days. A population of differentiated ESCs expressed GFP, and these cells were analyzed by RT-PCR and immunocytochemistry. The GFP positive cells showed the expression of germ cell markers: *Oct4, Vasa,* meiotic specific gene-*Scp3* and oocyte marker-*Gdf9*, *Zp3* and *Figla*, indicating that this method could be used for the purification and selection of female germ cells. Our study establishes a new selective system of female germ cell derivation and offers an approach for further research on the development and the differentiation of germ cells derived from stem cells.

Key words: embryonic stem cells; germ cells; retinoic acid (RA); Figla; mouse

Isolation and differentiation of germline stem cells from the postnatal mouse ovary

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It is widely believed that in most female mammals, all germ cells enter meiosis to form primary oocytes at the end of fetal development, and as a result, the postnatal mammalian ovary harbors a limited supply of oocytes that cannot be regenerated. However, this idea has been challenged by the demonstration of the existence of female germline stem cells (GSCs) in postnatal mammalian ovaries in recent studies. We isolated ovarian GSCs from neonatal and adult mouse ovaries and expanded them in the same culture conditions as embryonic stem cells (ESCs). The ovarian GSCs cultured for 1-3 passages formed compact round colonies with unclear borders, maintained embryonic stem cell characteristics and telomerase activity, and expressed germ cell markers *Vasa* and stem cell markers *Oct4, Klf4, C-myc, Nanog, CD49f, Sox2, CD133, SSEA1* and *SSEA4*. These cells had ability to form embryoid bodies (EBs), which expressed specific markers of three germ layers. Then we induced EBs to differentiate into cardiomyocytes, pancreatic cells and germ cells, which showed the expression of specific markers cardiac *a-actin, Glut2* and *Scp3* respectively. This study shows the existence of GSCs in postnatal mouse ovary with multipotent characteristics and could help provide a better understanding of the causes of ovarian infertility, its prevention and potential treatment.

Key words: germline stem cells (GSCs); ovary; mouse

Standardization study of G-CSF mobilized peripheral blood stem cells for the treatment of patients with lower limb ischiaemia

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One hundred fifty patients with LASO were randomised to either group A (76 cases implanted with mobilized peripheral blood-mononuclear cells) or group B (74 cases implanted with bone marrow-mononuclear cells), and followed up for 12 weeks. No transplantation related complication or adverse effect was observed in both groups. Comparative analysis revealed that at 12 weeks after cell implantation, improvement on ABI (difference0.06 [95%CI 0.02 to 0.10]; p=0.002) and rest pain (-0.57[-0.26 to -0.87]; p<0.0001) was significantly better in group A patients than group B patients. Autologous transplantation of either M-PBMNC or BM-MNC significantly promotes improvement of limb ischaemia in patients with LASO. In another trial, sixty patients with CLI were enrolled and randomized to either the transplant group or the control group. At the end of the 3-month follow-up, the main manifestations were significantly improved in the patients of the transplant group. Their laser Doppler blood perfusion of lower limbs increased from 0.42±0.13 to 0.59±0.12 perfusion units (P < 0.001). Mean ankle-brachial pressure index increased from 0.47±0.22 to 0.59±0.27 （P<0.001）. A total of 28 of 40 limb ulcers (70.0%) of transplanted patients were completely healed after cell transplantation, whereas only 26.8% of limb ulcers (11 of 41) were healed in the control patients (P=0.012). One patient received a lower limb amputation in the transplanted patients. In contrast, five control patients had to receive a lower limb amputation (P=0.005). Angiographic scores were significantly improved in the transplant group when compared with the control group (P = 0.002). No adverse effects specifically due to cell transplantation were observed in the transplant group at least 3 years after treatment.

Key Words: peripheral vascular disease • angiogenesis • cells • therapy

A new method of type 1 diabetes treatment：T lymphocytic series clearance and autologous hematopoietic stem cell transplantation（with 2 cases analysis）

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Objective The objective of this case analysis is to report the safety and effects of T lymphocytic series clearance and autologous hematopoietic stem cell transplantation (AHSCT) in two type 1 diabetes (T1D) cases.

Methods Two newly diagnosed T1D patients were managed by a low dose immuno- suppression regimen followed by AHSCT.

Results At 14 days and 20 days after AHSCT, the insulin injection was stopped and their blood glucose level can be maintained at normal rage without insulin for 7 months up to the date of this report.

Conclusion The clinical outcome showed that low-dose T lymphocytic series clearance and AHSCT were performed with safe in two small patients with prolonged insulin independence.

Improving reconstitution of human-blood lineage cells in humanized mice by ex vivo culture of hematopoietic stem cells with mesenchymal stem cells expressing angiopoietin-like-5 and in vivo expression of human cytokines

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Adoptive transfer of human hematopoietic stem cells (HSCs) into mice lacking immune cells leads to development of human-blood lineage cells in the recipient mice (humanized mice). First, we report a robust HSC co-culture system wherein cord blood CD34+ CD133+ cells were co-cultured with mesenchymal stem cells engineered to express angiopoietin like-5 in a defined medium. After 11 days of culture, SCID-repopulating cells were expanded ~60-fold by limiting dilution assay in NOD-scid Il2rg-/- (NSG) mice. Furthermore, the expanded cells supported a robust multi-lineage reconstitution of human blood cells in recipient mice, including a more efficient T cell reconstitution. Finally, we show that the poor reconstitution of NK cells and myeloid cells in mice is mainly the result of a deficiency of appropriate human cytokines. When plasmid DNA encoding human IL-15 and Flt-3/Flk-2 ligand were delivered into humanized mice by hydrodynamic tail-vein injection elevated levels of NK cells were induced for more than a month. Similarly, expression of human GM-CSF and IL-4 resulted in significantly enhanced reconstitution of dendritic cells, monocytes/macrophages. Thus, the newly established co-culture system and the human cytokine gene expression by hydrodynamic delivery are efficient methods to improve reconstitution of specific human-blood cell lineages in humanized mice.

Protection of mice against irradiation injuries by the post-irradiation combined administration of p38 inhibitor and G-CSF

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p38 mitogen-activated protein kinases (MAPKs) can be overactivated in hematopoietic stem and progenitors cells after ionizing radiation. In the present study, we examined if combined therapy with p38MAPK inhibitor SB203580(SB) and Granulocyte colony-stimulating factor (G-CSF) is more effective than individual agent alone against ionizing radiation(IR)-induced BM suppression. Mice were divided into five equal groups and designated as I-non-irradiated, non-treated control mice(control), II-total body gamma- irradiated mice(IR), III-IR mice treated with G-CSF by ip injection twice every day (1ug/each mouse) for six successive days, IV- IR mice administered SB (15mg/kg) by ip injection every other day after TBI for 10 days and V- IR mice undertaken combined therapy with SB and G-CSF. On the 30th day after 7.2Gy irradiation, 6.7% and 40% of mice remained alive in G-CSF and combined therapy groups, respectively. The femoral bone marrow cells (BMs), peripheral white blood cells (WBC) and CFU-GM in SB, G-CSF and SB+G-CSF groups exposed to 2Gy, 4Gy and 6Gy doses irradiation were all higher than those in IR group. In addition, the CAFC in SB and SB+G-CSF groups were also higher than that in 4Gy irradiation group. In conclusion, these data indicated that post-irradiation administration of p38 inhibitor and/or G-CSF may protect the TBI-induced acute bone marrow injury.

Targeting on the hematopoietic stem cell niche to protect stem cell from chemotherapy and G-CSF

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The majority of HSPC reside in the bone marrow surrounded by specialized bone-shielded environment. The specialized microenvironment or niche not only provides a favorable habitat for HSPC maintenance and development but also governs stem cell function. Here we investigated the potential role of bone remodeling osteoblasts and osteoclasts in homeostasis and stress-induced mobilization of hematopoietic progenitors, then further tested the hypothesis that targeting the niche might improve stem cell–based therapies using six mouse models to mimic the multiple rounds of chemotherapy followed by autologous HSC transplantation in a clinical setting. Herein, we show that

1. Short term G-CSF treatment leads to decreased number and activity of endosteal and trabecular osteoblasts in mice as well as in humam beings.
2. G-CSF treatment induced osteoblasts retraction is partly due to osteoblast apoptosis but not inhibits osteoblast differentiation inhibition through Wnt pathway.
3. G-CSF acts through an indirect pathway to suppress osteoblasts.
4. G-CSF administration stimulates osteoclast activity
5. Pharmacologic use of PTH or RANKL increases the number of HS cells mobilized into the peripheral blood for stem cell harvests and protects stem cells from repeated exposure to cytotoxic chemotherapy.

These data provide evidence that targeting the HSC niche may enhance stem cell–based therapies.

Stem cell transplantation in ataxia patients

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From June 2009 to September 2010 we treated 7-ataxia patients using stem cell transplantation. The report is as follows.

Objection: To study the effective of stem cell transplantation in ataxia patients.

Materials: There were 7 patients in total who were diagnosed as ataxia received stem cell transplantation. Among these 7 cases, age from 4 years old to 73 years old, there were 3 Friedreich's ataxia and 1 spinocerebellar ataxia type 3 (all of them have DNA reports). The left 3 cases were cerebellar ataxia patients whose MRI showed cerebellar atrophy. In 7 cases 3 female and 4 male patients. Their ICARS scores before treatment ranged from 23.5 to 91. The case history ranged from 1year to 17 years.

Method: The patients were assessed neurologically using a series of set criteria to evaluate kinetic (52 points), posture and gait (34 points), speech (eight points) and ocular (six points) symptoms according to the ICARS [International Cooperation Ataxia Rating Scale] before and after the transplantation. Give every patient regular examination to exclude cases with cancer and tumor before the transplantation and monitor kidney function, liver function, and communicable disease before and after transplantation. Patients were given 6 injections umbilical cord blood derived stem cells to transplant, 1 injection by intravenous to the veins and 5 injections by lumber puncture to subarachnoid cavity. The treatment term was one month.

Result: All patients got improvement a after transplantation. Their ICARS score decreased compare with that before transplanting, and the change of scores ranged from 27 to 10. Statistical analysis showed a significant difference between before and after treatment, p=0.0005. Nobody had liver function and kidney function damaged and no infection of communicable disease after transplantation.

Long-term Survival of Embryonic Stem Cells in Adult Bone Marrow

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Current protocols for embryonic stem cell (ESC) differentiation do not ensure complete or synchronized conversion of ESCs into a specific functional tissue type for therapeutic purposes. Thus, un-differentiated ESCs, though few, may be still contained in the therapeutic ESC derivatives. However, it is unknown whether un-differentiated ESCs can survive in adult organs/tissues for a long-term *in vivo*. To address this important question, we directly implanted GFP marked ESCs (driven by either Oct4 or chicken β-actin promoter) into adult mouse bone marrow (BM) *via* in-tibia injection and then tracked the cells in the animals by two-photon microscopy. The $Oct4^+$ cells could be detected in recipient's BM for more than 100 days post-transplantation. Moreover, the $Oct4^+$ cells could be recovered at various time points post-transplantation and became cell lines again. Those recovered cell lines expressed all markers known for ESC and were able to differentiate into cells from three germ layers. Gene expression profiling showed time-dependent epigenetic alterations in the cell lines. This study provides definitive evidence for long-term survival and differentiation capacity of exogenous ESCs in adults, thereby having important implications for the physiological maintenance of ESCs *in vivo* as well as the therapeutic use of ESC-derived products.

Key words: embryonic stem cells, adult, bone marrow, transplantation

An Oct4/Sox2 Co-Expression Vector Can Activate Endogenous Nanog Expression In Human Embryonic Kidney 293 Cells

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The technology of induced pluripotent stem (iPS) cell derived from somatic cells was born in 2006. The patient-orientated iPS cells are extremely useful for the drug discovery and regenerative medicine. However, the applications of viral-mediated iPS technology increase the risk of tumorigenicity and highly limited the utility of iPS cells in both developmental biology and clinical application. Thus, vector-mediated non-virus and nonconformable induction system have been considered although it may produce the low transfection efficiency. Here, we construct an Oct4/Sox2 co-expression vector (pOct4/Sox-GFP) with EGFP marker, which is controlled by CMV and EF1a promoters, respectively. With transfecting the construct of pOct4/Sox-GFP into HEK 293 cells, the positive clones showing the green fluorescence simultaneously express exogenous Oct4 and Sox2 gene. The immunocytochemistry analysis showed that exogenous Oct4 and Sox2 proteins were colocalized in a cell. In 48 hours after the transfection, the transcripts of Nanog gene was detected, while no Nanog signal in control HEK 293 cells. Thus, the result indicate that co-expression of Oct4/Sox2 can activate the endogenous Nanog gene expression, suggesting that the pOct4/Sox-GFP construct is a non-virus and nonconformable vector that can be used to induce iPS cells by itself or combined with other transcription factors and small molecules. Moreover, our study established the fundamental work for the next step research to utilize this plasmid to induce somatic cells into iPS cells.

Keywords: Induced pluripotent stem (iPS) cell, Oct4, vector

Amyloid beta peptide induces genetic programming in mesenchymal stem cells and neuronal PC-12 cells

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Abstract: Bone-marrow derived mesenchymal stem cells (MSC) are thought to be resistant to toxicity of environmental insults. Excessive exposures to environmental toxins or metabolic oxidants may induce chronic or acute neuronal damage that contributes to the late life onset of neurodegenerative disorders such as Alzheimer's disease. The neuronal damage may be linked to significant genotoxicity that cannot be repaired adequately, thus leading to development of AD. To investigate possible genetic modifications, we treated MSC cells with Amyloid beta peptide (A 1-40) associated with AD pathogenesis and determined DNA damage and subsequent DNA repair response. Our results showed that A 1-40 induced significant DNA damage by comet assay and cellular toxicity by Vybrant assay. Our studies also demonstrated that A 1-40 as well as environmental toxin cadmium caused strong DNA repair protein response with an increase in Rb, OGG1, CSB and p53 in MSC cells as determined using RT-PCR and western blotting analysis. Similar responses were observed in neuronal PC-12 cells. Since MSC cells are thought to be more resistant to genotoxic damage, further understanding of the molecular mechanism of genetic modifications and response in these cells may lead to novel therapeutic strategies for controlling neuronal diseases such as AD.

Inhibition of mTORC1 hyperactivity promoted self-renewal capacity of mouse hematopoietic stem cells during ex vivo expansion

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In certain organism the persistent target of rapamycin (TOR) C1 hyperactivity leads to increased proliferation but subsequent exhausting of the stem cells compartment, TOR C1 inhibition promotes their self-renewal. However, the role of TOR C1 is not yet clear during ex vivo expansion of hematopoietic stem cells (HSCs). In the present study, we found that the mouse lin-sca-1+HSCs/hematopoietic progenitor cells (HPCs) exhibited sustained increased activity of mammalian TOR C1 (mTOR C1) after sixth day of culture in serum-free medium supplemented with stem cell growth factors (SCF, Tpo and Flt3 ligand) .The mTOR C1 hyperactivity was accompanied by increasing expression of p16 and SA-β-gal positive senescent phenotype. The inhibition of mTOR C1 by Rapamycin between 7^{th} to 10^{th} day of the expansion resulted in a 1.75±0.37-fold increase in lin-sca-1+c-kit+ mouse HSCs and a 2.5±0.51-fold increase in sca-1+lin- mouse HSCs/HPCs than that without repamycin by flow cytometry and cobblestone area-forming cell (CAFC) assay, and reduced the expression of p16 protein to 58±16 % of the control cells and the frequency of SA-β-gal positive senescent cells to 3±1 % than 8±2 % in the control cells among sca-1+lin- mouse HSCs/HPCs. In colony forming unit(CFU) assay, the cells cultured with rapamycin generated 35±4% multilineage granulocyte, erythrocyte, monocyte, and megakaryocyte (GEMM) colonies than 20±5% in control cells, while the control cells produced 58±7% myeloid CFU than 24±2% in cells cultured with rapamycin. The western blot assay revealed rapamycin had not any influence on the phosphorylation of P38MAPK in the population of lin-sca-1+ mouse HSCs/HPCs, which was closely related to the senescence and differentiation of HSCs. All together, our primary results suggested that Rapamycin could promote self-renewal of HSCs during ex vivo expansion via senescence inhibition, which may be independent on activation of P38MAPK.

A novel approach to promote germ cell differentiation from mouse embryonic stem cells (mESCs)

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To our knowledge, ESCs can differentiate into male and female germ cells *in vitro* (Geijsen et al, 2004; Hübner et al, 2003; Nayernia et al, 2006; Toyooka et al, 2003). It represents a desirable experimental model and potential strategy for treating infertility and sterility. *In vivo*, sertoli cell synthesis and secret a variety of growth factors governing the spermatogenic cell proliferation, differentiation and mature. In the current study, we developed a novel approach to promote germ cell differentiation from mESCs. The Stra8-GFP mESC lines were established, co-cultured with mouse sertoli cells, and induced by RA（2×10^{-7} M） for 7 days. 1.8% GFP positive cells were obtained by FACs analysis and furthermore simulated microenvironment by adding testicular extracts *in vivo*. RT-PCR and immunofluorescent staining demonstrated that germ cell specific markers including OCT4, VASA, FE-J1 and SCP3 were expressed in both RNA and protein levels in the GFP positive cells. Expressions of Vasa, C-kit, Dazl, Stra8, Scp3 were increased after co-culture for 7 days and 14 days. Cells in G1 phase of cell cycle increased obviously reaching G1%=91.118%, approaching spermatogonia G1%=91.334%. Anline blue staining also indicated occurrence of nuclear concentration in the induced cells. At day 31, sperm-like cells were found. Our results suggested that co-culturing with mouse sertoli cells significantly promotes mESCs differentiation into germ cells. In conclusion, our results indicated that co-culturing of stra8-GFP mESCs with mouse sertoli cells significantly promote them differentiation into germ cells. Growth factors derived from sertoli cells, RA and testicular extracts may induce ESCs differentiation into sperms.

Human umbilical cord mesenchymal stem cells accelerate recovery of cisplatin-induced kidney injury and attenuate renal interstitial fibrosis in rats

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Abstract Acute kidney injury is main side effect of cisplatin. To elucidate the nephroprotective effects of human umbilical cord mesenchymal stem cells (hucMSCs), we transplanted hucMSCs into rats with cisplatin induced AKI. Our results showed that hucMSCs significantly accelearte the recovery of renal function by promoting proliferation and inhibiting apoptosis of renal tubular cells. Furthermore, the main mechanism of cisplatin nephrotoxicity has been considered mitochondrial dysfunction and activation of mitochondrial signaling pathways. In this study, the evidence of mitochondria-protecting effect of hucMSCs was showed through down-regulating the expression of Bax and up-regulating the expression of Bcl-2 as well as agonistic lipid peroxidation. Moreover, if the injured cells lose their ability to repair themselves in the process of AKI, irreversible renal interstitial fibrosis will occur because of persistent tubulointerstitial inflammation, proliferation of fibroblasts and excessive deposition of extracellular matrix. we observed remarkable renal interstitial fibrosis in rats in 4 weeks after cisplatin injected. Epithelial-to-mesenchymal transition (EMT) associated gene or protein such as α-SMA, E-cadherin and Vimentin also significantly changed in fibrosis kidney. hucMSCs transplantation significantly reduced renal interstitial fibrosis by reducing collagen accumulation, down-regulating vimentin protein and α-SMA gene expression and increasing E-cadherin gene expression. These findings indicate that hucMSCs may be a promising cellular therapy ways for treatment of cisplatin induced AKI and renal interstitial fibrosis, with a novel

mechanism of protecting mitochondria and inhibiting EMT.
Keywords: Human umbilical cord mesenchymal stem cells; Acute kidney injury; Cisplatin; Renal interstitial fibrosis

The Use of Bone Marrow and Fat Stem Cells in an Office Setting for the Treatment of Musculoskeletal Conditions

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The purpose of this talk is to present to the office based physician a simple and cost effective method of performing Stem Cell (both Bone Marrow and Adipose) and Platelet Rich Plasma injections in the office setting. The conditions which this paper discusses pertain to the musculo-skeletal system. These conditions include degenerative arthritis and many other soft tissue injuries of various joints. In addition to joint problems, the treatment of everyday tendon and muscle injuries are also discussed. The interested physician is shown the simple techniques of obtaining adipose and bone marrow Stem Cells in the office. The discussion includes the technique of harvesting fat cells from a simple liposuction technique. This fat tissue serves as both a scaffold for the stem cells and is also a source of stem cells. In addition to using fat grafts, we present a relatively simple technique of extracting fat stem cells (SVF) from fat tissue by enzyme digestion and centrifugation. The physician is given the indications of when to use each type of cell and when to use Platelet Rich Plasma as an adjunct with these cells. Also discussed is the use of various growth factors including Human Growth Hormone which seem to enhance stem cell mobilization, calcitonin which stabilizes articular cartilage and parathyroid hormone. In addition to these growth factors, the use of supplements, hyperbaric oxygen and the institute's results (over 3000 cases with results equaling 85% excellent relief) are also discussed.

Phosphorylation of ERK Contributes to Differentiation from Human Umbilical Cord Mesenchymal Stem Cells into Cardiomyocyte-like Cells Induced by 5-azacytidine

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5-azacytidine (5-Aza) promotes myogenic commitment of cultured mesenchymal stem cells (MSC), especially MSC derived bone marrow. However, the signaling mechanisms stimulating cardiomyocyte differentiation from human umbilical cord derived mesenchymal stem cells (hucMSCs) are not well understood. With 5-Aza treatment, hucMSCs were morphologically transformed into cardiomyocyte-like cells. Futhermore, 5-Aza induced the expression of cardiac-specific genes and proteins such as desmin, beta-myosin heavy chain, cardiac troponin T, ANP and Nkx2.5. 5-Aza treatment caused significant up-regulation of ERK phosphorylation whereas PKC showed no response. A specific MEK inhibitor, U0126 abolished 5-Aza-induced up-regulation of cardiac-specific genes and proteins, suggesting that ERK is necessary for cardiomyocyte differentiation of hucMSCs. Our results revealed that the expression of phosphorylated-STAT3, MEF2c and MyoD increased after 3 days with 5-Aza treatment and inhibited remarkably by U0126. These results suggest that the sustained activation of ERK contributes to the inducible function of 5-Aza on cardiomyocyte differentiation of hucMSCs in vitro.

Keywords: 5-azacytidine; hucMSCs; cardiomyocyte differentiation; ERK phosphorylation

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Competition between leukemic cells and normal hematopoietic cells in non-irradiated recipient mice

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Background: The biological mechanisms on the development of leukemia still remain nebulous. Our recent study demonstrated differential effects of T-ALL leukemic environment on normal hematopoietic stem (HSCs) and progenitor cells (HPCs) in the irradiated model (Hu X, et al. Blood 2009). Moreover, a previous publication showed that the leukemic cells created bone marrow niches and disrupted the behavior of normal HSCs/HPCs (Sipkins DA et al, Science 2008). To further understand the actual effects of the leukemic environment on normal HSCs and HPCs in a more clinically-relevant model, we have introduced a non-irradiated mouse model to better mimic the physiological condition.

Methods: The Notch1-over expression murine T-cell leukemic cells were established as previously reported (Hu X et al, Blood 2009). In our work, leukemic cells were injected into healthy C57BL/6J (CD45.2+) mice to establish the non-irradiated leukemic model. Flow cytometry was used to study the kinetics of HSCs/HPCs. Colony-forming cell (CFC) assay and competitive bone marrow transplantation (cBMT) were used to assess the impact of leukemic environment on HSCs/HPCs. In addition, confocal and two-photon microscopy imaging approaches were used to show the competition between leukemic cells and hematopoietic cells.

Results: T-ALL was induced with 100% penetrance in non-irradiated recipients. After 10 days of transplantation, hematopoietic suppression occurred to HSCs but not HPCs in the leukemia-bearing mice. In accordance with the increased frequency of primitive cells, the capacity of colony-forming *[CFU-GM (13±1.22 vs 3±0.35 CFCs/ 10^4 BMNCs), CFU-mix (8±1.13 vs 3±1.41 CFCs / 10^4 BMNCs)]* and repopulation *[(46±4.38)% vs (20±2.26)%, respectively]* of residual normal hematopoietic cells in leukemic condition were significantly

higher than that in control mice. The two-photon microscopy showed that the normal hematopoietic cells preferred to localize in the endosteum niches, while the leukemic stem cells (LSCs) firstly localized to spleen, and then migrated to bone marrow vascular niches where the LSCs created their own niche to increase their size of cell population. At last, the leukemic cells invaded and occupied the endosteum niches.

Conclusions: Our current findings demonstrates that normal HSCs/HPCs are also reversibly suppressed in leukemic environment in the non-irradiated model, which is similar as the results from irradiated model (Hu X, et al. Blood 2009). And the data from microscopy imaging approaches suggests a paradigm that the leukemic cells cause the suppression of normal hematopoietic cells by hijacking their normal niches.

Key words: T-ALL, Notch1, leukemic environment, HSC, niche

Vam3 stimulates phenotypic and functional maturation of murine bone marrow-derived dendritic cells

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Objective: To investigate the effects of Vam3 on the phenotypes and maturation of murine bone marrow-derived dendritic cells (BMDCs).

Methods: bone marrow-derived cells were cultured in granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL-4) for 7 days, then the maturate DCs were harvest. Cell phenotypes and antigen-capture were evaluated by flow cytometry. The antigen presenting by BMDCs was evaluated by mixed lymphocyte responses.

Results: Compared with the control, Vam3 could increase the expression of CD11c, CD86 of BMDCs. Though the phagocytosis of OVA-FITC by BMDCs stimulated with Vam3 was somewhat impaired, Vam3 was an efficient stimulator of naïve allogeneic T-cell proliferation.

Conclusion: Vam3 can promote not only the muturation of cultured murine BMDCs, but also the immune response initiation induced by BMDCs. This may be therapeutically useful in controlling some kinds of human diseases.

Human umbilical cord mesenchymal stem cells expand CD4+CD25+ forkhead boxp3(FOXP3)+regulatory T cells to improve survival and resolve experimental lung injury in mice

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Systemic and local inflammatory processes have a key, mainly detrimental role in the pathophysiology of acute lung injury (ALI). However UC-MSCs release of soluble factors, which limit inflammation and necrosis, may play an important role in conferring organ protection. Recent studies indicate that increased levels of CD4+CD25+ forkhead boxp3(FOXP3)+regulatory T lymphocytes(CD4+CD25+ Foxp3+Treg cells) were associated with a reduced risk of death from ALI causes. Among the population of MSC, UC-MSCs may have clinical advantages. Therefore, we hypothesize that UCMSC can expand Treg cells and the levels of CD4+CD25+ Foxp3+Treg differences in the ALI between administrating of UC-MSCs and PBS.—UC-MSCs were obtained from full-term caesarian section deliveries. As a model of ALI, mice were administered intratracheal (i.t.) endotoxin (lipopolysaccharide [LPS]) and received 1×106 UC-MSCs 4 hours after challenge. CD4+CD25+ Foxp3+Treg, survival, body weight, histology and lung injury scores was assessed after the UC-MSCs treatment. In addition, anti-inflammatory IL10 and pro-inflammatory mediator production such as tumor necrosis factor-a (TNF-a) , monocyte chemoattractant protein-1(MCP-1)and interferon-r (IFN-r) were detected. Differences were considered significant if $P < 0.05$. Results—UC-MSCs resulted in significant high level of CD4+CD25+Foxp3+Treg in ALI, which increased significantly anti-inflammatory factor IL-10 and decreased pro-inflammatory factor such as TNF-a, MCP-1and IFN-r than the control.

Conclusion—This study constitutes the first demonstration that UC-MSCs increased the diminished levels of alveolar CD4+CD25+Foxp3+Treg and do appear to protect and improve function of ALI.
KEYWORDS—UC-MSCs, CD4+CD25+ Foxp3+Treg, ALI, LPS

Impact of notch1-induced murine T cell leukemic environment on normal hematopoietic stem cells

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Objective: During the development of leukemias, leukemia stem cells outcompete hematopoietic stem cells (HSCs) and become dominant. Our recent study demonstrated differential effects of a leukemic environment on normal HSCs and HPCs (Hu X, et al. Blood 2009). It was previously reported that increased secretion of SCF by the leukemic cells played an important role in impeding normal hematopoietic cells by a leukemic environment (Sipkins DA et al, Science 2008). Our current work is to further explore the molecular mechanism of leukemic environment on normal HSCs.

Methods: The Notch1-induced murine T-cell leukemia model was established as previously reported (Hu X et al, Blood 2009). ELISA and real-time PCR were used to monitor the expression of SCF during the leukemia development. The expression difference between normal HSCs in leukemia and normal hematopoietic environment was studied by gene profiling experiment followed by real-time PCR verification. Exogenous SCF was added to HSCs to study its effects on gene expression.

Results: The serum SCF level in leukemia group mice was 2.2-fold higher than that in control mice. However, decreased SCF expression in total bone marrow, peripheral blood, spleen cells or purified leukemia cells was detected. In contrast, a 4.5-fold increase was observed in normal hematopoietic cells, which suggested that high level SCF was contributed by normal cells rather than the malignant cells. Gene profiling results showed significant difference in 169 genes (127 up and 42 down). Real-time PCR results further confirmed an increased expression of Hes1 and Fbxw11, IL-18R1 and Itgb3. In contrast, a decrease could be detected for the expression of CXCR4 and Mmp2. Moreover, exogenous SCF induced the up-regulation of Fbxw11, IL-18R1 genes in HSCs in vitro.

Conclusion: Our results suggested that leukemic environment have great impact on the kinetics of normal HSCs by affecting specific gene expression.

Inducing Pluripotency in Somatic Cells from the Snow Leopard (*Panthera uncia)*, an Endangered Felid

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Induced pluripotency is a new approach to produce stem cell-like cells from somatic cells for clinical and other uses, including potential applications in assisted reproduction.

Snow leopard iPS (SLiPS) cells were generated from dermal fibroblasts by retroviral transfection of genes encoding human transcription factors. Transduction efficiency was 96%. Initial attempts with 4 factors (Oct-4, Sox-2, Klf-4 and cMyc) resulted in the formation of small colonies, which could not be maintained beyond four passages. Addition of Nanog, to the transfection cocktail produced SLiPS colonies as early as Day 2, which were picked at day 5 and expanded *in vitro*. The resulting cells were alkaline phosphatase (AP) positive at P4. Colonies also tested positive for Oct-4, Nanog and Stage-Specific Embryonic Antigen-1 (SSEA-1), using immuno-histochemistry, both at P4 and P14. RT-PCR confirmed that all 5 human transgenes were transcribed at P4 but Oct-4, Sox-2 and Nanog transgenes were silenced at P14, suggesting reprogramming of the endogenous genes. Feline Oct-4 and Nanog were expressed by SLiPS cells from P4. On injection in the hind-leg muscle of a SCID mouse, SLiPS cells at P18 differentiated into cell types representative of the three germ layers in resulting teratoma. The cells also incorporated into the inner cell mass of developing murine blastocysts when injected into the perivitelline space at the 4-8 cell stage, providing a useful functional tool to assess pluripotency.

This study describes the first derivation of iPS cells from the endangered snow leopard and is also the first report on induced pluripotency in a felid.

Comparing liver differentiation potential of different source stem cells

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Aim: In order to compare and discuss the different liver differentiation capability of sourse stem cells. Proposed clinical treatment of stem cells for clinical transplantation for providing the theory basis for end-stage liver disease.

Method: 1. BMSCs and pBMSCs were obtained by Percoll gradient centrifugation technique, and ADSCs were digested by Trypsin. Immunofluoresce analysis results showed CD90, CD106 were positive and CD34 was negative for ADSCs and BMSCs. 2. The cells viability was evaluated with cell counting. 3. The forth-passage cells were induced by growth factors for 7 days，14 days，21 days. Cells were immunostained to detect the expression of liver markersas albumin、ALF and CK18. 4. The number of positive cells with fluorescent staining was analyzed by the chi-square statistics.

Results: 1.BMSCs and pBMSCs were obtained by Pecoll gradient centri-fugation successfully. 2. ADSCs have a higher proliferation capacity compared to the others. 3. These cells can be differentiated into cells which changed from long fusiform shape to polygon or similar round shape.

Conclusion: ADSCs have a higher proliferation capacity compared to the others, however the liver differentiation capacity of ADSCs, BMSCs and pBMSCs has no obvious difference, these results indicate that ADSCs have forpo-tential for stem cell transplantation.

Key words: Liver differentiation, Different source stem cells

Functionalization of poly (epsilon-caprolactone) surface with bioresponsive molecular components for accelerating in situ endothelialization

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There is a pressing clinical need for small-diameter vascular prostheses to bypass disease coronary and peripheral arteries. However, the current synthetic blood vessel materials, such as expanded polytetrafluoroethylene (ePTFE), can not be used for small-diameter (<5mm) prostheses because of acute thrombus formation and intimal hyperplasia.

In our group, we have been focusing on surface modification of synthetic materials with the potential biological responsive molecular components that can be incorporated into (and/or released from) biomaterial surfaces to obtain accelerated in situ endothelialization.

Fibrous poly (epsilon-caprolactone) (PCL) scaffold was prepared with electrospinning method. An endothelial cell specific peptide selected by phage display was grafted onto the surface of the PCL membrane. The surface was also modified with zwitterionic poly (carboxybetaine methacrylate) (PCBMA) by atom transfer radical polymerization (ATRP) method in order to improve the blood compatibility. Results show that modification with zwitterionic PCBMA and peptide resulted in a hydrophilic surface without changing the morphology of the PCL membrane. The material supported the adherence and growth of vascular cells and resisted platelets adhesion when exposed to platelet-rich plasma. The surface could specifically capture endothelial progenitor cells (EPCs) other than bone marrow mesenchymal stem cells (MSCs) and human umbilical vein endothelial cells (HUVECs). Based on these data we speculate that this functionized PCL graft may offer new opportunities for designing small diameter blood vessels.

In another approach, we synthesized a new molecule that contains short

peptide RGD: Nap-FFGRGD. The compound had an amphiphilic nature and could self-assembly. When the fibrous PCL scaffold was dipped into the solution of this compound, a thin layer was formed due to the self-assembly and self organization of Nap-FFGRGD, and resulted in a hydrophilic surface, RGD-PCL. This RGD-PCL surface significantly enhanced the adhesion and proliferation of NIH 3T3 cells. This functional surface can also inhibit platelet aggregation in the platelet rich plasma, but did not influence the clotting time through extrinsic, intrinsic and common coagulation pathways. Further AV-shunt experiment showed that when the RGD-PCL grafts contact with whole blood, there was no acute thrombus formation. Implantation of RGD-PCL or PCL grafts (1 mm in diameter) into rat abdominal aorta showed that four of five RGD-PCL grafts were patent after 1 month, whereas all of the five PCL grafts were totally occluded. Histological analysis showed that the grafts maintained their original morphological configuration and the luminal surface was fully covered with endothelium. In conclusion, this RGD modified PCL may be a suitable small diameter blood vessel material for further investigation.

The increased stability of p16 mRNA in murine hematopoietic stem cell after irradiation was mediated by p38 signal pathway

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Purpose: The present study was undertaken to examine the expression level of p16 mRNA in mouse hematopoietic stem cell after irradiation and to explore its molecular mechanism.

Methods and Materials: Bone marrow mononuclear cells was isolation from 30 C57 male mice and then lineage negative hematopoietic (Lin^-) cells was sorted using magnetic bead. The hematopoietic stem cell was divided into 5 groups, including control group, radiation group, p38 inhibition group (SB203580 (5 umol) was added into culture medium 30 minutes before radiation) and transcription inhibition group. Except the control group, the other four groups were exposed to γ ray at a dose of 4 Gy. The expression status of p16 mRNA was examined using Real-time PCR after 5 days. For the transcription inhibition group, actinomycin D (5ug/ml) was added into culture medium 3 hours before RNA extraction.

Results: Comparing with the control group, the expression of p16 in radiation group was significant increased, showing radiation can induce the expression of p16 in hematopoietic stem cell. In contrast, the p16 expression level in SB203580 group was not increased after radiation. These data suggested that the increased p16 expression in hematopoietic stem cell after irradiation was mediated by p38 signal pathway. Comparing with control group, the p16 expression level in transcription inhibition group was still increased, suggesting the stability of p16 mRNA was increased.

Conclusion: These data showed that the increased expression of p16 in mouse hematopoietic stem cell was mediated by p38 signal pathway.

Moreover, this kind of increasing was partly due to the increased stability of p16 mRNA.
Keywords: p16 mRNA, stability, p38, HSC

Trancriptome analysis of undifferentiated human ES cells and primary erythroid cells at different developmental stages

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Human hematopoietic stem cells (HSCs) are multipotent stem cells that give rise to all blood cell types, yet the mechanism for regulating HSC differentiation is unknown. Characterizing the transcriptome, the collective set of all RNA produced by a single cell-type, is an essential step toward characterizing the changes in gene expression during stem cell commitment and differentiation. We used next-generation DNA sequencing technology as a powerful and cost-efficient tool for high-throughput transcriptome analysis to identify candidate genes involved in erythroid differentiation. We generated a transcriptome profile by ribosomal RNA depleted (ribo-minus) RNA-Seq and miRNA-Seq of undifferentiated embryonic stem (ES) cells, erythroid cells derived from ES cells (ESER), human fetal erythroid liver cells (FLER), and peripheral $CD34^+$ derived erythroid cells (PBER).

We describe an approach we termed Principle Component Analysis (PCA) to analyze the transcript profiles and showed a large variance within the expression profiles among the four different cell types, especially the undifferentiated ES cells. Utilizing Gene Ontology (GO) terms, KEGG pathways and gene functional annotations we found up- and down-regulated networks containing many modules corresponding to many cell components, molecular functions, biologic processes as well as cell signaling pathways.

There are at least 65 miRNA that are expressed only in undifferentiated ES

cells suggesting their crucial role in maintaining pluripotency of ES cells. We identified erythroid specific miRNAs of which 17, 41 and 3 were found in ESER, FLER and PBER, respectively, suggesting their possible function in developmental regulation. It is interesting to note that some of these lineage or developmental specific miRNA have not been annotated in the miRBase database which suggests they are novel miRNAs. The specific target genes of each miRNA were analyzed by TargeScan, PicTar, miRanda, and our own in-house developed bioinformatics algorithms.

To delineate the functional networks responsive to erythroid differentiation, we used the concordantly up- or down-regulated genes as nodes and build up the networks according to GO clusters, KEGG pathways, miRNA targets and protein interactions prediction. Functional analysis is on going to prove our hypotheses.

Differentiation of Lymphatic Endothelial Cells From Bone Marrow Mesenchymal Stem Cells with VEGF

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Although many researches have demonstrated that bone marrow-derived mesenchymal stem cells (MSCs) have the potential to differentiate into mesenchymal tissues like osteocytes, chondrocytes, and adipocytes in vivo and in vitro, little information is available regarding its potential to differentiate into lymphatic endothelial cell. Thus, we investigated the differentiation of MSCs into cells of the lymphatic endothelial lineage. Rat MSCs were isolated from bone marrow aspirate of Sprague-Dawley rats as previously described, and flow cytometry to detect the surface markers: CD31, CD29 and CD90. Purified MSCs were then plated onto dishes at cell density of 1 to 1.5×10^4 cells/cm^2 in the presence of VEGF (20 ng/mL) and / or VEGF-C (50 ng/mL) cultured in differentiation medium for 10 days to media containing 2% FBS, then examined prox-1 and LYVE-1 by immunocytochemistry, RT-PCR, Western Blot. The immunocytochemistry, RT-PCR, Western Blot demonstrated that compared to control group, the groups of VEGF-A, VEGF-C and VEGF-A +VEGF-C express prox-1 and LYVE-1. Our results demonstrated that in differentiation medium, MSCs treating with VEGF-A, VEGF-C and VEGF-A + VEGF-C individually promote lymphatic endothelial cell formation in vitro, and express the marks of Prox-1and LYVE-1.

Analysis the advantage of autologous mobilized peripheral blood mononuclear cells transplantation on lower limbs ischemia diasease

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Objective: Through operating autologous mobilized peripheral blood mononuclear cells transplantation on 59 patients with lower limbs ischemia, and analyzing the correlation of therapeutic effect and species of transplanted cells, Demonstrated the advantage of mononuclear cells transplantation on clinical practice ;

Methods: Made the scores on the symptoms of all the patients before therapy, and then, injected the cells onto multiple points at muscle-rich body positions at equal intervals after mobilizing and collecting the peripheral mononuclear cells from all patients. After that, made the scores on the symptoms of all the patients at 7^{th} day and follow-up period. Compared the correlation of CD34+cells and mononuclear cells with therapeutic effect respectively, and made the relative formula between them and therapeutic effect;

Results: After mononuclear cells transplanting, the therapeutic effect at 7^{th} day and follow-up period improved to some extent, Demonstrated mononuclear cells had a higher correlation with therapeutic effect than CD34+cells (nimodipine value was on behalf of therapeutic effect); The correlation between CD34+cells and therapeutic effect was R=0.461（P=0.047）, the formula was nimodipine value =0.484+1.055×CD34+cells quantity; The correlation between mononuclear cells and therapeutic effect was R=0.473（P=0.000）,the formula was nimodipine value =0.288+0.401×mononuclear cells quantity;

Conclussion: autologous mobilized peripheral blood mononuclear cells have better therapeutic effect than CD34+cells transplantation.

Keywords: mononuclear cells, CD34+ cells, endothelial progenitor cells, transplantation, ischemia

Regulation of erythroid differentiation by skeletal and membrane proteins

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In the process of red blood cell maturation, hematopoietic stem cells (HSCs) undergo a series of differentiations, yet the mechanism for regulating HSC differentiation is unknown. Membrane and skeletal proteins can be asynchronously synthesized and assembled during erythroid differentiation, suggesting these proteins play a critical role in erythroid differentiation.

By using next-generation high-throughput sequencing techniques, we have comprehensively profiled mRNA expression patterns of all the skeletal and membrane proteins in undifferentiated human embryonic stem cells (ES), erythroid cells derived from ES cells (ESER, embryonic stage), human fetal erythroid liver cells (FLER, fetal stage), peripheral $CD34^+$ derived erythroid cells (PBER, adult stage). The results demonstrated the progressive accumulation of most major skeletal and membrane proteins exception of actin and tubulin, the principal components of skeletal proteins, and the protein components at the defined stages during erythroid differentiation. The mRNA expression level of the actin family proteins decreases rapidly compared to that of the tubulin that is almost retained stable during erythroid differentiation.

To further delineate the mechanism of one membrane or skeletal protein involved in erythroid differentiation, we have also accurately mapped the DNaseI hypersensitive sites (DHs) for each membrane and skeletal protein genomic regions in the above 4 cell lines and some non-erythroid primary cells or cell lines. Motif-based sequence analysis tools MEME were applied to analyze the potential transcription factor binding sites in these DHs. Dual luciferase reporter gene assay and Felsenfeld assay will be used to identify the regulatory elements regulating erythroid differentiation including promoters,

enhancers, insulators, boundary elements, locus control regions, and other *cis*-regulatory elements. Chromatin immunoprecipitation (ChIP) will be used to confirm if a transcription factor candidate or other proteins are capable of binding to the given motif. And chromosome conformation capture (3C) will be used to determine the interactions between the identified erythroid-specific regulatory elements and globin genes as well as other known erythroid regulators. Based on these works, we will further understand the regulatory mechanism for erythroid differentiation by the membrane and skeletal proteins.

Stem Cells Transplantation Clinical Therapy for Children Cerebral Palsy（Attach 40 patients report）

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Objective： to analyze and conclude the motor function of 40 cerebral palsy children patients undergoing umbilical cord mesenchymal stem cells transplantation.

Methods: after regular body check, 40 children patients were carried out randomly. Whole treatment course include umbilical cord mesenchymal stem cells transplantation once a week, four times operations; therapeutic approaches include: one time of venoclysis transplantation and three times of lumbar puncture subarachnoid space transplantation; neurotrophic factors, physical therapy, and specific treatment were necessary. Investigate the healing process in 6-12 month, average 8.5month. GMFM-88, Ashworth scale were applied for motor function assessment; SPSS statistical software used for analyzed the results.

Result: after one stem cell treatment course, the motor functions of the children patients improved at different levels comparing before transplantation; and the healing process were keeping improved after treatment 6 month and more.

Conclusion: stem cell transplantation can improve cerebral palsy children patients' motor function significantly, but therapeutic mechanism needs to investigate and research in depth in the further time.

Analysis of erythroid-specific enhancers in KLFS genomic regions

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More than 25,000 coding genes have been identified from the human genome sequence, but little is known about how most of them are regulated. Mapping DNase I hypersensitive sites (DHSs) is a powerful experimental method for identifying promoters, enhancers, insulators, boundary elements, locus control regions, and other *cis*-regulatory elements. We applied a novel approach of chromatin profiling DNase-tags, using next-generation high-throughput sequencing techniques, to accurately profile the DNase I hypersensitive sites in 16 KLF and 7 Sp genomic regions of undifferentiated human ES cells and three stages of human erythroid cell development: ES-derived erythroid cells; human fetal liver erythroid cells; and BFU-E culture derived erythroblast.

Sp1- and Krüppel-like factors (Sp1/KLFs) are highly conserved zinc-finger proteins that are important components of the eukaryotic cellular transcription machinery. By regulating the expression of a large number of genes with CACCC/GC-rich promoters, Sp1/KLF transcription regulators may take part in diverse cellular and physiological processes, including cell proliferation, differentiation, apoptosis, tissue development, tumorigenesis and determination of pluripotent stem cell fate. KLF1 and KLF3 are expressed specifically in erythroid cells, suggesting a more cell-type-specific function for these factors. Indeed, the knockout of KLF1 results in selective defects in erythropoiesis.

We have identified 9 KLFs gene specific enhancers from 27 erythroid specific DHSs using dual-luciferase reporter transient transfection assays. Motif-based sequence analysis tools MEME and Weeder were used to analyze the potential transcription factor (TF) binding sites in these DHSs. Electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP) are used to confirm if a candidate TF or other proteins are capable of binding to the given motif *in vitro* and *in vivo*. Chromosome conformation capture (3C) will be used to determine the interactions between erythroid KLFs enhancers and globin genes as well as other known erythroid regulators. The transcriptional regulatory networks of KLFs and their roles in erythroid differentiation will be further investigated.

Umbilical Cord Derived Mesenchymal Stem Cells Isolated by a Novel Explantation Technique Can Differentiate Into Functional Endothelial Cells and Promote Revascularization

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Background—Stem cells transplantation holds great promise for the treatment of ischemic diseases through functional revascularization. Umbilical cord derived mesenchymal stem cells (UC-MSCs) are also an ideal candidate for use in cell-based bioengineering. However, many issues remain to be further explored prior to their widespread application. Herein, we report on the development of a simple and effective protocol to isolate UC-MSCs, and confirm their potential to differentiate into the functional endothelial lineage, both *in vitro* and *in vivo*.

Methods and results—This study presents a novel explantation technique, used to successfully isolate MSCs from human umbilical cord. Morphological

assessment and proliferation analysis show that sufficient MSCs can be generated during a relatively short culture period and that lumen-like endothelial structures form *in vitro*. Increased expression of endothelial-specific markers (KDR, CD144, and vWF), and functional markers (ac-LDL uptake and UEA-1 binding), indicate that functional endothelial progenitor cells (EPCs) are induced after 9 days of *in vitro* culture. In an ischemic hindlimb mouse model, the ratio of ischemic/non-ischemic limb perfusion 4 weeks after MSC transplantation reached 0.84±0.09. The capillary density of this group was 2.57 fold greater than that of sham injected mice ($p<0.05$). Immunofluorescence and immunohistological analyses indicate that MSCs may act to salvage the ischemic tissue by incorporating into the local murine vasculature. *In vitro*, UC-MSCs were observed to incorporate into 3D Gel/MMT-CS composite scaffolds, to secrete extracellular matrix, to remain viable, and to retain their proliferation capacity.

Conclusion—UC-MSCs isolated by our novel yet simple explantation technique are well suited for application in the development of novel stem cell-based revascularization therapies.

Chemotaxis of AGM Mesenchymal Stem Cells Induced by Mouse Embryonic Circulation

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Ontogeny of mesenchymal stem cells (MSCs) in mammalian mid-gestation conceptus is poorly understood. Here, we identify canonical MSCs in the mouse E11.5 aorta with surrounding mesenchyme (AoM), the sub-region of aorta-gonad-mesonephros (AGM). They possess homogenous immunophenotype ($CD45^-CD31^-Flk\text{-}1^-CD44^+CD29^+$), perivascular cell markers ($\alpha\text{-}SMA^+NG2^+PDGFR\beta^+$), and differentiation potential (bone, adipose and cartilage). CFU-F assay reveals the frequency of tri-potent MSCs is very low. Interestingly, MSCs are also detected in E12.5-E14.5 circulation, implying the intravasation like hematopoietic stem cells. Functionally, E12.5 embryonic blood triggers efficient migration of AoM-MSCs through PDGFR-, TGF-β receptor-, but not bFGF receptor-mediated signaling. Moreover, JNK and PI3K signaling play important roles in embryonic blood- or PDGF-mediated chemotaxis of AoM-MSCs. Importantly, migration of human AGM-MSCs is also promoted by embryonic bloodstream via similar mechanisms. Thus, embryonic circulation, in addition to its conventional transporting roles, can modulate chemotaxis of MSCs during early embryogenesis.

ERK 1/2 signaling in hepatic differentiation of human umbilical cord-derived mesenchymal stem cells

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Human umbilical cord-derived mesenchymal stem cells (hUCMSCs) are expected to be a potential source for stem cells therapy. Although several research have shown that hUCMSCs can differentiate into hepatocyte-like cells, the efficiency is insufficient to produce enough functional hepatocytes for clinical purpose and the mechanisms is unclear. To define the important signals that roled in hepatic differentiation of hUCMSCs, hepatic differentiation was induced with hepatocyte growth factor (HGF)/oncostatin M (OSM)/fibroblast growth factor 2 (FGF2) and fetal liver-conditioned medium from embryonic days (ED) 9.5 at different time respectively. hUCMSCs can differentiate into hepatocyte-like cell in two kinds of induction and fetal liver-conditioned medium exert a more rapid and efficient influence than HGF/ OSM/ FGF2. We found activation of ERK1/2 signaling were found in fetal liver-conditioned medium treated hUCMSCs at 24 hours during the induction, which was not found until 84 hours in the group of HGF/ OSM/ FGF2 induction. Furthermore, u0126, a inhibitor of ERK 1/2, can significantly attenuate the expression of hepatocyte specific gene and hepatic function in fetal liver-conditioned medium group. The suppression of ERK1/2 decreased the differentiation of hUCMSCs into hepatocyte-like cells. Our findings suggest that ERK1/2 signaling plays a important role in hepatic differentiation of hUCMSCs.

Keywords: hucMSCs; hepatic differentiation; ERK signaling

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From stem cells to platelets: the role of cytokines, serotonin and herbal medicines

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Thrombopoiesis involves the proliferation and differentiation of haematopoietic stem/ progenitor cells into immature megakaryocytes (MK), and the differentiation of MK to produce platelets. The former is regulated mainly by thrombopoietin (TPO) and to a lesser degree by IL-1, IL-3 and PDGF. Several transcription factors have been implicated in the control of megakaryocyte differentiation. *GATA-1, FOG-1* and *Fli-1* are essential regulators in early- and mid-stages of megakaryocytopoiesis. *NF-E2* regulates late-stage of megakaryocytopoiesis and platelet production. However, the platelet release mechanism is poorly understood. TPO also have non-haematopoietic functions (Circulation, 2006). Our results showed that pretreatment with TPO significantly increased viability of DOX-injured H9C2 cells and beating rates of neonatal myocytes, with effects similar to those of dexrazoxane, a clinically approved cardiac protective agent. In the mouse model, administration of TPO significantly reduced DOX-induced cardiotoxicity. These data have provided the first evidence that TPO is a protective agent against DOX-induced cardiac injury. Serotonin is a monoamine neurotransmitter that has multiple extraneuronal functions. We previously reported that serotonin exerted mitogenic stimulation on megakaryocytopoiesis mediated by 5-HT2 receptors (Stem Cells, 2007). We further investigated effects of serotonin on ex vivo expansion of human cord blood CD34(+) cells, bone marrow stromal cell CFU-F formation, and antiapoptosis of megakaryoblastic M-07e cells. Our result showed that serotonin is a growth factor for hematopoiesis. Chinese Danggui *(Radix Angelicae Sinensis*) has been used for centuries to treat blood-deficiency related diseases. The hematopoietic effect of Danggui may be related to its constituent, polysaccharide. In this study, we specifically investigate the thrombopoietic effect of Angelica polysaccharide (APS) in a mouse model and its molecular mechanism. APS as well as TPO significantly enhanced the recovery of platelet and WBC count, and bone marrow CFU-MK

and CFU-GM formation. Morphological examination of bone marrows showed that APS treatment significantly increased the recovery of the megakaryocytic series. Addition of Ly294002 alone increased the percentage of cells undergoing apoptosis. However, additional of APS to Ly294002-treated cells reversed the percentage of cells undergoing apoptosis. Furthermore, addition of APS significantly increased the phosphorylation of AKT. APS promotes thrombopoiesis in a mouse model. This effect is likely to be mediated by the PI3K/AKT pathway.

Safety evaluation of allogeneic umbilical cord blood mononuclear cell therapy for degenerative conditions

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Background: The current paradigm for cord blood transplantation is that HLA matching and immune suppression are strictly required to prevent graft versus host disease (GVHD). Immunological arguments and historical examples have been made that the use of cord blood for non-hematopoietic activities such as growth factor production, stimulation of angiogenesis, and immune modulation may not require matching or immune suppression.

Methods: 114 patients suffering from non-hematopoietic degenerative conditions were treated with non-matched, allogeneic cord blood. Doses of 1-3×10^7 cord blood mononuclear cells per treatment, with 4-5 treatments both intrathecal and intravenously were performed. Adverse events and hematological, immunological, and biochemical parameters were analyzed for safety evaluation.

Results: No serious adverse effects were reported. Hematological, immunological, and biochemical parameters did not deviate from normal ranges as a result of therapy.

Conclusion: The current hematology-based paradigm of need for matching and immune suppression needs to be revisited when cord blood is used for non-hematopoietic regenerative purposes in immune competent recipients.

Induction of human bone marrow mesenchymal stem cells differentiation into neural-like cells using cerebrospinal fluid

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Objective: To optimize a technique that induces bone marrow mesenchymal stem cells (BMSCs) to differentiation into neural-like cells, using cerebrospinal fluid (CSF) from the patient.

Methods: *In vitro*, CSF (Group A) and the cell growth factors EGF and bFGF (Group B) were used to induce BMSCs to differentiate into neural-like cells. Post-induction, presence of neural-like cells was confirmed through the use of light and immunofluorescence microscopy.

Results: BMSCs can be induced to differentiate into neural-like cells. The presence of neural-like cells was confirmed via morphological characteristics, phenotype and biological properties. Induction using CSF can shorten the production time of neural-like cells and the quantity is significantly higher than that obtained by induction with growth factor ($P<0.01$).

Conclusion: The two induction methods can induce BMSCs to differentiate into neural-like cells. Using CSF induction, 30ml bone marrow can produce a sufficient number of neural-like cells that totally meet the requirements f orclinical treatment.

Human umbilical cord Mesenchymal stem cells affect the immunomodulation in autoimmunemyasthenia gravis rats

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Mesenchymal stem cells have been attracting people's attention for their immumodulatory potential. Here, we test human umbilical cord mesenchymal stem cells' (UCMSCs) effect on rats of experimental autoimmune myasthenia gravis rats. Intravenous administration of xenogeneic UCMSCs to EAMG rats on the day of their second immunization was effective in inhibiting the T cell proliferation in vivo. We also found that UCMSCs can significantly inhibit AchR-T cell proliferation in vitro. Through biochemical cytokines and cell-cell contact, UCMSC increased expression of ICAM-1 on CD4+ T cells and their production of IL-17, IL-6, IL-4 and IFN-r in vivo as compared with EAMG group. Furthermore, UCMSCs can increase the amount of Treg cells and the expression of foxp3 in these cells. Additionally, UCMSCs was able to migrate to lympho organs where they interacted directly with immune cells. High dose of UCMSCs also decreased the clinical scores of EAMG rats. In summary, systemic delivery of UCMSCs is beneficial for the treatment of EAMG animals, the underlying mechanisms may relate to their capability to regulate the immune system. Our data provide more understandings about the MSC-base cell therapy on MG and UCMSCs transplantation could be a potential strategy to treat MG.

Pancreatic Carcinoma Treated Using Dendritic Cells Pulsed by Cancer Cells with α-Gal Epitopesand DC Activated Naïve T cells

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Alpha-Gal epitopes were added onto pancreatic adnocarcinoma cell surface using neuraminidase and recombinant bovine -1,3-galactosyltransferase. -1,3-GT). The processed cancer cells in the presence of natural anti-Gal IgG antibody, resulted in the opsonization of effective phagocytosis by autogolous dendritic cells (DCs). They were co-cultured with naïve T cells, which was induced from autologous bone marrow heamatopoitic cells *ex vivo*. The primary goal of this new technique, cancer antigen pulsed DCs plus tumour antigen activated T cells, was to assess the safety and bioactivity of this novel therapy. The survival, adverse events and immune response against autogolous tumour associated-antigens (TAA) were measured. Twenty-nine patients with stage IV pancreatic ductal carcinoma or adnocarcinoma participated in the study, 15 for the control, 14 for the trial, ranging in age from 35 to 90 years, there was no significant difference of the age between the two groups. After the treatment, thirteen patients showed positive delayed-type hypersensitivity reactivity to the autologous tumour lysate, and robust systemic cytotoxicity as elicited by IFN- and IL12(70p) expression by peripheral blood mononuclear cells in response to tumour lysate, which was evaluated by the ELISPOT assay. Almost all of them had CD8, CD45RO and CD56-positive cells increasing in the peripheral blood lymphocytes. Laboratory test demonstrated CA19-9 and CEA decreasing in 8 of 14 patients. Clinical investigation showed that the procedure was safe, no serious side effects and no autoimmune disease were noticed. The therapy could significantly prolong the survival compared with the control, (106.6$\pm$33.7 vs 39.3$\pm$22.4 weeks, $p<0.0001$), four patients in the trial group having been survived with stable condition for nearly three years.

Resveratrol and its analogues - isorhapontigeni, heyneanol-A, protect mouse hematopoietic cells from ionizing radiation damage

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Background: Resveratrol (3,5,4'-trihydroxy-trans-stilbene), a kind of polyphenol compounds, which shows anti-oxidant, anti-apoptosis and lifespan extension effects, was reported in current studies. Here we investigate whether resveratrol (Res) and its analogues isorhapontigeni (ISO), heyneanol-A (VAM4) protect mouse hematopoietic cells from ionizing radiation damage.

Methods: Male C57BL/6 mouse bone marrow mononuclear cells (BMMNCs), separated by density gradient centrifugation, were incubated with Res, ISO and VAM4(from 10^{-3} mol/L to 10^{-9}mol/L) at 37℃ for 30 minutes, exposed to γ-ray at dose of 1Gy and 4Gy. Luminiscence assay was performed to evaluated cell viability that manifested drugs' radio protective effect at BMMNC level. Cloning-forming ability of BMMNCs is determined by CFU-GM in order to quantify healthy hematopoietic progenitors. Furthermore, competitive transplantation experiments were accomplished to assess the proliferation of hematopoietic stem cells.

Results: Compared with irradiation group, irradiated BMMNCs viability were increased by 33.32%, 22.8% and 37.21% respectively after incubation with the Res (1×10^{-8}M, $p < 0.05$), ISO (1×10^{-7M}, $p<0.05$) and VAM4 (1×10^{-6}-1×10^{-8}M, $p<0.05$). Irradiated cells exhibited a diminished ability to form CFU-GM, whereas treatment of cells with the three compounds caused a moderate but still significant recovery of the ability. CFU-GM rise by 32.4-88.2%，26.5%，32.4% ($p<0.05$) respectively after the three compounds treatment. Treated progenitors with VAM4 and Res have a competitive advantage over irradiated cells in competitive transplantation experiments and a diminished myeloid bias

given ameliorated lymphoid cell decline.

Conclusion: At BMMNC and HPC level, Res, ISO and VAM4 protect hematopoietic cells from radiation induced injury. In addition, Res and VAM4 may have the protective effect at HSC level in vitro experiments.

Regulation of erythroid differentiation by KLF3

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KLF3 (BKLF) is a member of the Sp1/Kruppel-like (KLF) zinc finger transcription factor family characterizing of a highly conserved DNA-binding domain at their carboxyl terminus that has three tandem Cys2His2 zinc-finger motifs. Together with KLF1, an erythroid-specific transcription factor, KLF3 is one of the most abundant CACCC-binding proteins in erythroid cells. The KLF3 deficient mice exhibit a myeloproliferative disorder which suggests that KLF3 plays an important role in haematopoietic differentiation.

By using next-generation high-throughput sequencing techniques, we have profiled KLFs mRNA expression pattern as well as DNaseI hypersensitive sites in the KLFs genomic regions in undifferentiated human ES cells (ES), erythroid cells derived from ES cells (ESER, embryonic stage), human fetal erythroid liver cells (FLER, fetal stage), peripheral $CD34^+$ derived erythroid cells (PBER, adult stage), and some non-erythroid primary cells or cell lines. The results demonstrate that KLF3 specifically participates in the regulation of erythroid differentiation.

To further delineate the molecular role and mechanism of KLF3 in erythroid differentiation we attempt to clarify the dynamic changes of transcription factor complexes by proteomics methodologies. We established stable cell lines K562 with KLF3 over-expressed and depleted using retrovirus- or lentivirus-mediated gene transfer system. The tandem affinity purification columns were used to isolate KLF3 associated protein complexes before and after induction by hemin which can accelerate the hemoglobin production and erythroid differentiation. The eluted proteins will be analyzed and identified by LC-MS/MS. The components of the protein complex will be analyzed by gel filtration chromatography. Electrophoretic mobility shift assay (EMSA) and

chromatin immunoprecipitation (ChIP) are used to confirm the candidate proteins that are capable of binding to the genomic regions of alpha- and beta-globin as well as other known erythroid regulators *in vitro* and *in vivo*. We will also observe the influences of the identified regulatory elements on erythroid differentiation. Chromosome conformation capture (3C) will be used to determine the interactions between KLF3 and other known erythroid regulators.

Based on these works, the similar strategy will be used in human embryonic stem cells as well as hematopoietic cells to address the detailed molecular mechanism of erythroid differentiation regulated by KLF3.

Derivation and characterization of ovine embryonic stem-like cell lines in defined conditions

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Domestic animal embryonic stem (ES) cells provide an invaluable research tool for genetic breeding and transgenic animal. Unfortunately, authentic domestic animal ES cells have not been established despite progress made over more than two decades. Here, we show that ovine embryonic stem-like (ES-like) cells can be efficiently derived and propagated in a fully defined medium that contains N2, B27, GSK3 specific inhibitor (CHIR99021) and basic fibroblast growth factor (bFGF). These ovine ES-like cells have a characteristic three-dimensional appearance, showed a dose-dependent feature of bFGF, and maintain for twenty-three passages. The cells express common molecular markers of stem cell pluripotency, Oct4, Nanog, Sox2, SSEA-1, SSEA-4 and alkaline phosphatase, but unexpectedly, the cell lines have a low capacity of differentiation in vitro and in vivo and fail to contribute to embryonic development upon blastocyst transplantation. To our knowledge, this is the first experiment to use a defined medium to derive ES-like cells from ovine blastocysts, and opens the door to deriving authentic ES cells from domesticated ungulates.

Key words: Ovine; Embryonic Stem cells; Self-renewal; Pluripotency; Chemical-defined medium

Isolation and Characterization of Cervical Cancer Stem Cells through Stem Cell-Specific Marker Sox2

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The cancer stem cells (CSCs) that are resistant to conventional cancer therapies and response for tumor recurrence and metastasis have recently attracted a great deal of attention, owing to the promise of a novel cellular target for the treatment of human malignancies. Although CSCs has been characterized in several solid tumors, CSCs in cervical cancer have not been well isolated and identified. Previously, we had observed the important role of Sox2, a stem cell-specific marker, in cervical carcinogenesis. To explore the relationship between Sox2 and cervical cancer stem cells, cervical cells were stably transfected with a plasmid containing the human Sox2 promoter elements driving an enhanced green fluorescent protein (EGFP) reporter, and were sorted into EGFP-negative and EGFP-positive populations by fluorescence-activated cells sorting (FACS). The tumor-forming capacity of and EGFP-positive cell populations were approximately 100-fold more tumorigenic ability than EGFP-negative cells, capable of forming tumors at doses of 100 cells, when injected subcutaneouly into NOD/SCID mice. Furthermore, the EGFP-positive cells were capable of regenerating tumors heterogeneous for Sox2 expression, resembling their parent cells. Altogether, all the data demonstrate that EGFP-positive cervical cancer cells have self-renewal capacity, differentiation capacity, and increased tumorigenecity, which are consistent with the CSCs model of tumorigenesis in other solid tumors, and implicate a functional link between the expression of endogenous Sox2 and cervical cancer stem cells. Our studies provide a useful tool to efficiently isolate viable populations of rare CSCs from the bulk of tumor cells based on their expression of nuclear protein Sox2, instead of cell surface markers.

Construction of RNAi lentiviral vector targeting mouse Islet-1 gene

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Object Construct and select RNAi lentiviral vectors targeting mouse Islet-1 gene.

Methods 3 groups of ShRNA oligo(Sh1,Sh2 and Sh3) of mouse Islet-1 gene were designed, synthesized and then inserted to the digested PLVTHM vector. Positive colognes were then selected and identified by PCR and sequencing. After being amplified by E.coli positive colognes were then infected into 293T along with the other helper plasmid to produce lentiviral vector. Test it's titer with plaque formation test. Infect the vector into $C_3H_{10}T1/2$ cells, testing its transfect efficiency with flow cytometry and its RNAi efficiency by Q-PCR.

Results sequencing and PCR showed that we inserted the right DNA fragment; titer values were 3.87×10^8TU/ml; the of its infect to $C_3H_{10}T1/2$ cells were over 90.36%; all the 3 ShRNA targets worked and the first group was the most obvious (76.8%), $P<0.05$ compared with normal cells.

Conclusion RNAi lentiviral vector that can silence mouse Islet-1 effectively was constructed and laid the foundation for further investigation of Islet-1.

Key words: Lentiviral vector; RNAi; Islet-1; MSCS differentiation

A Combinatorial Approach for Development of Synthetic Surfaces for Stem Cell Culture

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Development of scalable, reproducible, and regulatory-friendly synthetic surfaces for stem cell culture has been a challenge for cell therapy applications. Combinatorial chemistry and parallel screening approaches have been used to identify biological and biomimetic surfaces to culture stem cells with varying success. Here, we describe the combinatorial screening of a variety of acrylate polymers conjugated with different adhesion peptide epitopes with human neuronal stem cells. Interestingly, the hit surfaces shared a common sequence of RGD in their peptide epitope. Impact of the polymer properties and peptide epitopes on cell culture properties were also studied. The optimized hit from this approach was validated with long term propagation and terminal differentiation. Doubling time, fold expansion, cell morphology and cell surface markers were characterized on the hit and found to be similar to biological control. The same surface has also demonstrated multi-passage culture on multiple cell lines including human embryonic stem cells (hESC), human mesenchymal stem cells (hMSC) in animal-free, serum-free chemically defined media.

A novel mesenchymal stem cells can be obtained from pubertal goat testis

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With the objective of studying the functions of different populations of cells in goat testis, the CDDT (2mg/ml collagenase, 2mg/ml disperse, 20ug/ml DNase and 1mg/ml trypsin) compound enzyme was used to digest the pubertal testis. The cells were cultured on the Petri dishes treated with 2mg/ml gelatin at a low density, in the medium of DMEM added with 10% fetal bovine serum(FBS) and 10ng/ml basic fibroblast gowth factor (bFGF) at 38.5℃, 5% CO_2 and saturated humidity, the medium was changed 24 hours later and the medium would be changed every two days. After cultured 8 days, the cells were attained nearly 70%-80% fusion, and would be passaged after digested by 0.05% trypsin. These cells would be named as tMSCs. The cells appears to be typical fibroblast cells, and would be passaged every 2-3 days when the passage ratio was controled at 1:3, but the cells showed obvious phenominon of senescence after 8^{th} passage. When examined by Flow cytometry, the cells showed CD29, CD44 and CD166 positive, and CD71, CD34 negtive. Immunocytochemistry showed the cells expressed Oct4, Sox2, Klf4, PCNA, TERT, E-Cadherin and weakly expressed C-Myc, C-kit, and negtive for Nanog. The cells also showed negtive for Alkaline phosphatase (AP) and can be induced to form Embryoid bodies (EB) and differentiated into all three germ layers. These data proved the cells possess stemness and may provide the evidence that there are other stem cells existed in the testis beyond the germline stem cells, the functions of testis may not only dependent on germline stem cells. It may stimulate the studies of Reproductive Biology.

致 谢

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