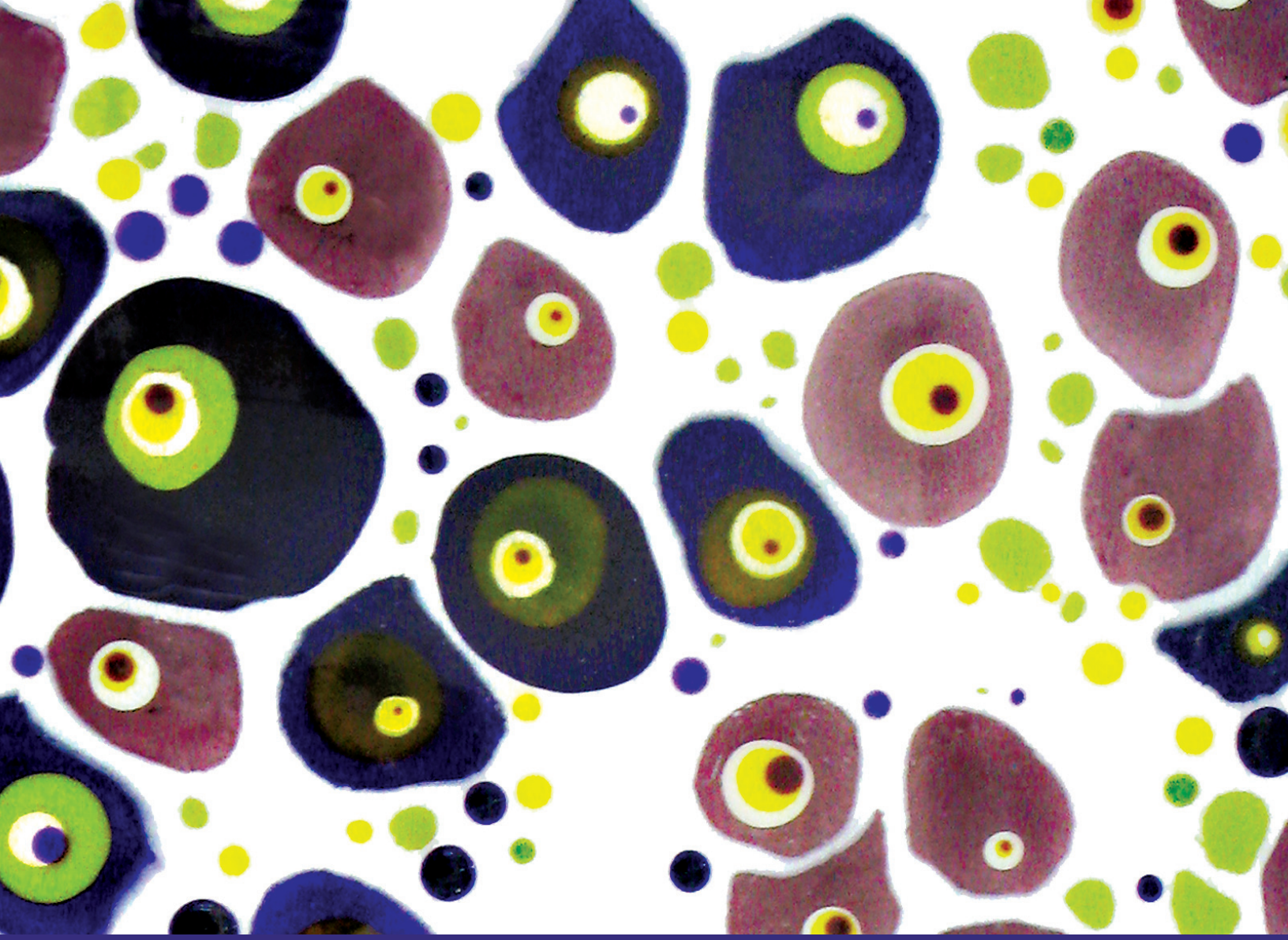

CIRM GRANTEE MEETING

2008

CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE

Dear CIRM grantees and guests,

Welcome to our first CIRM Grantee Meeting, which we want to be a different and productive experience for you. The purpose of the meeting is to build and consolidate an integrated stem cell research program in California that will rival any network of contemporary science anywhere in the world. This meeting will help introduce us to each other, improve our knowledge of the research capacity, special equipment and methodology available, and lay the foundations for developing working relationships that will help us achieve the maximum output possible.

The experiment of Proposition 71 is a critical model for funding innovation that is being closely examined worldwide as a new investment by the community in improving human social values and health at a time when reflection on the impact of consumerism is evident. Investment in quality of life through the support of stem cells and regenerative medicine needs to be demonstrated to have been a wise choice. Our grantees are the vanguard of this investment in science and we need to be able to clearly demonstrate the benefits this investment will make in the general community. In the end this will have to be shown as treatments in the clinic in the form of new drugs, cell therapies, gene therapies, tissue engineering etc., for some of the most debilitating diseases and injuries presently costing the society and individuals dearly.

The CIRM staff has organized an interesting mixture of presentations and breakthrough scientific sessions that will introduce you to several of your California colleagues and will address challenging issues that are evident in the stem cell research environment. We hope that in the course of this meeting you will have made many new friendships, talked to new interesting colleagues, worked out some new perspectives and collaborations, exchanged methods and materials, and generated new ideas that might grow into yet more CIRM granting opportunities. It should be a “cool” meeting and we want you to come away being proud of what is being done here, and what can be achieved.

We also want to get your feedback on this conference and how we might improve this meeting in the future to enhance the outcomes of your research by linkage with other scientists and projects.

Thank you, and enjoy the meeting!

Alan Trounson
President
California Institute for Regenerative Medicine

Agenda

WEDNESDAY SEPTEMBER 17th 2008

5:00 – 9:00 pm — **Poster Set Up** — **Parc 55 Atrium**

6:00 – 8:00 pm — **Registration** — **Contemporary Jewish Museum (CJM)**

6:30 – 7:30 pm — **Inaugural Event at the Contemporary Jewish Museum**: *Emerging issues in stem cell research* — **CJM**

Moderator: Monya Baker, Nature Reports Stem Cells
Alan O. Trounson, CIRM President
Arnold Kriegstein, University of California San Francisco
Lawrence Goldstein, University of California San Diego
Christine Mummery, University of Utrecht

7:30 – 9:30 pm — **Reception** — **CJM**

THURSDAY SEPTEMBER 18th 2008

7:00 – 8:00 am — **Breakfast, Poster Set Up** — **Parc 55 Atrium/Ballroom**

8:00 – 8:10 am — **Introductory Remarks** — **Parc 55 Ballroom**

Alan O. Trounson, CIRM President

8:15 - 9:00 am — **Keynote Presentation** — **Parc 55 Ballroom**

Irving Weissman, Stanford University
Normal and neoplastic stem and progenitor cells

9:15 – 11:30 am — **Functional Integration of Cells into Mature Tissue** — **Parc 55 Ballroom**

Chair: Fred Gage, Salk Institute for Biological Studies
CIRM representative: Rosa Canet-Aviles

Fred Gage, Salk Institute for Biological Studies
Functional integration of new neurons in the adult brain

Nobuko Uchida, Stem Cells Inc
Title TBD

David Schaffer, University of California Berkeley
Molecular engineering of synthetic stem cell microenvironments

Anthony Atala, Wake Forest University Baptist Medical Center
Organ engineering

Kenneth Chien, Massachusetts General Hospital
Title TBD

Agenda (continued)

THURSDAY SEPTEMBER 18th 2008 (continued)

11:45 am – 1:15 pm **Lunch** **Parc 55 Piazza**

1:15 – 2:45 pm **Plenary Session** **Parc 55 Ballroom**

Thea Tlsty, University of California San Francisco
Mechanisms of epigenetic plasticity in stem cells and tumor cells

Paul Fairchild, Oxford Stem Cell Institute
Taming the immune system in the pursuit of regenerative medicine

3:00 – 5:00 pm **Stem Cells, Cancer and the Immune Response** **Parc 55 Ballroom**

Chair: Stuart Orkin, Dana Farber Cancer Institute
CIRM representatives: Sohel Talib and Michael Yaffe

Julien Sage, Stanford University
The retinoblastoma gene family in stem cells and cancer initiation

Emmanuelle Passegué, University of California San Francisco
JunB limits hematopoietic stem cell (HSC) functions as a protective mechanism against initiation of myeloid malignancy

Richard Boyd, Monash University and Norwood Immunology
Thymus -based strategies for reconstituting immunity and inducing transplantation tolerance for the enablement of stem cell-based therapies

Jeff Bluestone, University of California San Francisco
Immune-modulation of human embryonic stem cell derived islet transplant rejection in mice

5:00 – 5:30 pm **Scientific Presentation**

Ron McKay, NIH
The importance of pluripotent, fetal and adult stem cells in Parkinson's disease

5:30 – 7:00 pm **Poster Session and Reception** **Parc 55 Atrium**

Agenda (continued)

FRIDAY SEPTEMBER 19th 2008

7:00 – 8:00 am **Breakfast** **Parc 55 Atrium/Ballroom**

8:00 – 9:45 am **Plenary Session** **Parc 55 Ballroom**

David Harlan, NIDDK, National Institutes of Health
Generating cells capable of physically-regulated insulin secretion: progress and remaining hurdles

Catriona Jamieson, University of California San Diego
Molecular events involved in initiation and progression of MPDs

10:00 am – 12:00 pm **Translating Cell Therapy** **Parc 55 Ballroom**

Chair: Marie Csete, California Institute for Regenerative Medicine
CIRM representative: Bettina Steffen

Thomas Rando, Stanford University
Stem cell therapy for muscular dystrophies

Tiziano Barberi, City of Hope
Finding the appropriate animal model in which to test hESC's therapeutic potential

Christine Mummery, University of Leiden
Cardiomyocytes from human embryonic stem cells in drug discovery and disease

Alice Tarantal, University of California Davis
In vivo imaging of stem and progenitor cells for translational research

Alan Lewis, Novocell
Engineering hESC to create a cell therapy to treat insulin-requiring diabetics

12:15 – 1:30 pm **Lunch** **Parc 55 Piazza**

1:45 – 3:15 pm **Plenary Session** **Parc 55 Ballroom**

Anne Brunet, Stanford University
Mechanisms of stem cell maintenance during aging

Konrad Hochedlinger, Massachusetts General Hospital
Understanding nuclear reprogramming

Agenda (continued)

FRIDAY SEPTEMBER 19th 2008 (continued)

3:30 – 5:30 pm **Pluripotency and Reprogramming** **Parc 55 Ballroom**

Chair: Peter Donovan, University of California Irvine
CIRM representative: Uta Grieshammer

Renee Reijo-Pera, Stanford University
hESC and iPSC pluripotency and germ line formation

Miguel Ramalho-Santos, University of California San Francisco
(De) Constructing pluripotency

Thomas Zwaka, Baylor College of Medicine
Embryonic stem cells: building alternative pluripotency networks

Jeanne Loring, The Scripps Institute and the Burnham Institute for Medical Research
Unraveling pluripotence with systems biology

Berta Strulovici, iZumi Bio
Accelerating therapeutic applications of iPS cell technology

For questions regarding the 2008 CIRM Grantee Meeting, please contact:

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No: 1

Engineering a cardiovascular tissue graft from human embryonic stem cells

Oscar Abilez, Beth Pruitt, Joseph Wu, Christopher Zarins
Stanford University

Cardiovascular disease (CVD) affects more than 71 million Americans and 1.7 million Californians. Recently, engineered cardiovascular tissue grafts, including one made from mouse embryonic stem cells (ESC), have shown promising results as a future therapy for CVD. The overall goal of our project is to extend these recent results to human ESC as follows. Aim 1: Electromechanically condition hESC-derived cardiomyocytes. Hypothesis: The in vivo electromechanical conditions that exist in cardiac development are required for the in vitro spatial-temporal organization of hESC-CM. Rationale: Current methods to differentiate hESC into cardiomyocytes (CM) do not result in the spatial and temporal organization required to generate enough force to support a failing heart and avoid arrhythmogenesis. To date, we have developed specially designed stretchable microelectrode arrays (sMEA) for mechanically stretching and electrically detecting and stimulating hESC-CMs. Aim 2: Engineer a hESC-CM based cardiovascular tissue graft. Hypothesis: The techniques established to engineer a mouse ESC-CM based tissue graft can be extended to hESC-CM and improved upon by the addition of biochemically and spatially defined extracellular matrices (ECM) and electromechanical stimulation. Rationale: Recently, mouse ESC-CMs, Matrigel, collagen I, and mechanical stimulation have successfully been combined to engineer a contractile tissue graft. To date we have combined hESC-CMs, ECM, and mechanical stimulation to engineer a cardiovascular tissue graft. Work is ongoing to add combined electromechanical stimulation in an effort to increase spatial-temporal organization. Aim 3: Assess tissue graft viability and function in a small animal model. Hypothesis: Our tissue grafts will remain viable and will exhibit sustained rhythmic contractions leading to improved cardiac function. Rationale: To date, most cardiomyocyte-based cellular/tissue grafts have been studied in experimentally created myocardial infarctions (MI). The limitations of these studies have been in assessing inherent graft function and fate. In our project we have proposed to first implant our tissue graft in rat aortas as a novel test-bed to assess inherent graft function. After characterization in this test-bed, we will implant the grafts over acutely infarcted rat myocardium and then use novel molecular imaging techniques, ultrasonography, echocardiography, and electrocardiography to non-invasively assess in vivo graft viability and function. To date we have implanted our cardiovascular tissue grafts subcutaneously and have been able to non-invasively follow their fate over two months via molecular imaging. By the end of our four year project we believe execution of the above aims will address questions relevant to hESC-based cardiovascular tissue engineering therapies and will provide vital information needed for safe and efficacious future pre-clinical and clinical translation.

No: 2

Expression of decidualization markers by human mesenchymal stem cells

Aghajanova L, Giudice LC
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Introduction: Human endometrium is a unique tissue that undergoes regular dynamic processes of growth, differentiation, sloughing, and renewal. To date, the cellular source(s) that contribute to the complete renewal of endometrium are still unknown. One possible source is stem cells, which have recently been demonstrated to be present in human endometrial tissue. However, the origin of these stem cells is still unclear. One potential stem cell source is mesenchymal stem cells (MSC), which are multipotent cells that reside in the bone marrow. Osteoblasts, adipocytes, muscle cells and chondrocytes originate from MSC in vivo and in vitro. It has been suggested that endometrial stem cells originate from bone-marrow derived MSC, with subsequent development into endometrial stromal and possibly even epithelial cells. Thus, we hypothesized that MSC treated with steroid hormones normally regulating endometrial function will differentiate into endometrial stromal cells.
Materials and Methods: MSC (Cambrex Bioscience) were expanded in DMEM (high glucose) containing 10% defined fetal bovine serum (FBS, HyClone), penicillin and streptomycin. Nearly confluent MSC were treated for 21 days in low-serum medium (2% FBS) with: (1) conditioned medium (CM) from human endometrial stromal fibroblast treated with or without estrogen/progesterone (E2/P4), or (2) 10nM E2/1?M P4, or (3) 0.5mM and 1mM 8-Br-cAMP (cAMP), or (4) BMP2 50nM and 100nM, or (5) cAMP+BMP2, or (6) cAMP+BMP2+E2/P4, or (7) FSH 50ng and 100ng, with vehicle controls at each time point. Each experiment was conducted in duplicate and repeated at least 3 times. Endpoint analyses assessed prolactin and IGFBP1 mRNA by QPCR and protein by ELISA in MSC lysates and culture supernatants respectively after 3, 7, 14 and 21 days of incubation. Stromal lineage of MSC was confirmed by vimentin immunostaining. Expression of several hormone receptors and steroidogenic enzymes by MSC was analyzed.
Results: We observed a dramatic MSC response to treatment with 8-Br-cAMP and activation of the PKA pathway peaking after 14 days of incubation, as revealed by prolactin and IGFBP1 (classical markers of endometrial stromal cell decidualization/differentiation) expression. Prolactin and IGFBP1 mRNA were significantly up-regulated (2600- and 2200-fold respectively) compared to vehicle control cultured for the same time period, while corresponding proteins in MSC culture supernatants were also increased significantly. After treatment with endometrial stromal fibroblasts' CM, E2/P4, or, BMP2, MSC differentiation was not observed. MSC expressed estrogen and progesterone receptors, as well as LH and FSH receptors without significant influence of 8-Br-cAMP treatment on their expression. Expression of some enzymes involved in the steroidogenic pathway (CYP11A1, StAR) was also up-regulated by 8-Br-cAMP. Treatment with recombinant FSH at low doses (50ng) significantly up-regulated mRNA for FSH and LH receptors, as well as ER beta (12.5, 2 and 2.5-fold respectively).
Conclusions: PKA activation in MSC with subsequent expression of classical markers of decidualization and the responsiveness of cells to FSH stimulation suggests the role of these bone-marrow derived cells in the regeneration of endometrial stromal cells after menses. Microarray analyses are ongoing to elucidate other genes involved in this process.

No: 3

BMI-1 in human neural progenitor cells and glioblastoma-derived cells

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Neural Stem Cell Research Center, UCLA, Los Angeles, CA 90095, USA

Brain tumors contain self-renewing, multipotent stem-like cells termed cancer stem cells. These cells, like neural stem cells express the polycomb transcription factor bmi-1, which had previously been shown to be crucial for the self-renewal of murine neural stem cells. Several lines of evidence indicate that its action varies during development and that it plays a larger role during postnatal ages and adulthood. Bmi-1 is expressed in different types of brain tumors (Hemmati et al, 2003, Leung et al, 2004). Recently, using transformed astrocytes, Bruggeman et al (2007) have shown that bmi-1 controls tumor formation (in vitro/in vivo) in an Ink4a/Arf independent manner. In this study, we aimed to analyze the role of bmi-1 in human embryonic and fetal neural progenitor cells (NPC), as well as human glioma primary cells. We propagated cells under neurosphere-forming or adherent conditions in the presence or absence bmi-1 shRNA. We observed that the loss of bmi-1 decreased sphere formation drastically in fetal progenitor cells (decrease of 80%) while having no effect on ES-derived NSC. These results correlate with the observation in murine cells that bmi-1 action is stage dependent and that bmi-1 is required for NSC self-renewal at later stages. In cancer cells, we observed that loss of bmi-1 did not affect unselected GBM-derived cells grown in serum (n= 4). In order to obtain a higher proportion of cancer stem cells, we utilized the neurosphere culture system (Hemmati et al., 2003). Knockdown of bmi-1 reduced the formation of secondary neurospheres in 3 out of 5 GBM patient samples in normoxia or under low oxygen concentration. We hypothesized that the different responses among the GBMs could be due to different genetic alterations of the tumor samples. We found that U87 cells that had high levels of expression of the EGFR variant III isoform (EGFRvIII) were highly dependent upon bmi-1 in vitro, while "standard" U87 cell lines were not. We tested whether this different sensitivity to bmi-1 was only an in vitro effect. An insensitive bmi-1 patient primary cells, "standard" U87 and U87vIII were transplanted intracranialy into nude mice. The in vivo data correlated with the in vitro observation. The loss of bmi-1 did not affect the tumor formation of the insensitive patient line; reduced tumor size of the sensitive lines was observed in the same proportion as the in vitro proliferation data. These preliminary data demonstrate that fetal human neural progenitor cells depend on bmi-1 for their self-renewal. However, ES-derived NPCs appear to be bmi-1-independent. In glioma, there is a heterogeneity of response, with some putative glioma stem cells dependent on bmi-1 for proliferation and some independent in vivo and in vitro. Some of which may be related to the mutation-status of the tumor.

No: 4

Identification of stem and progenitor cell miRNA signatures

Christopher Arnold, Ruoying Tan, Joe Biggs, Regis Doyonnas, Miao-Chia Lo, John M. Perry, Valérie M. Renault, Alessandra Sacco, Tim Somervaille, Anne Brunet, Michael L. Cleary, Linheng Li, Dong-Er Zhang, Helen M. Blau, Caifu Chen, Chang-Zheng Chen
Stanford University School of Medicine, Applied Biosystems, The Scripps Research Institute, Stowers Institute for Medical Research

Little is known about the role of post-transcriptional regulations in stem cell self-renewal and fate determination. MicroRNAs (miRNAs) are ~22-nt small regulatory RNAs that are thought to control gene expression at the post-transcriptional level by targeting cognate target mRNAs for either degradation or translational repression. MiRNA-mediated gene regulation represents a comprehensive layer of genetic programs at the post-transcriptional level, and has been shown to play diverse functional roles in animal development and in the pathogenesis of cancers. Intriguingly, several lines of evidence have implicated miRNAs in the developmental regulatory decisions of stem-cell maintenance and differentiation in flies and mice. To understand miRNA-mediated post-transcriptional genetic programs in stem cells, we first compared the miRNA expression profile of multiple tissue-specific stem cells from mice using miRNA quantitative PCR (qPCR) analyses. We have determined miRNA expression profiles in hematopoietic stem cells (HSCs), muscle stem cells (MSCs), and neural stem cells (NSCs). These analyses revealed a miRNA signature that is shared amongst these stem cell populations, termed "stem cell miRNAs (SC-miRNAs)," and the miRNA signatures that are unique to various tissue-specific stem cells, termed "tissue-specific stem cell miRNAs (TSC-miRNAs). It is likely that SC-miRNAs may play a role in stem cell self-renewal, whereas TSC-miRNAs may be important for the unique regulation that distinguishes the tissue-specific stem cell types. Furthermore, we also analyzed miRNA profiles in PTEN-/- HSCs and acute myeloid leukemia (AML) stem cells and compared their miRNA expression profiles to that of the normal HSCs. PTEN was known to regulate proliferation, survival, differentiation and migration of HSCs, and loss of PTEN results in enhanced HSC activation and short-term expansion which eventually leads to a myeloproliferative disorder (MPD). We have identified miRNAs that are upregulated upon deletion of PTEN, and miRNAs differentially regulated in the AML stem cells versus their differentiated progeny. Such analyses may reveal miRNAs that can account for the alterations in the self-renewal and proliferative capacity of these aberrant stem cells. Finally, using a murine embryonic stem (ES) cell competition assay, we have identified the SC-miRNAs and TSC-miRNAs that can either positively or negatively influence stem cell self-renewal. Amongst these, ectopic expression of the mir-181a-1 gene in ES cells negatively affects ES cell growth. Since mir-181a-1 has been shown to negatively regulate several inhibitors of the ERK signaling pathway in T cells, and ERK signaling is a key regulator of ES cell self-renewal, these finding suggest that mir-181a-1 may regulate the self-renewal capacity of ES cells by modulating ERK signaling. Collectively, systematic characterization of the miRNA expression and function in stem cells and elucidating the molecular networks controlled by these miRNAs would shed insights into the post-transcriptional networks that are required for stem cell self-renewal and fate determination.

No: 5

The role of CHD7, a chromatin remodeler, in human development and disease

Ruchi Bajpai and Joanna Wysocka
Stanford University

ATP-dependent chromatin remodeling is critical for many, if not all chromatin-templated processes such as gene expression regulation, replication, and DNA-damage response. Yet, little is known about the function or mode of action of a major class of chromatin remodelers- the CHD (chromodomain helicase DNA binding) family. Heterozygotic mutations in a gene encoding one of the CHD family members, CHD7, is a major cause of human CHARGE syndrome characterized by a complex set of malformations including coloboma of eye, heart defects, atresia of the choanae, growth retardation, renal and genital abnormalities, and ear defects. In vitro differentiation of human embryonic stem cells (hESC) can be used as a model to recapitulate various developmental processes occurring in the early embryo. We are using hESC and their differentiation to study the molecular function of CHD7 in early developmental decisions and mechanisms underlying defects observed in CHARGE patients. Since a large subset of the CHARGE syndrome phenotypes are likely due to aberrant neural and neural crest development, we have focused on the role of CHD7 in the neural commitment and differentiation of hESC. We have established models of hES differentiation into neural and neural crest cells in chemically defined conditions and demonstrated that these cells can be further differentiated into a wide repertoire of cell types. Our preliminary results suggest that downregulation of CHD7 perturbs proper neural and neural crest differentiation. To address the temporal requirement of CHD7 we have utilized a tetracycline regulatable lentiviral vector to induce the expression of the target shRNAs in narrow time windows during differentiation. We are currently using biochemical and developmental approaches to address the mechanism through which CHD7 participates in the gene expression regulation in the neural cell fate determination. We expect that these studies will contribute to understanding the role of chromatin remodeling in human early neural and neural crest development and shed light on the mechanisms underlying the CHARGE syndrome.

No: 6

Ontogeny strategies for differentiation of human embryonic stem cells toward renal precursors for kidney regeneration and repair

Cynthia A. Batchelder *, C. Chang I. Lee*, and Alice F. Tarantal**
*Center of Excellence in Translational Stem Cell Research, California National Primate Research Center, and **Departments of Pediatrics and Cell Biology and Human Anatomy, University of California, Davis, CA 95616

Human embryonic stem cells (hESC) are important cells to consider for the repair of kidneys damaged by disease if reliable and consistent differentiation strategies can be developed. A primate model of gene expression based on renal ontogeny from mesodermal differentiation through definitive kidney formation was developed and incorporated into these studies. Markers of metanephric mesenchyme (OSR1, PAX2, SIX2, WT1) were initially observed to increase over time during spontaneous differentiation followed by upregulation of kidney precursor markers (EYA1, LIM1, CD24). Directed differentiation was evaluated with the addition of Retinoic Acid, Activin-A, and BMP-4 ("RA4") or BMP-7 ("RA7") to cultures. Of the conditions tested, monolayer cultures on gelatin most closely recapitulated the developmental pattern of renal gene expression, and findings were similar when BMP-4 and BMP-7 were compared. The results of these studies indicate that hESC can be differentiated towards early kidney precursors using RA4 or RA7, and that differentiation efficiency is influenced by culture conditions and substrates. Further studies using combined markers and sorted precursor populations will be necessary to conclusively identify and collect cells that will be useful for kidney regeneration protocols in preclinical nonhuman primate models of human disease.

No: 7

Cellular reprogramming by microRNAs

Inbar Friedrich Ben-Nun[1], Candace Lynch[1], Pauline Liue[2], Jon Chesnut[2], Louise C. Laurent[1] and Jeanne F. Loring[1]
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The success of experimental reprogramming of somatic differentiated cells into embryonic stem cell-like cells has transformed our views of how pluripotence and differentiation are regulated. We have been investigating the molecular basis of pluripotence using an integrated comparative analysis of global mRNA transcription, DNA methylation, and microRNA expression, and have discovered that pluripotent stem cells (embryonic stem cells [hESCs] and induced pluripotent stem cells [iPSCs]) share unique molecular profiles that are linked to their qualities of pluripotence and self-renewal. We are using this knowledge of the molecular interactions underlying the pluripotent phenotype to devise new methods for reprogramming human somatic cells. Our recent comprehensive mapping of microRNA expression in hESCs suggests that these regulatory molecules may play an important role in maintaining the unique pluripotent phenotype. MicroRNAs are small, non-coding RNAs that regulate gene expression through post-transcriptional gene silencing, either by inhibiting translation, by mRNA cleavage or by mRNA destabilization. These small RNA's are involved in regulation of many critical biological processes, including cell proliferation, differentiation, apoptosis, morphogenesis and metabolism. We mapped 800 microRNAs in pluripotent and differentiated cells, and identified several that are consistently and specifically up-regulated or down-regulated in hESCs compared to fibroblasts ("hESC-specific" and "fibroblast-specific", respectively). Our strategy is to overexpress "hESC-specific" microRNAs and repress "fibroblast-specific" microRNAs in human fibroblast cells. We expect that the changes in microRNAs will alter the transcriptional profile, which we predict will lead to cell reprogramming toward pluripotency. In collaboration with Invitrogen, Inc, specific microRNAs and antago-miRs were cloned into a TET-inducible lentiviral vector, which we are using to manipulate microRNA expression in a fibroblast cell line that expresses the TET-repressor and a POU5F1/OCT4-GFP cassette reporter. One practical advantage of a microRNA approach is that the small size of the molecules may allow elimination of the vectors. After validating the approach using the lentiviral vectors, we will introduce the microRNAs directly into cells, with the goal of inducing cellular reprogramming without the requirement of permanent genetic changes in the cells.

No: 8

Understanding the molecular mechanisms of Rett Syndrome using phenotypic MeCP2 knockdown human neurons

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Rett Syndrome (RTT) is an X-linked neuro-developmental disorder that essentially affects girls and that is caused by mutations of the MeCP2 (Methyl-CpG binding Protein 2) gene. Mouse models of the disease indicate that MeCP2 function is essential for the maturation of post-mitotic neurons and/or for synapse formation and activity. Recent studies have shown that the neurological defects developed by MeCP2 deficient mice can be reversed by re-expressing MeCP2, suggesting that normal neuronal function might be restored in RTT patients. However, MeCP2 expression level must be tightly controlled to ensure normal neuronal activity which precludes direct use of MeCP2 ectopic expression as a possible treatment in human patients. It is thus essential to identify the molecular mechanisms which are responsible for the neuro-physiological defects of RTT downstream of MeCP2. Using human embryonic stem cells (hESC), we have developed MeCP2 deficient human neuronal cultures. Human MeCP2 knockdown neurons present an altered synaptic function characterized by a shift from a primarily excitatory glutamatergic activity in control neurons to a predominantly GABAergic activity in MeCP2 deficient neurons. In addition, we have observed abnormal expression of several microRNA and mRNA, some of them coding for synaptic proteins involved in Glutamate and GABA neurotransmissions, in MeCP2 deficient human neurons. Altered synaptic activity, as observed in this first human cellular model, might be responsible for some of the manifestations of Rett Syndrome. MicroRNA, mRNA and protein expression analysis will allow to better characterize the molecular mechanisms of this devastating disease and help to identify molecular targets for a future therapeutic intervention.

No: 9

Application of arrayed cellular microenvironment technology to define long-term maintenance conditions of hESCs

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The cellular microenvironment, composed mainly of extracellular matrix proteins (ECMPs), growth factors (GFs), glycans (GCs), and mechanical factors, interacts with cells in a complex manner to regulate their fate. Experimental manipulation of the microenvironmental conditions in which human embryonic stem cells (hESCs) are cultured is critical in affecting their proliferation and differentiation and is central to developing strategies for the production of defined cell types to treat a variety of disorders in which cells are either defective, damaged or dead. The exact microenvironmental components that promote long-term hESC self-renewal are unknown. HESCs can be expanded and maintained in a pluripotent state in poorly defined conditions, such as co-culture with mouse embryonic fibroblasts (MEFs) or with the assistance of commercially available substrates. An important step towards the application of hESCs in cell-based therapies is the development of defined and optimized conditions for hESC culture. Current technologies are inadequate to screen the vast number of combinations of factors that may influence hESC fate. We have developed an array platform for the real-time simultaneous screening of hundreds of physiochemical parameters on hESC attachment, proliferation, differentiation and gene expressions. We have used this novel approach to systematically assess and probe the complex relationships between hESCs and their microenvironment. Combinations of ECMPs, GFs, and glycans were monitored for their effects on hESC (Hues 1 and Hues 9) growth and survival. Using real-time microscopy and hESC lines expressing fluorescent proteins, we were able to monitor growth and survival over a period of 5 days. Statistical analysis showed that specific and fully defined microenvironments were able to support hESC proliferation at levels comparable to or exceeding current undefined culture conditions. A subset of these microenvironments was assayed for their ability to support the long-term maintenance (> 10 passages) of several hESC lines (Hues 1, Hues 9, and H9). The efficacy of the fully defined and optimized conditions to maintain hESC pluripotency in long-term culture was confirmed by quantitative PCR and immunostaining of known stem cell markers and by in vitro differentiation through embryoid bodies. In the future, we will derive new hESC lines from blastocysts in these defined conditions.

No: 10

hESC differentiation shows minimal yet distinct DNA methylation changes characterized by de novo methylation

Alayne L. Brown[1], David S. Johnson[1,+],Si Wan Kim[1] , Anton Valouev[2], Timothy Reddy[1,3], Devin Absher[3], Elizabeth Anton[1], Eric Chiao[1], Catherine Medina[1], Loan Nguyen[1], Chuba Oyolu[1], Norma Neff[1], Gary P. Schroth[4], Arend Sidow[2,1], Richard M. Myers[1&#] and Julie C. Baker[1#]
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DNA methylation in human tissues is thought to lock in a tissue-specific expression state. Although these signatures are known to be important, they remain virtually unexplored during early human development and in differentiating human embryonic stem cells (hESCs). To investigate the role of DNA methylation during human development, we developed Methyl-seq, a method that assays DNA methylation at more than 90,000 regions throughout the genome. Methyl-seq on hESCs, their derivatives and on in vivo human tissues allowed us to decipher the following. First, in vitro differentiation of hESCs results in DNA methylation changes at very few regions (<5%), and hESC differentiation is characterized by de novo methylation whereas in vivo fetal liver development is characterized by hypomethylation of sites. Second, hESC differentiation is uniquely characterized by de novo methylation at regions shown previously to be bound by the repressive histone mark, H3K27me3 or "bivalent domains" bound by H3K4me3 and H3K27me3, and by both de novo methylation and demethylation changes at low-density CpG promoters (LCPs). Third, hESC promoters that are bound by H3K4me3 are unmethylated, regardless of their CpG content. Taken together, our results suggest that hESC differentiation has a unique DNA methylation signature that is not indicative of in vivo differentiation.

No: 11

Stem cell derived retinal pigment epithelium for the treatment of age-related macular degeneration

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The retinal pigment epithelium (RPE) works with the rod and cone photoreceptors of the eye to form a functional visual transduction unit. RPE death and dysfunction in age-related macular degeneration (AMD) leads to death of the photoreceptors and loss of vision. AMD is the leading cause of blindness in elderly people in the western world, yet there is currently no cure. Recent studies have shown that autologous transplantations of peripheral RPE to the macula of AMD patients is beneficial. Our lab has derived RPE from human embryonic stem cells (hESC), providing a potential source of RPE for transplant. We have focused on characterizing the gene expression, protein expression and cellular function of these stem cell derived RPE. We have also looked at the stability of these cells over multiple passages. Stem cell derived RPE are highly similar to human fetal RPE at low passages, but by passage 5 have changed morphology and gene expression dramatically, resulting in a loss of cellular function. These studies demonstrate that stem cell-RPE are a promising cellular therapy for AMD, although the challenge of scalability still needs to be solved.

No: 12

Old muscle inhibits its own regeneration by inducing excessive TGF-□/pSmad signaling and up-regulating cell-cycle check point genes in satellite cells

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Organ regenerative capacity, and its age-associated decline, can be largely attributable to changes within the extrinsic cues that govern stem cell responses. Previous work demonstrates that the regenerative potential of skeletal muscle stem cells (satellite cells) fails to trigger in the old, due to a decline in Notch activation. Notably, this decline can be rejuvenated by forced local activation of Notch, by exposure to young circulation or undefined human embryonic stem cell-produced factors. Such evidence suggests that stem cells endogenous to old organs retain their capacity for continued maintenance and repair, but are unfortunately inhibited by the aged differentiated niche. Recent data from our lab reveals that physiological aging of the differentiated muscle niche can be defined by excessive TGF-β production, which interferes with old muscle stem cell regenerative responses by inducing heightened TGF-β/pSmad signaling and CDK inhibitor levels. These data also show that muscle repair success is determined by an antagonistic balance between TGF-β/pSmad signaling levels and Notch pathway activation, such that activation-inactivation of Notch determines the presence of pSmad3 on the promoters of CDK inhibitors. Such balance becomes unstable with age, in a way that assures the lack of tissue repair. Furthermore, attenuation of TGF-β/pSmad3 in old injured muscle can restore productive regeneration to satellite cells, in vivo. Comprehensively, this work advances our understanding of the aging process at the cellular and molecular levels, reveals specific biochemical mechanisms by which aging of stem cell niches attenuates stem cell performance, and introduces novel therapeutic strategies for the enhancement of organ regenerative potential.

No: 13

A novel engineered niche to explore the vasculogenic potential of embryonic stem cells

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Numerous pathologies are characterized by poor blood vessel growth and therefore inadequate nutrient and oxygen delivery. Therapeutic angiogenesis seeks to enhance vessel growth into ischemic tissues by delivering combinations of pro-angiogenic factors with precise spatial and temporal resolution to recruit host vasculature. An alternative approach is to deliver an appropriate cell type that can provide a more physiologic mixture of pro-angiogenic cues to accelerate the recruitment of host vessels in a paracrine fashion, or that can differentiate into a functional vasculature directly. It is possible that both embryonic and adult progenitors may act in either or both of these manners to stimulate new vessel formation. Consistent with the first possibility, we have utilized a three-dimensional (3D) in vitro cell culture model to demonstrate that pre-committed (mesenchymal stem cells, MSCs) or more highly committed (fibroblasts) mesenchymal cells stimulate capillary formation from vascular endothelial cells via distinct mechanisms involving differential dependence on proteases that degrade the extracellular matrix (ECM). Specifically, our findings suggest that MSC-stimulated capillary formation relies solely on matrix metalloproteinases (MMPs, specifically MT1-MMP), whereas both MMPs and plasmin, a potent serine protease, mediate fibroblast-stimulated capillary morphogenesis. With respect to the second possibility, the focus of our research funded by a CIRM New Faculty Award is to better understand the factors that govern the direct differentiation of embryonic stem cells (ESCs) into vascular progenitors. To facilitate this effort, we have transferred our existing 3D model system into a novel engineered stem cell niche to investigate how soluble and insoluble morphogens, and cues from other cell types, are integrated to coordinately govern the endothelial differentiation of ESCs. A better understanding of these factors may transform efforts to use stem cells for ischemic conditions and other pathologies, and facilitate our long-term goal of developing "instructive" biomaterials and strategies to direct tissue repair.

No: 14

Molecular characterization of the human NANOG protein

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NANOG is a key transcriptional regulator of pluripotent stem cell (PSC) self-renewal. NANOG occupies promoters that are active and others that are repressed, however, the mechanisms by which NANOG regulates transcriptional repression and activation are unknown. We hypothesized that individual protein domains of NANOG control its interactions with the promoters its co-regulators. We performed a detailed characterization of the functional domains in the human (h) NANOG protein, using a panel of deletion and point mutant constructs. We determined that six amino acids in the homeodomain (136YKQVKT141) are sufficient for the nuclear localization of hNANOG. We also determined that the tryptophan-rich region (W) of hNANOG contains a CRM1-independent signal for nuclear export, suggesting a possible cellular shuttling behavior that has not been reported for hNANOG. We also show that at least four tryptophans are required for nuclear export. We also determined that similar to mNANOG, the W region of hNANOG contains a homodimerization domain. Finally, in vitro transactivation analyses identified distinct regions that transactivate or repress the Oct4 promoter. Specifically, the N-terminal region has dominant repressive transcriptional activity and removal of this region produced a 'super-active' hNANOG with enhanced transcriptional activity. We also confirmed that the transcriptional activator in hNANOG is contained in the C-terminal region, similar to murine NANOG. In summary, this study has characterized the structure and function of hNANOG protein leading to an increased understanding of the mechanism by which hNANOG regulates both transcriptional activation and repression during PSC self-renewal.

No: 15

Self-renewal and differentiation in ectodermal derivative adult stem cells

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Stem cells hold promising prospects for regenerative medicine. Yet not enough is known about the mechanisms by which they self-renew and differentiate, and whether there are conserved mechanisms amongst different adult stem cells. It is not clear if stem cells in different systems employ the same or different factors to promote maintenance or differentiation. We attempt to answer these questions by comparing, in epidermal and neural progenitors, the factors responsible for self-renewal and the initial steps of lineage determination. We will screen known pathways as well as novel factors identified in a screen for their role in self-renewal and differentiation.

No: 16

Electrical monitoring and stimulation of embryonic stem cell cardiac differentiation and integration

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The success of stem cell therapy for cardiac repair requires a thorough understanding of the response of stem cells to the in-vivo environment and the properties of the resulting tissue. While many studies have helped elucidate the chemical and mechanical contributions, electrical factors and properties remain poorly understood. Of particular interest is the influence of the electrical environment on the differentiation of grafted stem cells, the functional integration of the graft into native tissue, and the electrical properties of the graft (risk of arrhythmogeneicity). This work aims at developing technologies and models to investigate these effects and properties in vitro. A platform for the study of the effect of the electrical environment on stem cell differentiation has been built and tested with mouse embryonic stem (ES) cells. The system uses microelectrode arrays to deliver localized currents (point-source stimulation) to the ES cells, mimicking the electrical environment in-vivo¬. Cells were stimulated and then screened for a cardiomyocyte cell fate using real-time PCR and immunostaining. A range of responses were revealed that demonstrated a high sensitivity dependant on the initial differentiation stages and stimulation strength. Optimal conditions (pacing amplitude, duration of continuous pacing) that best encouraged cardiomyocyte differentiation without increasing embryonic stem cell proliferation were determined. A six-fold increase in the cardiac marker Troponin-T has been observed at optimal conditions. To further investigate transcriptome changes after pacing, whole genome microarray analyses are being carried out. While differentiation of ES cells into cardiomyocytes is a necessary step for cardiac repair, the functional integration of these cardiomyocytes in a native myocardial tissue is equally important. In order to study this integration, a technology based on spatially-patterned co-cultures of embryonic stem cells (differentiated or not) and mature cardiomyocytes on microelectrode arrays is being developed. This approach enables the monitoring over time of the integration process as measured by the presence of electrical activity. In addition, measurement of conduction properties in the graft and at the interface graft/host will be used to evaluate the propensity to arrhythmia caused by conduction mismatch. Such a platform will also allow the testing and development of pharmacological and electrophysiological manipulations to improve this integration process.

No: 17

Myocyte enhancer factor 2C (MEF2C)-directed neurogenesis in human embryonic stem cells (hESCs)

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Potential embryonic stem cell (ESC)-based repair of the central nervous system faces several hurdles, including massive cell death, failure of appropriate neuronal differentiation/migration/ incorporation into host brain, and tumor formation after transplantation. As a part of our efforts to overcome these problems, we used forced expression of the transcription factor MEF2C to obtain lineage-specific differentiation of hESCs into neurons. MEF2C, which was originally cloned and characterized some years ago in our laboratory, is the predominant isoform of this family of transcription factors in developing cerebrocortex. We and others have shown that MEF2 is a calcium-dependent neurogenic effector that enhances survival and affects synaptic morphogenesis of neurons. Previously, we showed that expression of constitutively active MEF2C (MEF2CA) under the nestin enhancer produced pure 'neuronal' (as opposed to neural) progenitor cells from mouse ESCs, which differentiated into neurons, both in vitro and in vivo, and, following stroke damage, functionally integrated into the endogenous nervous system to improve animal behavior (Li et al., J. Neurosci. 2008). Here we report that expression of hMEF2CA triggers virtually 100% efficient neuronal differentiation of hESC-derived neural progenitor cells (NPCs). Histological and electrophysiological analyses demonstrate that MEF2CA-programmed hESC/NPCs differentiate into functional neurons, which exhibit fast sodium currents and robustly respond to glutamate and GABA application in vitro. In contrast, knockdown of endogenous MEF2C decreases the number of cells expressing neuronal-specific markers such as microtubule associated protein-2 (MAP-2), and results in fewer neurons and smaller dendritic trees. In preliminary experiments, following transplantation of hESC-derived NPCs into a NOD/SCID mouse model of focal cerebral ischemia, we found that MEF2CA-programmed hESC/NPCs do not form rosettes, representing hyperproliferative tumor-like masses, but instead differentiate to express neuronal markers. Unlike MEF2CA-programming, hESC-derived NPCs not expressing MEF2CA form such rosettes. These data strongly suggest that MEF2C instructs hESC-derived NPCs to differentiate into functional neurons and prevents tumor formation after transplantation.

No: 18

"Open Science": Can open source principles solve ip and data sharing?

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In the wake of Federal funding restrictions on human embryonic stem (hES) cell research, publicly funded programs in eleven states emerged to fill the gap. However, the promise of cures and speedy delivery of novel therapeutics from "bench to bedside" may be slowed by problems in intellectual property bottlenecks caused by patent and licensing thickets around foundational stem cell tools and reagents. This problem has a particularly high impact on researchers in academia and other non-profit settings; however, it also presents a unique opportunity to implement a progressive solution. Through the application of principles from software's successful Open Source model, so-called "Open Science" may facilitate the data and material sharing between researchers that are hindered under the present IP regime. Moreover, Open Science can, and should, be complimentary to the current patent-based biotechnology landscape. This poster examines why Open Science approaches are appropriate for stem cell technology, describes the community and project characteristics likely needed for Open Science success, and explores the many benefits of Open Science and if these advantages outweigh costs for non-profit researchers and their host institutions. This poster also describes several creative "patent friendly" mechanisms and "patent preemptive" strategies to promote and enable data sharing and tool access. This poster concludes with recommendations for the California Institute for Regenerative Medicine (CIRM) and other funding agencies on how to implement, incentivise, and enforce Open Science. Such a paradigm shift to Open Science will likely help mitigate the identified problems that hinder progress in stem cell research: technology barriers can be lowered by better, more transparent and organized sharing, communication, and collaboration among researchers; IP bottlenecks can be mitigated by creative solutions to the patent thicket problem such as patent pools, patent pledges, and preemptive acts; and, ethical and regulatory complexities can be improved on a shorter time scale and with increased transparency by open communication and non-legislative solutions to problems with the Federal patent law system.

No: 19

Vegf-mediated cross-talk within the neonatal murine thymus

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Although the complex mechanisms of cross-talk that regulate the hematopoietic and epithelial compartments of the thymus have been intensively studied, relatively little attention has been paid to the interactions of these compartments with the thymic vascular endothelium. Current understanding of the architecture and function of the thymic vasculature is based on studies of adult thymus. We show for the first time that the neonatal period represents a unique phase of postnatal thymic growth and differentiation. Relative to adult thymus, the neonatal thymus has greater thymocyte proliferation (neonates: 10.8%; adults: 6.8%, p = 0.01), and a predominance of immature CD4-CD8- "double negative" (DN) thymocytes (neonates: 9.63 ± 2.63%; adults: 2.93% ± 0.61, p = 0.0009) and cortical thymic epithelium (cTEC). Moreover, the neonatal thymus contains endothelium that is organized as primitive, dense networks of capillaries and is dependent for its growth on vascular endothelial growth factor (VEGF). VEGF dependence of the neonatal thymic endothelium during the first week of life is mediated by significantly higher levels of both VEGF production and VEGF receptor 2 (VEGF-R2) expression than in the adult thymus. We found that VEGF is expressed locally in the neonatal thymus, both by immature DN thymocytes and by thymic epithelium. Inhibition of VEGF signaling during the neonatal period using VEGF-Trap, a soluble fusion protein containing the extracellular domains of both VEGF-R1 and VEGF-R2, results in a rapid loss of the dense capillaries in the thymus and a marked reduction in the number of thymocytes, particularly those in the immature CD4+CD8+ "double positive" (DP) stage of differentiation (VEGF-Trap: 0.95 x 10E7 cells; control: 3.5 x 10E7 cells, p < 0.0001). These data demonstrate that, during the early postnatal period, VEGF mediates complex mechanisms of cross-talk between the thymocyte, epithelial and endothelial compartments of the thymus.

No: 20

Early Acquisition of Neural Crest Competence During hESCs Neuralization

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We present evidence that neural crest (NC) competence can be acquired very early during human ES cell (hESC) neuralization. The model employed here takes advantage of the intrinsic cell migration that occurs from hESC-derived neurospheres that are plated on fibronectin. Emigrating cells resemble the early in vivo population and uniformly acquire SOX10, a critical NC marker within 8 days of neuralization, in the absence of feeders or exogenous BMPs. Early Migratory Neural Crest Stem Cells (emNCSC) cells give rise in vitro to all NC lineages including glia, peripheral neurons, myocytes, melanocytes, chondrocytes and adipocytes. Consistent with known neural crest specification signaling, the addition of the BMP-inhibitor Noggin completely abolishes SOX10, p75 and HNK-1 expression. Differentiating emNCSCs also respond appropriately to extrinsically applied developmental cues, such as BMP2, TGFβ and FCS/Forskolin. emNCSCs can colonize aganglionic embryonic gut cultures derived from Ret.k- null mice, which completely lack an enteric nervous system, and differentiate into neurons. Furthermore, they incorporate into neural crest-derived structures when microinjected into neural crest migratory pathways of stages 7-11 chick embryos. Grafted emNCSCs migrate laterally and ventrally into endogenous NC areas, and contribute specifically to proper NC derivatives, differentiating into neurons and glia in the cranial ganglia, glia along nerves, mesenchyme and connective tissues in cranial regions. emNCSCs do not incorporate or differentiate into non-NC derivatives. Our findings reveal broad developmental potential of early migratory neuroepithelial cells during hESCs neuralization. We describe a straightforward model for the generation of emNCSCs with broad developmental potential, which may provide a framework for studying early human neural development.

No: 21

Establishment of indivdualized stem cells for regenerative medicine therapy

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Abstract Somatic cell nuclear transfer (SCNT) and induced pluripotency have become important methods in reprogramming the terminally differentiated genome of somatic cells into an embryonic stem cell and stem cell-like epigenetic state. These technologies have attracted much attention because they can be used to derive stem cells for regenerative medicine therapy, thereby preempting immunorejection after autologous transplantation. However, to be therapeutically-useful, somatic cell-derived stem cells must be shown to be safe and effective. In this project, we sought to demonstrate whether derived stem cells with a genetic mutation could be corrected in vitro which could subsequently be used for autologous transplantation to correct the genetic defect in vivo. From ApoE-knockout (ApoE-/-) mouse, we generated nuclear transfer-derived embryonic stem cells (NT-derived ESC) by performing SCNT using nuclei from cumulus cells, and we generated induced pluirpotent stem cells (iPSC) by expressing 3 defined pluripotency factors (Oct3/4, Sox2 and Klf4) in tail tip fibroblasts cells. We then corrected the ApoE-/- genetic defect in vitro, determined integration and expression of restored gene, conducted in vitro and in vivo characterization of pluripotency makers, determined the ability of NT-derived ESC and iPSC to differentiate into all three germ layers and ultimately determined chromosomal stability and germ line transmission. We observed that, after correction of the genetic defect, both NT-derived ESC and iPSC from ApoE-/- mice could be maintained in an undifferentiated and pluripotent state, and could undergo targeted differentiation into all 3 germ layers in vitro, and could successfully transmit through the germline line in vivo. This project using a mouse model demonstrates the steps to successfully generate genetically-corrected customized NT-derived ESC and iPSC that can be used effectively for individualized regenerative medicine. The next step in this research is to demonstrate similar success in using somatic cells from humans with genetic defects.

No: 22

Mitochondrial regulation of human embryonic stem cell growth and differentiation

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We are testing the hypothesis that changes in mitochondrial function are central to determining the developmental status of human embryonic stem (hES) cells. Mitochondria oxidative phosphorylation (OXPHOS) entails the oxidation of calories (reducing equivalents) with oxygen to generate a mitochondrial inner membrane electrochemical gradient. This gradient can then used to synthesize ATP or regulate cytosolic calcium, cellular pH, and redox status. The mitochondria also generate most of the endogenous reactive oxygen species (ROS). Mitochondrial manipulation of ROS and cellular redox than regulate cellular proliferation, differentiation, and apoptosis, the later via the activation of the mitochondrial permeability transition pore (mtPTP). Evidence in support of our hypothesis has been obtained by treatment of the hES cell line, H9, with the mitochondrially active superoxide and catalase mimetics Mn-TBAP and MnTE-PyP for two days. This caused the cells to become quiescent and enlarged. Additionally, an antioxidant cocktail was able to reduce the levels of superoxide anion induced in hES cell by paraquat. Reduced ROS would be expected increased reduced glutathione levels, which we have found can be manipulated by the antioxidant N-acetyl cysteine or buthionine-sulfoxamine, which prevents the synthesis of reduced glutathione. The effects of these treatments on cell proliferation, differentiation, and death are beginning correlated with changes in the expression of nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) encoded mitochondrial genes and their proteins. These insights are being used to determine if the different growth characteristics of different hES cell lines or of the same line at different passage numbers might be the product of changes in mitochondrial function. The hES cell line H1 grows more slowly than its companion line H9. Moreover, as the passage number of both H1 and H9 increases from < 25 to > 60 passages the incidence of spontaneous differentiation on matrigel increases. Consistent with a mitochondrial modulation of these features, H9 cells were found to produce several fold more ROS than H1 cells. Moreover, as the passage number of the hES cells increased, the mitochondrial distribution changed from a perinuclear to a more widely dispersed throughout the cytoplasmic. We are now attempting to manipulate these intracellular and proliferation characteristics by treatment with various pro- and anti-oxidants. Our studies on hES cell mitochondria promise to advance our understanding of the mitochondrial system biology of hES cells. In the process, we should development novel approaches to modulate the growth and differentiation of hES cells.

No: 23

Development of a hESC model system to examine processing and secretion of Amyloid beta in human neurons

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Alzheimer's disease (AD) is a neurodegenerative disease characterized by amyloid plaques, neurofibrillary tangles and a massive loss of neurons in affected brains. The major constituent of amyloid plaques is extracellular Amyloid beta peptides, which are processed from beta-Amyloid Precursor Protein (APP) by gamma- and beta-secretases. APP is transported on vesicles in axons, and is also intricately involved in regulation of axonal transport. The localization of APP processing as well as the details of Abeta secretion have yet to be fully elucidated. To date, the majority of this work has been performed in non-human cells, and the specifics in human neurons remain unclear. We have developed a system using neurons derived from human embryonic stem cells (hESCs) in a novel microfluidic culture device. This device allows us to assess the biochemistry of APP and Abeta in axons independent of the soma in these neurons. This, as well as the development of additional assays, will help determine the levels of endogenous axonal APP processing and Abeta secretion, as well as from disease model cell lines.

No: 24

Targeting the source: evaluating the utility of combinatorially derived peptides in targeting tumorigenic stem cells

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Introduction: ALDH1, CD133, CD44, and CD24 have been associated with a stem/progenitor cell population in human breast cancers. As a relatively quiescent subpopulation, breast cancer stem cells may be resistant to cytotoxic therapies. Specific therapies targeting these cells may circumvent this limitation. Hypothesis: Synthetic peptides are capable of targeting breast cancer stem cells. Methods: The luciferase-transfected cell lines MDA-MB-231-luc-D3H2LN and MCF-7-luc-F5 (Xenogen) were grown to subconfluence, harvested using enzyme free dissociation buffer (Gibco) and treated as below. To compare ALDH1 expression with CD44 and CD24 expression, an Aldefluor (Aldagen) reaction was performed according to manufacturer`s protocols on both MDA-MB-231-luc-D3H2LN and MCF-7-luc-F5. The cells were then stained with APC-anti-CD44 and PE-anti-CD24 (BD). Preparative and analytic flow cytometry was performed using a Cytopeia inFlux Cell Sorter. The CD44+/CD24-/ALDH1br population of MDA-MB-231-D3H3LN and the CD44+/ALDH1br population of MCF7-luc-F5 were separated from the remainder of the cell mass; the separated populations were collected. TentaGel beads conjugated to the novel combinatorially derived compounds LXY1 and crRGDylc were prepared for analysis by serial washes with PBS and media, and incubated in the presence of sorted cells using a 2 mL final volume in a 12-well culture plate (Corning). Cultures were continued for 35 days without addition or changing of growth medium. Results: Preparative flow cytometry on MDA-MB-231-luc-D3H2LN using ALDH1, CD44, and CD24 demonstrated 57% CD44 expression, whereas 96% of these cells were CD24- and ~0.8% were ALDH1br overall. The majority of ALDH1br cells were in the CD44+/CD24- population. However, ALDH1br and ALDH1- cell populations as assigned by negative-control-derived gating regions appeared merged on fluorescence distribution plots without clear separations. In contrast, 93% of the MCF-7-luc-F5 cells were CD44+/CD24+, with ~0.2% in the CD44+/CD24- region. The ALDH1br population comprised 0.9-1% of the cells, however, a merged fluorescence distribution was observed as in MDA-MB-231-luc-D3H2LN. Nearly all MCF-7-luc-F5 ALDH1br cells were in the CD44+/CD24+ population. Binding assays using LXY1 and crRGDylc in MDA-MB-231-luc-D3H2LN confirmed that the ALDH1-/non CD44+/CD24- cells in this population bound to ligand-conjugated beads; ALDH1br/CD44+/CD24- cell yield was insufficient to assess binding. In MCF-7-luc-F5, binding was observed for beads conjugated to these ligands in all cell subpopulations, although low ALDH1br/CD44+ cell numbers were observed. After ten days on MCF-7-luc-F5, cell proliferation was observed in both subsets. At 35 days, only the ALDH1br/CD44+ subset had persistently viable colonies surrounding beads. Conclusions: The novel ligands LXY1 and crRGDylc are capable of targeting MDA-MB-231-luc-D3H2LN and MCF-7-luc-F5. In addition, these compounds are capable of binding to a subpopulation with increased signal for ALDH1 relative to the remainder of the cell mass in MCF-7-luc-F5. The majority of the MDA-MB-231-D3H2LN cells were CD44+/CD24-, whereas MCF-7-luc-F5 exhibited a primarily CD44+/CD24+ phenotype. ALDH1 signal did not appear to correlate reliably with the CD44/CD24 phenotype. Further study is indicated to determine whether ALDH1, CD44, and CD24 are capable of defining a true tumorigenic stem cell population in these cell lines.

No: 25

FoxO-1 plays a role in regulating the beta-cell precursor pool in the adult human pancreas

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It has long been controversial whether maintenance of beta-cell mass under normal conditions and beta-cell regeneration following damage stimuli arises from the replication of preexisting beta-cells or from neogenesis from endocrine precursors. In the adult human pancreas, our laboratory demonstrated that nonendocrine pancreatic epithelial cells, comprising both duct and acinar compartments, could differentiate into beta-cells under the influence of as yet unknown factors present in the human fetal pancreas. Because PDX-1 is the first marker of beta-cell neogenesis, it is important to understand the mechanism by which its expression is controlled in the ductular epithelium. The transcription factor FoxO1 has been shown to play an important role in controlling PDX-1 therefore we studied FoxO-1 expression following pancreatic duct ligation, a known stimulus of beta-cell neogenesis in mice. While in the non ligated portion of the pancreas very few ductal cells exhibited FoxO-1 expression, the ligated portion showed a substantial number of FoxO-1 positive ductal cells. Most of these cells were also positive for PDX-1 staining representing potential beta-cell precursors. Interestingly, using our in vitro model of human nonendocrine pancreatic epithelial cells (NEPECs), we found that most duct cells from monolayer cultures express high levels of FoxO-1. Moreover, downregulation of FoxO-1 induced cell proliferation in monolayer cultures of NEPECs. We propose that the forkhead protein, FoxO1, plays a central role in the regenerative potential of adult beta-cell progenitors.

No: 26

CDK2AP1 is required for epigenetic silencing of Oct4 during murine embryonic stem cell differentiation

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Oct4 is a know regulator of stem cell renewal and differentiation. Expression of Oct4 during stem cell differentiation is regulated by promoter methylation through the NuRD complex. When these complexes bind to promoters containing CpG islands, it results in transcription repression. MBD3, one of important NuRD components, is required for pluripotency of embryonic stem cells. Knockout of MBD3 resulted in LIF-independent maintenance of ES cells and a reduced differentiation potential of ES cells upon LIF withdrawal. However, the mechanism by which the NuRD complex regulates Oct4 promoter is still not clear. In this study we show that Cdk2ap1, a negative regulator of Cdk2 function and cell cycle, is required for murine ES differentiation. Cdk2ap1-/- mES cells are lack of methylated promoter of Oct4. Furthermore, we show that Cdk2ap1 interacts with Mbd3 to regulate methylation of Oct4 promoter. Therefore, our data support that Cdk2ap1 play an important role in the function of NuRD complex regulating methylation status of Oct4 promoter and mES cell differentiation.

No: 27

Exogenous LIF promotes oligodendrocyte progenitor cell proliferation and remyelination in vivo

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The development of therapies that enhance the repair capabilities of the adult brain by stimulating the proliferation of endogenous neural stem/progenitor cells and/or directing their subsequent differentiation into functional neurons and glia is a relatively neglected area of current stem cell research. We have found that injection of an adenovirus expressing leukemia inhibitory factor (LIF) into the adult brain promotes neural stem cell (NSC) self-renewal and stimulates the proliferation oligodendrocyte progenitor cells (OPCs). Based on these findings, in particular the effect of LIF on OPCs, we hypothesized that if LIF could enhance the OPC response in the context of chronic demyelination it may, in turn, promote the generation of new oligodendrocytes and aid remyelination. To test this, mice were given a diet containing cuprizone that induces demyelination in the corpus callosum (CC), hippocampus, and cortex and then injected with either a LIF- or lacZ-expressing adenovirus (Ad-LIF or Ad-lacZ) in the lateral ventricle. Three weeks after adenovirus injection and removal of cuprizone from the diet, mice that received Ad-LIF exhibit an increase in the number of proliferating OPCs that expands the total number of OPCs in the demyelinated hippocampus. Many of these OPCs survive and differentiate so that by 6 weeks after removal of cuprizone, the number of mature oligodendrocytes in LIF-treated mice is restored to near normal numbers in the CA3 region of the hippocampus, where LIF-induced Stat3 activation is the greatest. Remarkably, remyelination in the CA3 region is more extensive in LIF-treated mice and is accompanied by the reformation of nodal, paranodal, and juxtaparanodal domains on a subset of axons. Our finding that LIF can promote oligodendrocyte generation and remyelination in vivo taken together with its known ability to protect oligodendrocytes from death in the EAE model of MS, as well as following spinal cord injury, suggests that LIF has multiple activities that could be of therapeutic benefit for demyelinating diseases such as MS.

No: 28

Analysis of genes involved in restricting pluripotency in the C. elegans embryo

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The efficient direction of differentiation of stem cell populations is hampered by the heterogeneity of gene expression profiles present in a given culture. Knowledge of gene regulatory networks that are involved in guiding cells toward differentiation and restricting pluripotency would allow researchers to greatly increase the affectivity of techniques designed to direct the differentiation of embryonic stem cells. While large scale expression analyses have identified candidates, functional analysis is needed in order to develop new strategies. Model organisms such as C. elegans provide platforms for genome wide functional analysis. We have completed an RNAi screen for genes involved in the switch from pluripotency to committed differentiation. The genes identified restrict the ability of cells in the developing C. elegans embryo to be directed towards fates they would not otherwise adopt. They encode functions spanning from signal transduction to regulation at the level of transcription, and chromatin modification. We are now focusing on genes that are representative of these activities: lin-26 is a transcription factor involved in hypodermal differentiation; glp-1, a homologue of NOTCH is involved in contact mediated communication between cells; and set-16 is a homologue of yeast Set-1 which is involved gene silencing at the level of chromatin modification. Knocking these genes down has allowed us to direct cell lineages which would normally make skin, neurons and muscle into making only endodermal cells. We are also developing a strategy for the primary culture of C. elegans embryonic cells in order to understand how our candidate genes may be acting within isolated cell lineages and perhaps within cells that have been directed to differentiate into a specific cell type. With already available interaction databases, we have constructed an interaction network that will aide in the analysis of the relationships between our candidate genes. We will look for regulatory interactions and seek the most relevant nodes for further study. The final phase of our study involves looking at the function of the human homologues of the most robust candidate genes in Human or Murine Embryonic Stem Cell Culture. This will involve the use of siRNAs to temporarily knock-down a candidate or group of candidates while using established culture methods to direct the differentiation of a cell culture. The efficiency by which we are able to obtain cells of a desired type will be used as a measure for the usefulness of a candidate as a target in future strategies for directed differentiation.

No: 29

Kismet regulates transcription elongation and histone H3 lysine 27 methylation

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Factors that regulate chromatin structure play important roles in development. Members of the Polycomb group (PcG) of repressors and their antagonists, the trithorax group (trxG) of activators, act at the level of chromatin to maintain patterns of gene expression and cellular identities in multicellular organisms. Kismet is an ATP-dependent chromatin-remodeling factor of the trxG that facilitates an early step in transcriptional elongation. Recent work has been focused on understanding how Kismet regulates transcription elongation and counteracts PcG-mediated transcriptional repression. Using Drosophila melanogaster as a model organism, we found that Kismet opposes the methylation of histone H3 on lysine 27, a repressive histone modification catalyzed by PcG proteins that is required for stem cell self-renewal and the maintenance of pluripotency. Since the function of PcG and trxG proteins has been highly conserved during evolution, these findings suggest the human counterpart of Kismet - CHD7 - regulates stem cell development by counteracting PcG repression. Current work is focused on testing this model in the human embryonic stem cell-like cell line Ntera2. Even if our hypothesis is incorrect, characterization of the function of CHD7 will shed light on the molecular nature of CHARGE syndrome, a serious developmental disorder linked to mutations in human CHD7.

No: 30

The effects of intraspinal transplantation of adult rat spinal cord neural stem cells on the rat ventral root avulsion model of cauda equine/conus medullaris syndrome

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Transplantation of neural stem cells (NSC) is an emerging therapy for spinal cord injury. The potential benefits of NSC transplantation include neuroprotection of injured neurons, promotion of axonal regeneration, and replacement of lost neurons. The Havton laboratory has developed a rat model of cauda equina (CE)/conus medullaris (CM) injury involving lumbosacral ventral root avulsion(VRA) that produces progressive loss of motoneurons(MN) and preganglionic parasympathetic neurons(PPN), bladder dysfunction, and neuropathic pain. This proposal will investigate the effects of NSC transplant into the spinal cord following VRA. Novel techniques have made it possible to isolate and cultivate neural stem cells from the adult rat spinal cord. These cells have several potential advantages compared to other stem cell sources including: 1) same species (i.e. rat to rat) transplantation may reduce immunologic reaction to transplanted cells, 2) cells derived from the spinal cord may have an intrinsic advantage to repair the spinal cord compare to NSCs obtained from elsewhere in the neuraxis, 3) adult derived neural stem cells may have less oncogenic potential, and 4) the potential for harvesting autograft stem cells, which would offer a great clinical advantage. The Havton laboratory has developed a technique to successful transplant these cells into the spinal cord. The current proposal will investigate the potential for transplanted adult rat spinal cord NSCs to survive, protect injured neurons from death, and differentiate into neurons in the spinal cords of rats that have undergone ventral root avulsions. The success of NSC transplantation following SCI has been limited by the observation that although transplanted NSCs proliferate and migrate to the site of SCI, most of the cells differentiate to an astrocytic lineage, with minimal replenishment of motorneurons. To enhance motoneuron differentiation, in-vitro techniques have been developed to prime stem cells toward a motoneuron fate. This proposal will investigate whether motor-priming increases motoneuron differentiation of grafted adult rat spinal cord NSCs. Further we will investigate impact of motor-priming on the potential neuroprotection conveyed by NSC transplantation. Neuropathic pain is a common occurrence following SCI and has been demonstrated to develop in rats following VRA. Several studies have demonstrated amelioration of pain following stem cell transplantation. However, other studies have shown that neuropathic pain develops above the level of injury following intraspinal neural stem cell transplantation. This may be related to the degree of astrocytic differentiation of transplanted cells. We will investigate the effects of NSC transplantation on VRA-induced neuropathic pain as well as determine whether transplantation produces neuropathic pain. Further experimentation will investigate effect of motor-priming adult rat spinal cord NSCs prior to intraspinal transplantation on the alleviation and production of neuropathic pain. Novel therapies are needed for functional recovery and pain relief following CE/CM injury. These studies may have direct translational potential for patients with CE/CM, as well as, SCI, brachial plexus avulsion, and motoneuron disease.

No: 31

Definitive hematopoiesis from human Embryonic Stem (hES) cells

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hES cells offer unique opportunity to study human development and possible source of therapy if desired tissue can be generated from them. hES cells are capable of differentiating into all 3 germ layers viz. Ectoderm, Mesoderm and Endoderm during in vivo development, which can to a certain extent be recapitulated in vitro. Blood arises from mesoderm during development. We studied the development of blood derivatives in a stromal free serum free system to understand the morphogens and signals involved in blood formation. In presence of Bone Morphogenetic Protein-4 (BMP-4) and basic Fibroblastic Growth Factor (bFGF), mesoderm development was evident by expression of brachyury followed by hematopoietic specification as seen by expression of SCL, GATA-1 and Hox B4 genes. CD34+ cells (putative hematopoietic) could also be generated. We compared these CD34+ cells to CD34+ cells obtained from Umbilical Cord Blood (UCB) phenotypically, by imaging and functionally by stroma based cultures. Our results indicate that hES derived CD34+ cells have the ability to differentiate into GlyA+ erythroid cells, CD66b+ granulocytes, CD14+/HLA-DR+ macrophages and to a certain extent CD56+ Natural Killer (NK) cells, similar to UCB CD34+ cells. However unlike UCB CD34+ cells no B-lymphoid development was seen from hES derived CD34+ cells. Phenotypically, hES CD34+ are KDR+ (receptor for Vascular Endothelia Growth Factor) and CD45neg (a pan leukocyte marker) while UCB CD34+ cells are KDRneg CD45+. This indicates the former have a more yolk sac like hematopoiesis rather than definitive hematopoietic (erythroid, myeloid and lymphoid) ability. Image stream analysis also highlighted critical differences in size and morphology. This is the first detailed study of hES derived v/s adult HSCs to identify similarities and critical differences between these two origins of cells.

No: 32

Identification of human embryonic stem cell-derived blastocyst- and epiblast-stage progenitors

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The potential of human embryonic stem cell (hESCs) to differentiate into any type of adult tissue makes these cells a unique model for studying early human development and at the same time a source of cells for regenerative medicine. To address the challenge of isolating lineage committed progenitors that are specified during the earliest stages of differentiation, we used flow cytometry in conjunction with libraries of commercial and novel monoclonal antibodies that we prepared against surface markers of hESCs. We sorted identified subpopulations that are present either in undifferentiated cultures or emerge during early stages of differentiation and evaluated their lineage by analyzing the expression of developmentally regulated genes. Of over 30 different subsets defined by specific cell surface markers we discovered only four distinct precursor profiles, two of which likely correspond to early visceral endoderm cells and later-stage mesododerm progenitors. Purification of these precursor types may improve the derivation of desired lineages and facilitate study of early differentiation programs with unprecedented resolution.

No: 33

Characterization of X-Chromosome inactivation in early passages of human embryonic stem cells

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X chromosome inactivation (XCI) is essential for X-linked gene dosage compensation in female cells. It has been demonstrated that different lines of human embryonic stem cells (hESCs) lines and different passages of the same line exhibited different XCI patterns. Recent study from our lab demonstrated that XCI in established lines of female hESCs (>25 passages) is non-random and prone to epigenetic alterations upon long-term culture. However, it is still unclear when and whether XCI takes place in the earliest passages of female hESCs during derivation. Towards this goal, we characterized XCI patterns in four lines CSES1, CSES3, CSES5 and CSES6 in the earliest passages available [between passages (P) 5-13]. By real-time PCR, we found that only one (CSES1) out of the four lines express detectable levels of XIST mRNA at the undifferentiated state and this expression is increased upon differentiation. XIST expression in CSES1 is correlated well with the presence of XCI markers such as punctuate immunostaining of Histone H3 lysine 27 trimethylation (H3K27me3) and XIST RNA coating of X-chromosome. Our results suggest that variations of XCI in different lines of female hESCs can take places during the earliest passages during derivation. To directly explore whether XCI in early hESCs is random or non-random, we assayed polymorphic X-linked cDNAs from CSES1 cells. We found that most of X-linked alleles were subject to XCI in a non random pattern and X-linked gene expression is primarily from paternal alleles, suggesting that non-random XCI patterns were initiated during the earliest stages of hESC derivation (from passage 5 to 14). Finally, we also observed the loss of XIST expression and H3K27me3 markers in early passages of CSES1 hESCs, suggesting that the instability of XCI could underlie the absence of XCI markers in certain lines of female hESCs.

No: 34

Identification of the critical extracellular matrix proteins required for human embryonic stem cell aggregation

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The assembly of multi-cellular units into complex 3-dimensional (3D) tissues requires structural and functional connections between stromal and epithelial cells through extracellular matrix (ECM) proteins. During early embryonic development, components of the ECM are expressed by cells within the inner cell mass (ICM) of the blastocyst. In the present study we hypothesized that ECM proteins and their specific receptors are expressed either by undifferentiated hESC or the feeder layers that sustain them in culture, and that these ECM regulate assembly and growth of ESC colonies. Our studies demonstrated that undifferentiated hESC express high levels of the ECM proteins collagen IV, laminin and nidogen. Dissociated hESC colonies, depleted of mouse embryonic fibroblasts, failed to re-aggregate into 3D structures after centrifugation, suggesting an intrinsic defect in self-assembling potential of hESC. However, addition of a complex of purified human laminin and nidogen was sufficient to permit robust reaggregation of hESC cell colonies and subsequent production of EBs. Other ECM such as collagen and fibronectin had no effect on aggregation. RT-PCR analysis demonstrated that the specific ECM isoforms expressed by undifferentiated hESC are laminin 511 and nidogen 1. hESC also express the laminin receptor integrin beta-1 and inhibition of integrin beta-1 blocked formation of EBs demonstrating the crucial role of integrin-laminin interactions in the hESC re-aggregation. Finally, we showed that EBs generated with the laminin-nidogen complex ("matrix EBs") recapitulate the properties of the ICM to undergo synchronous differentiation into endodermal, ectodermal and mesodermal derivatives, and were highly efficient in generating hematoendothelial progenitors. In summary, our data reveal the crucial role of specific ECM in hESC aggregation and provide a novel practical tool to investigate hESC differentiation in a chemical and xenogenic cell-free microenvironment.

No: 35

Effects of mitochondrial gene mutations on mouse stem cell biology

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The mitochondria regulate cellular energy production, redox status, and cell death, all factors central to the developmental biology of stem cells. To determine the role of mitochondria in stem cell biology, we have studied the physiology of mouse embryonic stem (mES) cells in which we have introduced various mitochondrial gene mutations and then examined the effects of these mutations on cellular physiology and the ability of the mutant cells to generate functional tissues in animals. The mitochondrial genome encompasses genes encoded by both the mitochondrial DNA (mtDNA) and the nuclear DNA (nDNA). Seven mtDNA gene mutations have been isolated in cultured mouse cells and introduced into female mES cells. The mutations included: (1) two severe mtDNA frameshift mutations in the ND5 and ND6 genes causing > 90% reduction in complex I dependent respiration and specific activity and mildly increased ROS production, (2) four milder mtDNA ND5 and ND6 missense mutations causing partial complex I defects and markedly increased ROS production, and (3) a COI missense mutation that causes a 50% reduction in complex IV activity and substantially increased ROS production. Introduction of the ND6 frameshift mutation into the mouse yielded only one female with about 50% mutant mtDNAs and evidence of myopathy. This frameshift mutation was directionally lost over three generations indicating intra-ovarian selection against the frameshift mutant mtDNAs. The COI missense mutation resulted in mice that developed progressive myopathy and cardiomyopathy, as well as neurological decline and increased cancer risk. Therefore, mildly to severely deleterious mtDNA mutations in ES cells which reduce mitochondrial energy and increase ROS production can have severe deleterious consequences on the usefulness of ES cells for cell replacement therapy. Mutations in nDNA encoded mitochondrial genes can also affect the developmental potential of stem cells. The adenine nucleotide translocator (Ant) proteins exchange mitochondrial ATP for cytosolic ADP and help regulate apoptosis. Mice have two Ant genes: Ant1 specific for heart, muscle and brain and Ant2 for all tissues except muscle and testis. Inactivation of Ant1 results in mice which develop a progressive myopathy and cardiomyopathy. Inactivation of Ant2 kills mES cells, and conditional inactivation of Ant2 results in fetal loss due to cardiomyopathy. Muscle satellite cells derived from both Ant1 or Ant2 mutant animals exhibit defects in myoplast to myotube differentiation in vitro. Therefore, mitochondrial defects, whether mtDNA or nDNA encoded can have profound effects on the developmental potential of ES cells for cell replacement therapy.

No: 36

hESC-derived hemangioblasts induce angiogenesis in a rodent model of myocardial infarction

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Recent clinical studies demonstrate that stem cell therapy can lead to improvement in left ventricular (LV) function following a myocardial infarction (MI). In a xenogeneic rat model of MI, we have demonstrated the beneficial effects of both direct injection of hematopoietic CD34+ stem cells (HSCs) and a bispecific antibody (BiAb) targeting approach of CD34+ HSCs. We hypothesized that human embryonic stem (hESC) cell-derived hemangioblasts can be an alternative allogeneic source of cells for myocardial regeneration after ischemic injury. To test this hypothesis, we are now studying the capacity of transplanted hESCs to induce angiogenesis in a rodent ischemic-reperfusion model of MI. Adult female nude rats (150~200g) underwent ligation of the left anterior descending artery for 30 min followed by reperfusion. Different hES-derived hemangioblast cell lines (2×106 cells in 50 µl media, Advanced Cell Technologies) were directly injected into the myocardium via a thoracotomy 10 min post MI or via echo-guided injection 1 day post-MI. Media vehicle served as the control. Hearts from each group were excised, fresh frozen and sectioned 4 weeks after injection. Vessel numbers, vessel density and infarct size were quantified in each immunohistochemistry stained section. We compared four hES-derived hemangioblast cell lines. In the following table, H7, MAO1, and MAO1+HUVEC cells show a trend of increased angiogenesis. But it is not statistically significant due to the small sample size. Infarct Area (mm2) No. of Vessels Density (vessels/mm2) Medium (n=4) 9.2±3.1 86.5±46.4 8.6±2.0 H7 (N=5) 13.9±1.0 142.4±30.0 10.4±2.6 H3 (N=4) 11.0±2.9 103.6±61.0 8.7±3.1 MAO1 (N=4) 10.7±2.6 92.27±23.3 9.2±3.7 MAO1+HUVEC (N=4) 11.7±1.5 112.5±27.8 9.8±3.0 Our preliminary data suggests an increased angiogenesis induced by the H7 and MAO1+HUVEC hES-derived hemangioblast cell lines. Conformation of these findings with a larger sample size is in progress. Neovasculature continuity will be be tested via fluorescent microbead perfusion. The functional aspects of hES-derived cell induced angiogenesis are to be measured by SPECT nuclear imaging for perfusion and LV function.

No: 37

Regulation of self-renewal and pluripotency by Sox2 in human embryonic stem cells

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Human embryonic stem (hES) cells, derived from blastocysts, are capable of unlimited self-renewal and differentiation into all cell lineages of the body. Because of their pluripotent nature, hES cells are valuable tools for understanding human development and advancing the field of regenerative medicine. However, one key to harnessing the therapeutic power of hES cells for biomedical applications begins with determining how these cells maintain their pluripotent and undifferentiated state. Studies in mice have implicated three factors in regulating pluripotency in embryonic stem cells, Oct4, Nanog, and Sox2. However, significant differences in growth regulation between mouse embryonic stem and hES cells have been identified, suggesting a need to determine when and how factors work in hES cells. To date, the transcription factors Oct4 and Nanog have been identified as critical regulators of stem cell fate by functional studies in hES cells. To determine the role of Sox2 in maintaining hES cell pluripotency and self-renewal, we used RNA interference to specifically knock down Sox2 gene expression. Reduction of Sox2 expression in hES cells results in loss of the undifferentiated stem cell state, as indicated by a change in cell morphology, altered stem cell marker expression, and increased expression of trophectoderm markers. In addition, knockdown of Sox2 results in reduced expression of several key stem cell factors, including Oct4 and Nanog, linking these three factors together in a pluripotent regulatory network.

No: 38

Differentiation of T cell progenitors from human embryonic stem cells in a feeder free system

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Human embryonic stem cells (hESC) have the potential to revolutionize certain medical treatments, including T cell based therapies. However, optimal approaches to develop T cells from hESC are lacking. In this report, we show that T cell progenitors can be derived from hESC cultured as embryoid bodies (EB), and that these cells give rise to phenotypically and functionally normal cells of the T lineage when transferred into human thymic tissue implanted in immunocompromised mice. Importantly, the EB system eliminates the previous need for murine co-cultures, a key impediment towards developing a protocol for T cell progenitor derivation suitable for clinical use. Furthermore, following lentiviral-mediated introduction of a vector expressing GFP into hESC, stable transgene expression was maintained throughout differentiation, suggesting a potential for gene therapy approaches aimed toward the augmentation of T cell function or treatment of T cell disorders

No: 39

Kip2 uncouples cell cycle control from differentiation state in a model of human pancreatic endocrine progenitor cells

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The shortage of tissue available for transplantation into diabetic patients could be alleviated by insight into the mechanism by which replication is blocked in insulin producing ß-cells, a normally quiescent population. We determined that Kip2 (p57) is the major cyclin dependent kinase inhibitor differentially expressed in the islet fraction of the human pancreas. Interestingly, Kip2 deficiency in developing human (but not rodent) ß-cells is speculated to cause ß-cell hyperplasia. To facilitate eventual clinical application, and because human and rodent ß-cells differ substantially in potentially important characteristics such as the pattern of cyclin dependent kinase inhibitor (CDKIs) expression, we have focused our efforts on the study of human islets and their precursors. One of the human model systems we developed is T6PNE is a cell line we derived from embryonic islets which expresses the ß-cell transcription factors PDX-1, NeuroD1, and an inducible form of E47. We discovered that E47 elicited cell cycle exit in T6PNE, concurrent with potent induction of insulin gene expression. Thus, the data suggest a role for E47 in coordinating the differentiation (insulin) /cell cycle (Kip2) axis in human ß-cells and their progenitors. Gene array studies revealed that E47 significantly upregulated expression of Kip2. Further, quantitative PCR studies revealed that Kip2 expression was induced one thousand fold by E47. We examined a series of nested deletion mutants of the Kip2 promoter, identifying a 900kb region of the promoter critical for E47 mediated activity. Chromatin precipitation, followed by site directed mutagenesis revealed the Kip2 promoter element for which E47 binding is critical. In order to determine whether Kip2 was sufficient to induce growth arrest in T6PNE, Kip2 was expressed in the absence of E47. BrdU uptake was attenuated in all Kip2 expressing cells, suggesting that Kip2 plays the major role in E47 mediated growth arrest. Conversely, in E47 growth arrested cells, inhibition of Kip2 by shRNA promoted proliferation, notably, without affecting insulin expression. We conclude that manipulation of Kip2 in primary pancreatic cells and progenitors is a promising approach to ß-cell regeneration which does not compromise differentiation state.

No: 40

Ependymal Stem Cells Divide Asymmetrically and Transfer Progeny into the Subventricular Zone when Activated by Injury

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Abstract—Evidence is presented to show that cells of the ependymal layer surrounding the ventricles of the mammalian (rat) forebrain act as neural stem cells (NSCs), and that these cells can be activated to divide by a combination of injury and growth factor stimulation. Several markers of asymmetric cell division (ACD), a characteristic of true stem cells, are expressed asymmetrically in the ependymal layer but not in the underlying subventricular zone (SVZ), and when the brain is treated with a combination of local 6-hydroxydopamine (6-OHDA) with systemic delivery of transforming growth factor-alpha (TGF_), ependymal cells divide asymmetrically and transfer progeny into the SVZ. The SVZ cells then divide as transit amplifying cells (TACs) and their progeny enter a differentiation pathway. The stem cells in the ependymal layer may have been missed in many previous studies because they are usually quiescent and divide only in response to strong stimuli.

No: 41

The APOBEC3 gene family as guardians of genome stability in human embryonic stem cells

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One major threat to genome stability in human embryonic stem cells (hESCs) is the retrotransposition of resident mobile genetic elements. It is likely that metazoans have evolved mechanisms to circumvent the mobilization and integration of these transposable elements. DNA methylation represents one important protective mechanism. Recent studies further suggest a role for the APOBEC3 family of deoxycytidine deaminases. Based on nonsynomous mutations, the APOBEC3 family ranks as one of the most rapidly evolving set of genes in the entire mammalian genome. Over the past 100 million years of evolution, the APOBEC3 locus has expanded from a single gene in mice to seven genes (A3A-A3H) in humans. The need to control retroelement retrotransposition likely played a major role in this remarkable expansion. In somatic cells, retroelement retrotransposition is chiefly suppressed by DNA methylation. These epigenetic changes result in tight chromatin packing of the retroelement DNA and greatly diminished production of retroelement RNA. In contrast, embryonic stem cells exhibit very dynamic changes in DNA methylation. During periods of hypomethylation, retroelement RNA production is likely to occur. Accordingly, hESCs must deploy additional post-transcriptional defensive strategies to constrain these mobile genetic elements. We hypothesized that the APOBEC3 family might be centrally involved in this defense. During the first year of CIRM funding we have found that six out of the seven human A3 genes (A3B-A3H) located in a tandem array on chromosome 22 are expressed in hESCs. A3A, which in prior studies was suggested to exert the greatest anti-retroelement effects in tissue culture assays, surprisingly is not expressed in hESCs. During hESC differentiation into embryoid bodies, A3 protein levels rapidly change with some A3's increasing and other decreasing. Next, a retrotransposition assay was established in hESCs that allows us to visualize genetic jumping of various "marked" retroelements. Using this assay we have clearly shown that Line-1 elements effectively retrotranspose, or jump, in hESCs. To test our central hypothesis, namely that A3 enzymes guard the genome of hESCs, we have established experimental conditions for RNAi knock-down of all expressed the A3 genes. Currently we are combining these knock-downs with the retrotransposition assay to assess the impact of each of the A3 proteins for maintaining hESC genomic stability.

No: 42

Multi-genetic events collaboratively contribute to Pten-null leukaemia stem-cell formation

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Cancer stem cells, which share many common properties and regulatory machineries with normal stem cells, have recently been proposed to be responsible for tumorigenesis and to contribute to cancer resistance. The main challenges in cancer biology are to identify cancer stem cells and to define the molecular events required for transforming normal cells to cancer stem cells. Here we show that Pten deletion in mouse haematopoietic stem cells leads to a myeloproliferative disorder, followed by acute T-lymphoblastic leukaemia (T-ALL). Self-renewable leukaemia stem cells (LSCs)are enriched in the c-Kit(mid)CD3(+)Lin(-) compartment, where unphosphorylated beta-catenin is significantly increased. Conditional ablation of one allele of the beta-catenin gene substantially decreases the incidence and delays the occurrence of T-ALL caused by Pten loss, indicating that activation of the beta-catenin pathway may contribute to the formation or expansion of the LSC population. Moreover, a recurring chromosomal translocation, T(14;15), results in aberrant overexpression of the c-myc oncogene in c-Kit(mid)CD3(+)Lin(-) LSCs and CD31 leukaemic blasts, recapitulating a subset of human T-ALL. No alterations in Notch1 signalling are detected in this model, suggesting that Pten inactivation and c-myc overexpression may substitute functionally for Notch1 abnormalities, leading to T-ALL development. Our study indicates that multiple genetic or molecular alterations contribute cooperatively to LSC transformation.

No: 43

Regulated pre-mRNA splicing during myogenesis and osteogenesis

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While the importance of regulated gene expression during development has been well-documented, the role that post-transcriptional regulation of gene expression, such as pre-mRNA splicing, plays remains largely unexplored. We are examining how global pre-mRNA splicing regulation changes during early myogenesis and osteogenesis, and how these changes translate to key developmental decisions within the cell. Using our splicing-sensitive microarray technology, we identified hundreds of splicing events that become altered during myogenesis or osteogenesis. Interestingly, gene ontology analysis identifies subsets of genes with expression or splicing changes that are unique to each pathway or are shared by both pathways, suggesting, for example, common mechanisms for common processes like exit from the cell cycle and unique mechanisms for differentiation. Additionally, we were able to identify an ACUAA motif present downstream of myogenically-regulated cassette exons. We show that this element functions as a splicing enhancer element in a reconstituted minigene system in mouse myoblast cells. Using RNA affinity chromatography and subsequent MudPIT analysis, we identified a complex of proteins associating specifically with the ACUAA elements in mouse myoblast nuclear extract. These proteins include specific heterogenous nuclear ribonucleoproteins, serine-threonine rich proteins, and most interestingly Quaking 1 (Qk1), which has been implicated in regulation of translation, mRNA stability, and pre-mRNA splicing and is known to bind ACUAA elements. Our current efforts are focused on assigning specific functions to proteins associating specifically with the ACUAA elements.

No: 44

Focal remyelination following intraspinal transplantion of human embryonic stem cell-derived oligodendrocyte progenitor cells into a viral model of multiple sclerosis

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Demyelination of intact axons is a prominent secondary degenerative process in many central nervous system disorders and transplantation of oligodendrocyte progenitor cells (OPCs) has been shown to aid in remyelination. Here, we examined the ability of human embryonic stem cell (hESC) -derived OPCs to survive and promote remyelination in an immune-mediated viral model of demyelination. Intracranial inoculation of C57BL/6 mice with the neurotropic strain of mouse hepatitis virus (JHMV) results in an acute encephalomyelitis followed by a chronic immune mediated-demyelinating disease similar in pathology to the human demyelinating disease multiple sclerosis. In the present study, hESC-derived OPCs were transplanted into spinal cords of JHMV-infected mice at 14 days post inoculation and were kept under daily immunosuppression with cyclosporine A (120 mg/kg) for the duration of the experiment. Results show that human cells present at 1 and 2 weeks did not migrate from the implantation site, and by 3 weeks post-implantation human cells were not present within any animals. Examination of animals at 2 weeks post-transplant revealed limited accumulation of CD4+ and CD8+ T cells with a large presence of macrophage and endogenous OPC (NG2+ cells) surrounding implanted human cells. Interestingly, at 3 weeks post-transplant increased remyelination accompanied by muted demyelination was also evident at the injection site but not at distal sites suggesting the local activation of endogenous OPC by our hESC-derived transplants. These data highlight the complexities and difficulties of transplanting human OPCs into areas of demyelination where robust immune mediated pathology is present.

No: 45

Identification of distal transcription factor binding sites in the hESC genome

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Pluripotency and the ability to self-renew are hallmarks of embryonic stem cells (ESCs). Therefore, these cells hold great promise for regenerative medicine and present an interesting biological phenomenon. Yet, our scope of transcriptional regulation in ESCs remains predominantly incomplete, as gene expression studies and regulatory networks for a few select transcription factors have only begun to elucidate the regulatory mechanisms that underlie these properties. To further our understanding of the role of various cis-regulatory elements and gene networks in the regulation of gene expression, we determined the genome-wide location of enhancers, based on chromatin modifications, and CTCF bound insulator sites in human ESCs. Collectively, they constitute the cis-regulatory blocks necessary for the coordinated control of gene expression with cell type-specific enhancers enriched in blocks with cell type-specific gene expression. Many of these ESC-specific enhancers contain pluripotent transcription factor binding motifs. To validate the chromatin-based enhancer predictions, we have begun determining the genome-wide binding sites of OCT4, SOX2, NANOG, and p300 using high-throughput sequencing of chromatin immunoprecipitated DNA bound by these factors. Identification of these distal binding sites will provide a starting point for determining how CTCF-restricted promoter and enhancers are regulated.

No: 46

Maternal embryonic leucine zipper kinase in mouse mammary tumor initiating cells

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Maternal embryonic leucine zipper kinase (Melk) is a member of the AMPK family of kinases and is highly expressed in a variety of human cancers including poorly differentiated breast cancers. We are investigating the role of Melk in the stem and progenitor cells of the mouse mammary gland and mammary tumors. The expression of a GFP reporter gene driven by the mouse Melk promoter (MelkGFP) gene is elevated in proliferating normal mammary epithelial cells and in mammary tumors caused by oncogenic transgenes. The MelkGFP reporter gene provides a method for rapid isolation of subsets of normal and tumor cells. Preliminary results of the characterization of MelkGFP expressing mammary cells, their culture and their differentiation potential indicate Melk is associated with proliferative progenitor cells in both normal mammary gland and mammary tumors. The ability to monitor the proliferation, differentiation and tumorigenicity of subsets of tumor cells, both in culture and by transplantation, has great potential for identifying key regulatory targets.

No: 47

A genome-wide FlipTrap screen in zebrafish for novel players involved in the maintenance of the stem cell-like state of progenitor cells

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Stem cells are functionally defined as able to self-renew and capable of generating several distinct differentiated cell types. Embryonic stem cells are totipotent cells derived from the inner cell mass of the blastocyst, and can give rise to all cell types. More restricted in their developmental potential, but with great significance for maintenance of tissue homeostasis, stem cell-like populations have been identified in association with differentiated tissues or organs, such as skeletal muscle, heart, skin and hematopoietic tissue. These tissue- or organ-specific populations of cells constitute important local sources of multipotent, proliferating cells, which are activated and recruited in response to tissue damage. In addition, these precursors play a constitutive role in replenishing differentiated tissue with newly generated cells throughout life. Nonetheless, a limiting factor in understanding the mechanisms for the maintenance of stem cell-like properties is the lack of a thorough molecular characterization of tissues or populations with these attributes. To shed light on the mechanisms involved in the maintenance of the stem cell-like state of tissue-specific progenitor cells, we have devised a genetic approach, using a transposon-based gene trap and protein fusion screen (FlipTrap) in zebrafish. Using two heterotypic pairs of loxP sites and Cre-mediated unidirectional recombination, it consists of a construct with two possible conformations: in the initial conformation, the FlipTrap cassette forms a functional fusion protein with citrine (an EYFP variant). In the second conformation, addition of Cre causes the trap to be flipped and its cherry portion is positioned into a functional orientation, as the citrine segment is deleted. In this case, the FlipTrap cassette produces a cherry (RFP variant) gene trap, and the polyA signal causes the transcript to be truncated, thus generating a mutant allele for the trapped gene. Therefore, each FlipTrap line can be used to reveal the gene expression pattern as well as the fusion protein's subcellular localization (citrine fusion trap). Additionally, it also allows assessment of the mutant phenotype (cherry gene trap). Using this approach we have identified a number of uncharacterized genes in zebrafish. Of particular interest, FT57a has been determined by 3' RACE PCR to have trapped a novel member of the musashi family of genes. In this line, citrine is expressed in the olfactory placode, epiphysis and ventral neural tube. Interestingly, in the neural tube, citrine is highly expressed by a subset of ventral cells closely associated with the midline, while differentiated neurons in the dorsal-lateral region of the neural tube, as determined by expression of HuC/D and Islet-1, display lower levels of expression. Subsequent molecular and functional assays will determine the nature of these cells and its importance for the development of neural tube cell types. In conclusion, the unbiased insertion of the trap into the genome will expand the possibilities of identifying novel important genes and pathways in the maintenance of the stem cell-like state of precursor cells, thus helping advance comprehension of its mechanisms and contributing to development of therapies for diseases affecting tissues that contain tissue-specific stem cells.

No: 48

Regulating stem cell differentiation by expressing an engineered Gs-coupled receptor

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Understanding the regulation of tissue differentiation is essential for developing stem cells into new therapies or into disease models. Although G-protein coupled receptors (GPCRs) are strongly implicated in development, their precise functions in differentiation have not been well defined. Several human genetic diseases involving abnormal G-protein signaling affect tissues derived from the mesenchymal lineage, such as bone, cartilage, fat, and muscle. We are developing complementary in vivo and embryonic stem cell models to study the effects of altered Gs signaling in the formation of these tissues. To assess whether Gs signaling alters cellular differentiation in vivo, we used a tetracycline-transactivator system ("Tet-Off") to express an engineered GPCR ("Rs1") that constitutively activates the Gs pathway. Expression of Rs1 in maturing osteoblasts from gestation was sufficient to induce a dramatic anabolic skeletal response with a 2-5 fold increase in femoral bone volume as compared to age and sex-matched littermate controls. The affected mice also displayed decreased muscle mass as well as altered bone marrow structure and adiposity. These results indicate that Gs-GPCR signaling in osteoblasts can produce a progressive increase in bone formation and bone mass, possibly at the expense of other tissues derived from mesenchymal progenitors. We are creating an in vitro model of mesenchymal tissue development using embryonic stem (ES) cells to study the role of Gs signaling in early developmental events, such as the formation of the skeletal system. We have developed a new single-construct tetracycline regulated vector that is custom designed for ES cell expression of Rs1 and other signaling molecules. These constructs have been introduced into mouse embryonic stem cells by directed targeting into the Rosa26 locus. Preliminary studies demonstrate doxycycline-regulated Rs1 expression. Increased intracellular cAMP levels are detected in the Rs1-expressing ES cells. Finally, teratoma studies suggest that tissues representing the three germ lineages are present. The results of these studies will provide complementary in vivo and in vitro models for understanding the roles of G protein signaling in stem cell differentiation.

No: 49

Investigating the role of BMI-1 as a cooperative oncogene in Ewing Sarcoma family tumors

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Ewing's Sarcoma Family Tumors (ESFT) are highly aggressive bone and soft tissue tumors of unknown histogenesis that primarily affect children and young adults. ESFT express EWS-FLI1, a highly toxic chimeric oncogene that induces cell cycle arrest or death in most primary cells. The polycomb group family protein BMI-1 is frequently deregulated in cancer and has been shown to promote stemness and tumorigenicity, largely through transcriptional repression of the CDKN2A locus which results in inhibition of the cell-cycle inhibitors p16INK4A and p14ARF. In these studies we are investigating the hypothesis that BMI-1 may confer tolerance to EWS-FLI1 and thereby function as an important cooperative oncogene in the initiation and maintenance of ESFT. We have shown that BMI-1 is highly expressed by both primary tumors as well as ESFT cell lines and that its expression promotes anchorage-independent growth in vitro and tumorigenicity in vivo. Surprisingly, gain and loss of function studies revealed that BMI-1-mediated promotion of tumorigenicity in ESFT is independent of CDKN2A repression. To further identify novel CDKN2A-independent mechanisms of BMI-1 action, we performed gene expression profiling of ESFT cells following BMI-1 knockdown and demonstrated, significant modulation of genes involved in development as well as in cell adhesion. These data suggest that BMI-1 may mediate ESFT tumorigenicity by influencing tumor cell differentiation as well as cell:cell and/or cell: matrix interactions. Indeed, BMI-1 knockdown in ESFT cells induces expression of genes associated with neural/neural crest differentiation and adversely affects the adhesion capacity of ESFT cells in vitro. In ongoing and future studies we aim to further characterize and validate the functional significance of these novel downstream effectors of BMI-1 oncogenic activity. In addition, we are investigating whether BMI-1 can cooperate with EWS-FLI1 to induce malignant transformation of human neural crest and/or mesenchymal stem cells - putative cells of origin for ESFT. These studies are offering crucial insights into the molecular origins of ESFT and elucidating the role of disrupted stem cell pathways in the pathogenesis of this highly malignant family of human tumors.

No: 50

Inducing pluripotent cells from retinal pigment epithelial cells

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Various ocular diseases are characterized by a loss of specific types of ocular cells. One of the promising therapies is to transplant functional ocular cells. Human embryonic stem cells (hESC) have potential for curing ocular diseases by differentiating to ocular cells. However, current methods are not suitable for developing patient-specific, immunologically matched hESC. We reprogrammed the retinal pigment epithelial (RPE) cells to induced pluripotent stem (iPS) cells via expressing a specific set of transcription factors. Reprogrammed iPS cells exhibited the similar morphologies as hESC, expressed stem cell markers, and were able to differentiate to three germ layers. We hypothesize that these iPS cells contain epigenetic molecules, so that iPS cells may intend to redifferentiate to original cell types. Thus, iPS cells from RPE cells may be applied to generate ocular cells. These may facilitate the development of iPS cell based transplantation therapies for ocular diseases.

No: 51

Exploring the roles of Eph receptors and ephrins during neuronal differentiation of embryonic stem cells.

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Eph receptors and ephrins are bi-directional signaling molecules that form the largest family of receptor tyrosine kinases and their cognate ligands in mammals. Ephs and ephrins are required in many stem cell related processes, including development of the pancreatic and intestinal stem cell niches and neuronal stem cell function (Holmberg et al. 2006; Depaepe el al. 2005). These observations suggest that Eph/ephrin signaling can influence neural development via multiple mechanisms, including promoting exit from the cell cycle, elimination of unwanted cells via apoptotic mechanisms or by signaling with transcription factors such as STAT3 (Bong et al. 2007). An important goal for the stem cell field is to understand how embryonic stem cells can be differentiated into specific cell and tissue types for use in regenerative and transplant therapies. I am investigating the roles of Eph/ephrin signaling during the differentiation of mouse embryonic stem cells (mESCs) to neurons. Using feeder-free E14Tg2A and 46C stem cell lines, I have characterized EphR and ephrin expression profiles for the entire gene family, initially by reverse transcription - polymerase chain reaction (RT-PCR) and more recently by quantitative RT-PCR. Surprisingly, all members of the murine EphR/ephrin gene families are expressed during a 13-day mESC to neuron differentiation protocol. However, the expression levels of some genes vary widely; ephrin-A1, EphA3, EphA6 and EphA7 show zero or low transcript levels in mESCs but increase markedly at the neuroblast stage (day 8). In contrast, EphA1 mRNA levels are initially high in mESCs but diminish by day 13 of differentiation. We have also used EphR and ephrin-Fc fusion proteins to identify cognate binding partner cell populations via flow cytometry. EphA3-Fc, EphA7-Fc, ephrin-A1-Fc and ephrin-A5-Fc bind 100% of mESCs and showed similar staining levels across all cells. In contrast, EphA5-Fc and EphB2-Fc bound mESCs variably. Some cells were refractory to EphA5-Fc binding whereas EphB2-Fc bound all cells, but with highly variable intensity. We are currently using fluorescence-assisted cell sorting of EphA5-Fc marked cells to see if these sub-populations show differential developmental programs. We are also examining if Eph-Fc and ephrin-Fc labeled neuroblasts derived from mESCs show differing developmental potential. Our ultimate goal is to take our Eph/ephrin-based discoveries from the mouse stem cell system and apply them to human stem cell differentiation. It is our hope that Eph-Fc and ephrin-Fc tagging will provide a powerful tool for identification and purification of particular neuronal lineages that can ultimately be used in transplantation therapies.

No: 52

Ly6d bisects common lymphoid progenitors into T cell and B cell biased subpopulations

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Early hematopoietic development proceeds in a stepwise manner through progenitor intermediates, which sequentially divide and lose potential for alternate lineages. These progenitors were initally identified and isolated by their expression of unique combinations of surface markers; however, many progenitor populations are considered impure and heterogenous. As a result, one of the key challenges in stem and progenitor cell biology is how to identify novel markers to separate functional progenitor cells from lineage-committed downstream populations. Using the Boolean analysis of over 2000 publicly-available mouse expression arrays developed by Sahoo et al., we identified 27 unique surface proteins with predicted differential expression during the transition from fully multipotent to lymphoid-restricted to committed B cell progenitors. Importantly, no arrays from these populations were present in the analysis. We identified one marker, Ly6d, which bisects common lymphoid progenitors (CLP) into two distinct populations. In vivo, Ly6d- CLP have robust B and T output, while Ly6d+ CLP produce primarily B cells. In the thymus, Ly6d- and Ly6d+ CLP are T cell and B cell biased, respectively. These results highlight the utility of the Boolean analysis algorithm developed by Sahoo et al to identify developmentally regulated genes, even when intermediate populations are unknown.

No: 53

Threshold of a key regulator specifies the number of transit amplifying divisions in an adult stem cell lineage

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In high throughput adult stem cell lineages, such as blood, skin and the germ line, progenitor cells destined for differentiation commonly undergo several rounds of mitotic transit amplifying (TA) divisions before initiating terminal differentiation, allowing small numbers of adult stem cells to produce many differentiated progeny. Although defects in the regulation of TA divisions can have serious consequences, such as faulty tissue replenishment or cancer, the mechanisms that count the number of TA divisions and regulate when precursor cells stop dividing and differentiate are not known. I am using a model stem cell lineage, the Drosophila male germ line, to investigate the counting mechanism that specifies the number of TA divisions. The Drosophila Bag of Marbles (Bam) protein is required for male germ cells to cease TA spermatogonial division and initiate differentiation. My results suggest that the Bam protein must reach a critical threshold in late TA cells to trigger differentiation, that this accumulation is independent of the cell cycle, and that the timing of Bam accumulation with respect to cell cycle progression determines the number of TA divisions in the Drosophila male germ line. I am now investigating what Bam is doing biochemically at threshold levels. Because a known binding partner of Bam, Bgcn, has a proposed RNA binding motif, we are using a global approach and testing specific candidates to determine whether Bam may be regulating translation or stability of specific RNAs. One candidate RNA which Bam may negatively regulate is meip26. meip26 RNA but not protein is expressed in cells expressing Bam protein. We hypothesize that Bam may be required to keep meip26 RNA translationally repressed in TA cells, and that Meip26 protein is subsequently required to be re-expressed upon Bam downregulation for differentiation.

No: 54

The CD133 cancer stem cell marker is cell cycle dependent

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Background: Cells displaying a stem cell like phenotype have been proposed in several different types of cancer. A variety of markers have been used to characterize these cells but recently, it has been suggested that one protein in particular, the glycosylated form of the cell surface protein CD133 (AC33), is highly expressed in cancer stem cell like cells, including medullablastomas, glioblastomas, prostate and colon carcinomas. The function of AC133 is yet unknown, but it has been shown that cells that express AC133 are more likely to form tumors in vivo than cell that are AC133 negative. In the present work we investigated AC133 expression in correlation with the cell cycle profile of human embryonic stem cells, colon cancer and melanoma cells. Results: Fluorescence activated cell sorting (FACS) analysis and immunofluorescence staining of H9 hES cells, colon cancer cells (Caco2) and melanoma cells (WM115) detected high levels of the AC133 epitope. For all three cell lines, FACS analysis of the DNA contents of AC133 reactive cells revealed that cells with greatest AC133 reaction were enriched for cells with DNA content of 4N, or even greater. The least reactive cells were in the G1/G0 portion of the cell cycle. Continued cultivation of cells sorted on the basis of high and low AC133 reactivity results in a normalization of the cell reactivity profiles indicating that cells with low AC133 reactivity can generate highly reactive cells as they resume proliferation. Gene expression profiles revealed remarkable similarity of cells expressing high or low amounts of the antigen with the notable exception of CD133. Conclusions: Our study indicated that reaction with the AC133 antigen is cell cycle dependent with the highest expression associated with cycling cells in G2/M and the lowest level of expression in cells in G1/G0. Furthermore, we show that cultivation of cells with the extremes of AC133 reactivity resulted in a redistribution of the antigen expression which suggests that high and low expressing cells do not belong to stable distinct populations. AC133 reactivity may be valuable for identify cells with increased tumorgenicity. However, the basis of this utility may be due to the distinction between proliferative and quiescent cells and therefore AC133 should be used cautiously as a putative marker of a stable, distinct stem cell like population.

No: 55

Human embryonic stem cells as tools to investigate the neural crest origin of ewing's sarcoma

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Ewing's sarcoma family tumors (ESFT) are the second most common bone and soft tissue malignancy of children and young adults. Although their histogenesis remains elusive, ESFT display varying degrees of neural differentiation and nearly all tumors express characteristic fusion oncogenes, most commonly EWS-FLI1. The EWS-FLI1 fusion gene behaves as an aberrant transcription factor and is believed to initiate ESFT development. Paradoxically, despite its action as on oncogene in ESFT, EWS-FLI1 is toxic to most primary cells. Recent data suggest that somatic stem cells may be uniquely tolerant of ESFT fusion oncogenes and amenable to EWS-FLI1-mediated malignant transformation. In these studies we are using model organisms and human embryonic stem cells (hESC) to investigate the consequences of EWS-FLI1 expression on neural crest development. It is our goal to determine if neural crest stem cells (NCSC) may be the cells of origin for ESFT. Using the developing chick embryo as a model system, we have shown that mis-expression of EWS-FLI1 or wild-type FLI1 in the neural tube leads to significant aberrations in NCSC migration and neural crest development. To determine if EWS-FLI1 similarly disrupts human neural crest development and may thereby initiate ESFT genesis we have exploited the pluripotency of human embryonic stem cells (hESCs). Using the stromal-derived inducing activity (SDIA) of PA6 fibroblast co-culture we have induced hESC to differentiate into terminally differentiated neural crest cells within three weeks. Within one week of co-culture, cells that express the early neural crest markers p75 and HNK1 as well as numerous other genes associated with neural crest induction such as SNAIL, SLUG and SOX10 are readily detectable and can be seen migrating away from the hESC colonies. FACS-based isolation of this p75 positive population enriches for cells with genetic, phenotypic and functional characteristics of NCSC. These p75-enriched cells readily form neurospheres in suspension culture, self-renew to form secondary spheres, and give rise under differentiation conditions to multiple neural crest lineages including peripheral nerves, glial and myofibroblastic cells. Thus, SDIA co-culture combined with FACS-based enrichment can be used to successfully and efficiently isolate early human NCSC from hESC in vitro. We are now assessing if these hESC-derived cells integrate and migrate as neural crest cells in vivo. In addition, we are using lentiviral-mediated transduction to express EWS-FLI1 in hESC-derived NCSC. This innovative system will, for the first time, permit evaluation of the consequences of EWS-FLI 1 expression in human NCSC and early neural crest progenitors and provide crucial insights into the cellular and molecular events that underlie ESFT tumorigenesis.

No: 56

Comparative analysis of messenger RNA and microRNA expression profiles of human embryonic stem cells, primary hepatocytes, and hepatocellular carcinoma cells

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Recent emergence of literature has described the apparent parallels between the molecular traits of tumor cells and stem cells. The implications of such understanding would enhance the applicative potential of stem cells for therapeutics and the detection of oncogenesis in cancer diagnosis. However, much focus has been centered on demonstrating the enrichment patterns of gene sets that are associated with embryonic stem cell (ESC) identity in the expression profiles of tumor cells, and it is unclear, whether these patterns are common to all tumor cells, or tumor type dependent. Here, we have undertaken a comprehensive analysis of global mRNA and miRNA expression signatures in hESCs, primary hepatocytes (hPHs), and two lines of hepatocellular carcinoma cells (HepG2 and Hep3B). Strong correlation and hierarchical clustering were observed between the gene expression activities of HepG2 and Hep3B ($r = 0.8511$), while a marked decrease in similarity was noted between hESCs and hPHs ($r = 0.5654$), suggesting that similar alterations in gene expression accompanied the transformation of the two carcinoma cells, while a very different set of genes were required for the differentiation of pluripotent stem cells into hepatocytes. When we examined the relationship between hESCs and the two carcinoma cells, we observed r values that were in between 0.8 and 0.5, and notably similar to that of r values that were derived from the correlational analyses of hPHs with HepG2 and Hep3B. Further analysis of the differentially and similarly expressed genes between and within these groups using the Gene Ontology functional categories revealed that a large fraction of genes that were commonly expressed between hESCs and the carcinoma cells were involved in metabolism, ion transport, and protein biosynthesis. In contrast, stem cell specific genes (e.g., Nanog, Oct4, and Sox2) were highly expressed only in hESCs, while genes that are indicative of fully differentiated mature hepatocytes (e.g., ALB) remained unchanged between hPHs and the hepatocellular carcinoma cells. Interestingly, genes that are associated with earlier stages of hepatic differentiation (e.g., AFP) were dramatically up-regulated in HepG2 and Hep3B, and remained down-regulated in hPHs, suggesting that these carcinoma cells may have partially re-acquired the functional properties of less differentiated, hence more stem cell-like, hepatic progenitor cells. The expression patterns of miRNAs in the same set of cells revealed a stronger correlation between hESCs and the carcinoma cells, wherein, a dramatic up-regulation of miRNAs that are hESC specific were frequently observed in HepG2 and Hep3B, but not in hPHs. On the other hand, specific mRNAs that are essential for miRNA biosynthesis (e.g., Drosha, DGCR8, Dicer, and Argonaut) were slightly up-regulated in hESCs, but similarly expressed in hPHs, HepG2, and Hep3B. Hence, our analysis revealed that the two hepatocellular carcinoma cell lines possess traits that were in general, somewhere in between those ascribed to stem cells and hepatic cells. As such, subtle differences and similarities between tumor cells and stem or differentiated cells may play an important role as markers of oncogenicity.

No: 57

Survival and differentiation profile of HUES7 and HUES9 cells after spinal grafting in rats with spinal ischemic paraplegia

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In previous studies, we have demonstrated long term survival and neuronal differentiation of human spinal neural precursors when grafted spinally in rats after spinal ischemic injury. The goal of the present study was to characterize the survival and differentiation profile of human embryonic HUES7 and HUES9 cells when grafted spinally at different densities (500-5000 of viable cells/injection) in rats with ischemic paraplegia. Three different cell preparation protocols were employed before cell grafting: 1) a single cell suspension was prepared from proliferating ES colonies by using collagenase (Group A); 2) previously frozen cells were thawed, washed and viability tested (Group B); or 3) cells were differentiated using a standard 3 step differentiation protocol and the "rosettes" like structures collected and dissociated with trypsin (0.05%). Cells were then cultured in non-adherent 6 well plates to form neurospheres. After 7-14 days, undissociated neurospheres (40-80 µm in diameter) were used for spinal grafting (Group C). In groups A and B, animals received 5 bilateral injections (500 µm apart) targeted into intermediate zone (lamina VII) of L2-L5 segments. Each animal received only one concentration of cells, i.e., 500, 2500 or 5000 cells per injection. In group C, animals received 15-25 neurospheres per injection. After grafting, animals survived for 2-6 weeks and the presence and phenotype of grafted cells confirmed with standard immunofluorescence staining protocol and human specific antibodies. In colocalization studies, confocal analysis was performed. All animals were immunosuppressed with Prograf (1-3 mg/kg/day; i.p.) for the duration of study. Results: In groups A and B, a clear development of teratomas was seen. In contrary to previous reports, the occurrence of teratomas was independent of the density of cells injected. In group C, no teratomas were formed in any of the grafted animals. By using human specific antibodies, the majority of grafted cells showed immunoreactivity for nestin or colocalization of NUMA-positive cells with DCX antibody (early postmitotic neuronal marker). A clear ingrowth of host derived TH-immunoreactive processes into the graft was also identified. These initial data demonstrate that hESC-derived NPs can represent a readily accessible cell source for cell replacement therapy in patients with spinal ischemic injury. Characterization of long term survival and synapse formation between grafted cells and the host neurons is currently in progress. Support: (CIRM: RC1-00131)

No: 58

Genetic modification of the human genome to resist HIV-1 infection and/or disease progression

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Background: The successful modification of human genes to forms that provide resistance to HIV-1 infection will not only serve as a proof of principle for human embryonic stem cell (hESC) genetic therapies for AIDS, but also serve as a model for other diseases in which modification of the genome in hESC would have therapeutic benefits. We recently expressed an siRNA directed against chemokine (c-c motif) receptor 5 (CCR5) using lentiviral mediated transduction of CD34+ rhesus hematopoietic stem/progenitor cell and found stable reduction of the CCR5 expression in rhesus T-lymphocytes. We test stable expression of shRNA in hematopoietic CD34+ progenitor cells and H1 hESC cells, and down-regulation of CCR5 expression in differentiated hematopoietic progeny cells, particularly macrophages, which are a major player for HIV transmission. Results: We constructed a lentiviral vector expressing CCR5 shRNA (1005), previously shown to downregulate CCR5 in human T-lymphocytes (PNAS. 2007, p13110). We first compared the transduction efficiency by monitoring expression of the EGFP reporter in the vectors in CD34+ cells derived from fetal liver (FL-CD34+) compared to CD34+ cells derived from H1 cell embryoid bodies (hESC-CD34+). Transduction efficiencies in FL-CD34+ and hESC-CD34+ was similar (62.8% and 66.6%, respectively). Maintenance of the vector was monitored in vitro in the FL-CD34+ cells and following differentiation into macrophages using IL-3, MCSF, and SCF after cell sorting by EGFP expression. EGFP expression was maintained at 89 % in the populations after one month. Quantitative real-time PCR for CCR5 mRNA indicated 7-fold reduction of CCR5 mRNA levels in CCR5 shRNA transduced population. Conclusion: We successfully transduced our lentiviral vector expressing CCR5 shRNA in hematopoietic progenitor cells derived from fetal liver and hESC, and confirmed the reduction of CCR5 mRNA levels in the macrophages derived from FL-CD34+. We are currently in the process of assessing the effect of shRNA expression on CCR5 expression in the macrophages derived from hESC-CD34+ in comparison to those from FL-CD34+.

No: 59

Establishing a role for DNMT3L in directing dna methylation in embryonic stem cells

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In mammals, epigenetic regulation has emerged as a critically important process necessary for the proper regulation of gene expression during embryonic development. Such epigenetic control is ensured through the modification of histones, and the methylation of DNA, which represent two inter-connected layers of epigenetic information. The DNMT3A and DNMT3B cytosine DNA methyltransferases (DNMTs), which are highly expressed during the blastocyst stage, catalyze the transfer of methyl groups to CpG dinucleotides de novo and are required for proper cellular differentiation. The DNMT3L protein by contrast, is inactive on its own yet serves as a potent stimulatory factor for de novo methylation. In addition, DNMT3L was recently shown to bind to the histone H3 tail and to respond to specific histone post-translational modifications. Here, we address the possibility that DNMT3L can serve to target DNA methylation to specific genomic loci in murine embryonic stem cells (mESCs). In order to characterize potential DNMT3L targets, we have combined methylated DNA immunoprecipitation (MeDIP) and chromatin immunoprecipitation (ChIP) techniques using both wild-type and Dnmt3L-/- mESCs. Our data so far indicates that a subset of genes, strongly enriched for developmental and transcriptional regulators, rely on the presence of DNMT3L for proper promoter methylation. We are currently expanding this approach to the human Ntera2 pluripotent testicular carcinoma cell line. This will allow us to identify human DNMT3L genomic targets and to provide insights into the emerging role of DNMT3L over-expression on the progression of testicular tumors from benign carcinomas-in-situ to aggressive nonseminomas.

No: 60

Mechanisms of automaticity of human embryonic stem cell-derived cardiomyocytes

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Human embryonic stem cells-derived cardiomyocytes (hESC-CMs) have been proposed for cardiac repair after myocardial infarction or for generating biological pacemaker cells. However, most of hESC-CMs generate spontaneous action potentials (automaticity) and display various electrophysiological phenotypes. The molecular basis of automaticity of these hESC-CMs during differentiation and maturation remains poorly understood.

Methods: The H9 hESC line was transduced with a lentiviral vector containing the Puromycin-resistant gene under the control of the cardiac-specific □-MHC promoter. Embryoid bodies at various stages of differentiation from this hESC line were treated with Puromycin to isolate clusters of pure cardiomyocytes (termed cardiospheres). These cardiospheres were then plated for calcium imaging study with Fluoro-4 and pharmacological characterization.

Results: Calcium imaging and pharmacological analysis of purified hESC-CMs showed that early hESC-CMs have different mechanisms of automaticity from the more differentiated hESC-CMs. The mechanisms of dominant automaticity of hESC-CMs evolved from an intracellular Ca2+-mediated pulse-generation to a mature and complex automaticity during hESC-CM differentiation. These results suggest that future research on molecular signaling pathways governing automaticity development of hESC-CMs is needed to develop proper types of cardiomyocytes for cell-based therapy.

No: 61

Directed differentiation of hues9 human embryonic stem cells into forebrain neurons

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Human embryonic stem cells have the potential to serve as an unlimited source of neuronal tissue for cell replacement therapies that may ameliorate many devastating neurodegenerative diseases such as Alzheimer's Disease and Parkinson's Disease. However, there are important caveats for their use. First, the transplanted cells must be a pure population of a desired cell type. Second, the cells have to retain the cell fate specified under culture conditions after transplantation. Finally, the cells have to be functionally connected in a physiologically relevant way to existing circuitry. Therefore, it is critical to develop strategies that will yield efficient differentiation and enable purification of specific cell types. We are attempting to develop a strategy to direct the differentiation of hESCs into forebrain neurons by exposing them sequentially to developmentally relevant signals. HUES9 hESCs lentivirally transduced to express GFP (HUES9-CMV-GFP), were induced to form embryoid bodies using forced aggregation in V-bottom 96-well plates. When these "spin EBs" were cultured in suspension in the presence of the BMP antagonist Noggin and then allowed to attach to a matrigel substrate, numerous neuroepithelial rosettes appeared. The early neuroectodermal marker Sox1 expression was increased with Noggin treatment compared to non-treated controls. These rosettes were subsequently dissected, replated, and expanded in the presence of FGF2 and EGF as neural progenitor cells (NPCs). At the rosette and NPC stage, forebrain markers such as Pax6 and Emx2 were upregulated, whereas the more caudal marker HoxB4 was undetectable by RT-PCR. Terminal differentiation of the NPCs was initiated by replating them at a lower density on a glial feeder layer and withdrawal of FGF2 and EGF. After 3-4 weeks in the co-culture, the NPCs differentiated into numerous nestin-positive and TuJ1-positive cells with neuronal morphologies. Patch clamping was used to characterize the electrophysiological properties of these cells. The average cell capacitance (Cm) was 16.3±1.8 pF (n=15), membrane resistance (Rm) was 0.9±0.2 GO (n=15), and the resting membrane potential (Em) -57.7±4.7 mV (n=13). GFP+ differentiated hESCs showed a varied range of voltage-dependent sodium and potassium currents (INa and IK), sensitive to inhibitors TTX and 4-AP, respectively. The relative magnitude of INa and IK within a particular cell correlated well with changes in membrane depolarization in current clamp mode. Cells with large INa and small IK tended to elicit an initial depolarizing spike with a sustained depolarization. Cells with INa >1nA and IK >400pA showed spontaneous firing at resting membrane potential with clearly defined bursts of action potentials. These currents typically reflected those recorded from mature neurons. Cells with currents smaller then 200pA had no significant depolarization in response to current injection. These observations suggest that hESCs exposed to developmentally relevant signals can give rise to cells that display a range of characteristics typical of forebrain neurons.

No: 62

Engineering anti-HIV immunity from human embryonic stem cells

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The human immunodeficiency virus (HIV) has a profound effect on lymphoid cells in an infected individual. A therapeutic strategy that contributes to the antiviral immune response and allows protection and reconstitution of these cells could have important implications in the treatment and clinical prognosis of an infected individual. We are interested in exploring the potential of human embryonic stem cells (hESCs)to allow hematopoiesis and further engineering these cells to combat HIV infection. We have previously shown that hESCs are capable of differentiation, following various manipulations, into cells of multiple hematopoietic lineages. In particular, hESCs are capable of differentiating into thymocytes following the introduction of hESC derived hematopoietic stem cells into human thymic implants residing in SCID mice. We are currently utilizing this system to examine the ability of hESC derived hematopoietic progenitors to generate mature CD8+ cytotoxic T lymphocytes (CTLs) that express a transgenic anti-HIV T cell receptor (TCR). In preliminary studies, we have utilized a TCR specific for the SL9 peptide, a conserved region in the HIV gag protein sequence. This TCR is restricted for this peptide presented in the context of the HLA-A2.01 molecule. To initially show proof-of-principle that a “transgenic” TCR can function in the context of human cells, we transduced this vector into CD34+ human hematopoietic progenitor cells derived from fetal liver. These were introduced into SCID-hu mice bearing human thymic implants that expressed the restricting HLA-A2.01 molecule, to allow positive selection and lineage commitment of the transduced cells during thymopoiesis. We determined that the expression of the anti-HIV TCR appears to allow gag-specific tetramer binding, and induces preferential lineage commitment towards CD8+ mature thymocytes. These studies suggest that proper differentiation of these “TCR transgenic” cells is occurring. This system should allow us to pursue in vivo modeling of this approach, and extend it beyond HSC into hESC.

No: 63

Core stemness: a homolog perspective

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Stem cells are defined by their ability to generate and maintain tissues and organs and therefore hold a great potential in regenerative medicine. There are several types of well-studied adult and embryonic stem cells in both mouse and human. As these different types of stem cells share functional properties, it has been hypothesized that stem cells express a core set of genes that regulate stem cell function. Various studies have been performed with the aim to identify such "stemness" genes: genes required for the maintenance of the stem cell state. However, few stemness genes have been identified at the single-gene level. In particular, the studies in mouse of Ivanova, et al, Ramalho-Santos, et al and Fortunel, et al surprisingly identify only a single common stemness gene, Itga6. In this analysis, we are taking a more global approach to the stemness problem. Because of the robustness and redundancy encoded in the genome, we hypothesize that stem cells may employ common pathways even though they express distinct repertoires of genes. To uncover core pathways utilized in stem cells, we use gene expression data to search for stemness homolog families. Each stemness homolog family is identified as a group of evolutionarily related genes that are upregulated reproducibly in various stem cells and across multiple independent studies. Initially, we characterize mutually exclusive groups of highly similar genes based on protein sequence similarity. We then determine if the family as a whole is upregulated in stem cells using a sensitive statistical test that integrates the gene expression data across all genes in the family. Preliminary data identified several significant groups of interest, including the myc, the integrin alpha, the map kinase and the melanoma antigen families. Each family contained at least one gene that showed upregulation in at least five of the nine stem cell populations investigated in this global survey, while some other family members were upregulated in a single population. On average, family members were upregulated in 3.3 cell types in the study. These preliminary findings implicate a selection of gene families that are shared between different types of stem cells and may point to common mechanisms of stem cell function.

No: 64

The effect of Hedgehog signaling on BMP and BMP antagonists in intestinal development

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Mesenchymal-epithelial interactions are critical to developmental growth. Investigation of intestinal development has identified several key signaling families involved in this process including the bone morphogenetic protein (BMP), Wnt/beta-catenin, and Hedgehog pathways. How these pathways coordinate to generate and maintain the intestine is not completely understood. A potential source and target of these signals are intestinal subepithelial myofibroblasts (ISEMFs). Our recent study revealed ISEMFs express BMP antagonists that are capable of activating Wnt signals within intestinal epithelial cells. Interestingly, BMPs, which have been shown to inhibit Wnt signals are also expressed by the same myofibroblast cell population. While the roles of BMPs and BMP antagonists in intestinal development have been established, we know little about how BMP signals are regulated in the intestine. Recent evidence suggests Hedgehog signals target ISEMFs, which make these signals promising candidate regulators of the BMP pathway in the intestine. To determine whether Hedgehog signals are involved in the regulation of BMP pathway components, we measured the expression levels of BMPs and BMP antagonists following manipulation of Hedgehog signals in vitro and in vivo. We show that N-Sonic Hedgehog (N-Shh) treatment of human ISEMFs resulted in a 5-fold decrease in gene expression of the BMP antagonist gremlin 1 and elicited nearly a 2-fold increase in BMP2 gene expression. Utilizing Ihh -/- mice, we found that loss of Hedgehog signaling increased gremlin 1 expression 2-fold in E17.5 intestine. These results suggest that Hedgehog signaling negatively controls BMP antagonist expression, and may act in collaboration with the BMP pathway to drive differentiation of intestinal epithelial cells. Additional experiments using an intestine-specific Ihh knockout mouse (villin-Cre;Ihhflox/flox) suggest that the formation of myofibroblast cells at intestinal crypts may be dependent on Indian hedgehog signaling. These results are an important step toward understanding how mesenchymal-epithelial interactions regulate intestinal stem cell self-renewal and differentiation.

No: 65

Smad 6 and Smad 7 modulation of BMP and TGFß pathways

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Bone morphogenetic proteins (BMPs) are the only growth factors that can commit mesenchymal stem cells (MSCs) to the osteoblast lineage. The predominant mode of signaling for BMP and transforming growth fator-beta (TGF-beta) is through R-Smads 1/5/8 (BMP) or R-Smads2/3 (TGF-beta/activin). Smad activity is regulated intracellularly by inhibitory Smads (I-Smads), Smads 6 and 7. Smad 6 inhibits BMP pathways, whereas Smad7 inhibits BMP and TGF-beta pathways. Smads 6 /7 recruit the ubiquitin ligases Smurf1 and Smurf2 to BMP/TGF-beta receptors, inducing receptor degradation. The promoters of I-Smads 6 /7 contain Smad binding elements. Thus, Smads 6 /7 form negative feedback pathways that limit the intensity and duration of BMP and TGF-beta signaling. Smads 6 /7 also mediate crosstalk with other signaling pathways. These I-Smads are induced by proinflammatory mediators and inhibit activation of NF-kappa B. Through a knockout mouse model we have observed that Smad6-/- mice develop heterotopic ossification of blood vessels. Smad7-/- mice exhibit inflammatory defects due to hyperactive TGF-beta activity in B cells. We have shown that growth plate chondrocytes and osteoblasts of Smad6-/- mice demonstrate elevated BMP activity, evidenced by increased proliferation and increased levels of pSmad1/5 activity in cartilage and periosteum where osteoblasts reside. Western analyses of lysates from long bones confirms that active BMP Smad levels are elevated, and also reveals a compensatory decrease in levels of active TGF-beta Smads (pSmad2/3). Subsequently, we have further characterized in vitro the transcription of genes downstream in the TGF-beta signaling cascade as well as those genes involved in osteoblast differentiation (Runx2, ALP) using both wild type and Smad 7-/- calvarial osteoblasts. We have also demonstrated an increase in osteopontin expression among the Smad 7-/- mutants. In conclusion, inhibitory Smads (Smad 6 /7) are negative regulators of the BMP and TGF-beta pathways and loss of these regulatory elements can lead to an increase in the molecular machinery driving toward osteogenesis. Further studies are currently in progress to further characterizing these in vitro expression patterns in Smad 6-/- calvarial osteoblasts and to evaluate the feasibility of blocking Smad6 and Smad7 activity in mesenchymal stem cells in order to augment fracture healing. Effects on the NF-kappa B pathway are also being analyzed in these murine Smad 7-/- cells. The theoretic enhanced response to BMP and accelerated differentiation of these Smad 6-/- and Smad 7-/- calvarial osteoblasts as well as Smad 6-/- and Smad 7-/- bone marrow stromal cells will likely provide us with essential information for improving osteogenesis and fracture healing in the future.

No: 66

The role of protein prenylation in epidermal stem cell function

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Regulation of stem cell proliferation is a key step in development, tissue homeostasis, and diseases such as cancer. Recent work has identified small protein GTPases like Rac1, Kras and Eras as regulators of stem cell function. A key feature of those proteins is a carboxyl-terminal CaaX motif, which triggers posttranslational modification by an isoprenyl lipid-either a 15-carbon farnesyl or a 20-carbon geranylgeranyl group. These lipids are added by the cytosolic enzymes farnesyltransferase (FTase) and geranylgeranyltransferase-I (GGTase-I). This lipid modification, generally called prenylation, is important for the targeting of proteins to the plasma membrane and for proper protein function. Thus, posttranslational modifications such as protein prenylation could affect stem cell proliferation and the maintenance of tissue homeostasis. Recent studies of FTase and GGTase-I suggest that both enzymes are essential for cell proliferation. Specific protein substrates for FTase and GGTase-I are presumably important for normal cell proliferation, but the identity of these substrates is not clear. I hypothesize that specific proteins-substrates for FTase and GGTase-I-are important for regulating both transit-amplifying cell proliferation and stem cell self-renewal. In addition, there is evidence that stem cells in the epidermis utilize unique mechanisms to balance self-renewal and differentiation. Hair follicle stem cells enter and exit the cell cycle differently than their transit-amplifying progeny, as judged by cell-cycle profiles. Identifying the prenylated proteins that are crucial for cell proliferation should provide insights into mechanisms for regulating stem cell self-renewal in both health and disease. To begin to address this hypothesis, I will use mouse skin as a model system. The skin is a highly proliferative tissue with a well characterized stem cell population tractable for investigation. Our group has already generated conditional knockout alleles for both FTase and GGTase-I. With these mice and other genetic tools, I am poised to perform the experiments necessary to assess the functional importance of FTase and GGTase-I in epidermal stem cells. My first aim will be to knockout FTase and GGTase-I function in the epidermis including the follicular stem cell niche. My second aim will be to knockout FTase and GGTase-I function in only the follicular stem cell niche. Specifically, I will use constitutive and inducible transgenic Cre mice to generate skin specific FTase and GGTase-I knockout mice during development and adulthood. The proposed experiments will contribute to our understanding of the functional importance of FTase and GGTase-I in normal cell growth and epidermal stem cells.

No: 67

Directed differentiation of Oligodendrocytes from hESCs to treat White Matter Strokes

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Neuron-, or nerve cell, based therapeutic strategies to repair or replace damaged neuron cell bodies following gray matter stroke have been addressed by several groups. In contrast, therapeutic strategies for the treatment of white matter stroke that primarily affects the glial cell oligodendrocytes and neuronal axons have not been well studied, even though white matter stroke can cause severe disability in up to 150,000 patients per year in the US alone. In addition, Periventricular Leukomalacia (PVL), the most common single form of cerebral palsy, is caused in part by a loss of oligodendrocytes and their precursors due to hypoxic-ischemic damage to the cerebral white matter. Although oligodendrocytes ("oligos") that produce the lipid rich neuronal axon insulation called myelin are preferentially damaged during these events, oligos derived from either neural (somatic) or embryonic stem cells (ESCs) have not been tested for their efficacy in preclinical animal models of white matter stroke or PVL. Furthermore, robust and reproducible methods for generating human ESC-derived oligos are lacking. Thus, we seek to develop efficient and scalable methods for the production of human ESC-derived oligos and to determine their functionality and potential for the treatment of white matter stroke and PVL. In order to generate sufficient amount of oligos, we are expressing SoxE factors in human ESC-derived neural stem cells. The SoxE family of transcription factors (Sox8, 9 and 10) was recently reported to have a fundamental role in oligo-genesis. Sox10, in particular, is only expressed by oligo precursor cells (OPCs) and oligos in the CNS and is essential for the commitment and generation of mature oligos. Moreover, we have previously shown that exogenous expression of Sox10 can induce OPC development from multipotent neural precursor cells in mice. The ability of Sox10 to direct OPC differentiation from human ESCs and neural stem cells is now being examined. Additionally, the early specification of neural stem cells in the ventral spinal cord requires SHH-dependent patterning. SHH signaling also has potent post-patterning effects on the development of OPCs and oligos. Therefore, in addition to Sox10, we will explore the potential of extracellular morphogens such as Sonic Hedgehog (SHH) and WNT inhibitors for increasing the efficiency of ESC-derived oligo differentiation. White matter MCAo and neonatal hypoxic-ischemic injury animal models will be used to assess oligo survival, migration, engraftment, and subsequent myelin repair following the transplantation of ESC-derived oligos at different stages of in vitro differentiation.

No: 68

Human embryonic stem cell derived dopaminergic neurons respond to axon guidance cues

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An attractive therapy for Parkinson's disease is the transplantation of viable dopaminergic (DA) neurons to restore function, however human clinical trials have been mixed; some patients improve but others show intolerable dyskinesias. This, combined with difficulties in harvesting viable DA neurons from embryonic human fetuses has halted trials in humans. Recent advances in ES cell technology, allowing the in vitro production of DA neurons has renewed interest in such transplantation therapy. Of importance is whether the transplanted DA neurons make appropriate connections with their targets. The netrin and slit families of guidance molecules play critical roles in sculpting neural systems and both affect native midbrain DA neurons. However, their reported effects on mouse ES derived DA (ES-DA) neurons were not robust, suggesting that ES derived DA neurons may be fundamentally different than native DA neurons. To study this in more detail we examined human ES-DA neurons, mouse ES-DA neurons and native rat and mouse DA neurons for their ability to respond to netrin and slit in vitro. Each experiment was done in both the presence and absence of inflammatory cytokines, in order to mimic conditions of the diseased brain. Native embryonic rat DA neurons show a robust response to both netrin and slit, but surprisingly, native mouse DA neurons respond only weakly to either molecule. Consistent with this, mouse ES derived DA neurons (mES-DA neurons) respond only weakly to slit and netrin. In contrast human ES-DA neurons show robust responses to both netrin and slit, similar to native rat neurons.The addition of various cytokines did not blunt the effects but rather increased the intensity of tyrosine hydroxlyase staining in the induced DA neurons and caused a marked increase in DA axonal fasciculation in response to netrin. The axonal growth response to netrin increased over time in culture with a peak at about 2 weeks post differentiation.

No: 69

Characterization of synthetic copolymer with stem cell applications

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This study aims to develop functional and interactive polymeric biomaterials derived from saccharides and peptides for controlling stem cell differentiation that can aid in the engraftment and survival of cells to an injury site in vivo. Natural extracellular matrix (ECM) is expensive to extract, and has significant batch to batch variations. Therefore, synthetic ECM may be a better alternative where the product is more controlled and can easily be functionalized. These copolymers can also be designed to contain different charges, stiffness and peptide sequences that broaden their applicability. Having successfully developed a biocompatible polymer composed of Lys-Glu-Gly tripeptide, more efforts were devoted into the making of different variants of the polymer. Mono-amino acid and di-amino acid were efficiently made that greatly reduce polymer synthesis time. The initial part of this study involves synthesis of the polymer and characterization of the structure using NMR. Confirmation of the tyrosine incorporation level into the backbone of the polymer is done through comparison of the integration of hydrogen peaks of the methyl ester at to those on the phenol ring of tyrosine. Different tyrosine percentages were used to access different gelling behaviors. As expected, the greater the percentage of tyrosine, the shorter the gellation time. Gellation time varied from seconds to over half an hour. Hydrogel gellation time is also found to be inversely proportional to enzyme concentration. The cytotoxicity of the polymer was assayed using standard MTT test. Polymer solution exhibited minimal cytotoxicity to MSCs up to 10 mg/mL for 9, 17, and 24 percent tyrosine polymers, indicating that our polymer is feasible for MSC studies.

No: 70

Primordial germ cell specification from human embryonic stem cells

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Fertility in mammals requires the production of sufficient gametes by the sexually mature animal. This in turn requires the formation of a population of cells during embryonic and fetal development that will eventually undergo differentiation to form the gametes. The cells of the germline are derived from a pluripotent cell population of cells, the so-called inner cell mass, of the pre-implantation blastocyst-stage embryo. Primordial germ cells (PGCs) are the founder population of germ cells that are specified during early development. Failure of PGCs to form in the embryo or to differentiate correctly can result in infertility and predispose individuals to developing gonadal tumors. Embryonic stem (ES) cells derived from the inner cell mass can also give rise to cells of the germline when they are introduced into blastocyst-stage embryos. Male and female germ cells have also been generated from mouse ES cells when they are induced to differentiate in vitro, albeit at low efficiency. It has also been reported that differentiated human ES (hES) cells included a subset of germ cells. Here, we show that PGCs can be efficiently isolated and further differentiated to germ cells that enter meiosis. Two hES cell lines, H1 (XY) and H9 (XX) were stably transfected with an hOCT4-eGFP reporter construct, in which eGFP expression is controlled by a human OCT4 promoter. hOct4-eGFP reporter lines provide a non-invasive method for genetic analysis of germ cell differentiation from hES cells since both ES cells and PGCs express Oct4. When hES cells were induced to differentiate into embryoid bodies (EBs), genes characteristic of differentiating PGC and pre-meiotic germ cells, such as VASA, cKIT and STRA8 were up-regulated. Nonetheless, the expression of these genes was not sustained in older EBs, suggesting that these PGCs either differentiate or die. Hence, we reasoned that PGCs can be selectively induced in early EBs. To further study the mechanisms regulating germ cell development in human cells, we established a tet-on inducible system in hES cells for conditional expression of genes that might regulate germ cell development. These reagents should prove useful for studying the mechanisms that regulate germ cell development, which in turn could reveal important new information about the factors controlling fertility and gonadal tumor etiology in humans.

No: 71

Use of embryonic stem cells and embryoid bodies as novel models to study environmental toxicants

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New assays for studying prenatal toxicity are needed as developing cells are generally more sensitive to toxicants than adult cells, which traditionally are used in toxicity testing. Embryonic stem cells (ESC) and embryoid bodies (EB) provide new valuable models for assaying the effects of toxicants on early stages of development. Our prior work showed that cigarette smoke adversely affects adult reproductive organs. In this study, mouse embryonic stem cells (mESC), which are proxies for the inner cell mass, were treated with mainstream (MS) or sidestream (SS) smoke from commercial traditional or harm reduction cigarettes, and ESC attachment, survival, proliferation, and differentiation were evaluated over time. A traditional brand of commercial cigarettes (Marlboro Red) was compared to two harm reduction brands (Advance Lights and Marlboro Lights). For each brand, MS and SS smoke were made using a University of Kentucky analytical smoking machine. Smoke solutions were tested at 1PE, 0.1PE, and 0.01PE (PE = puff equivalent = smoke dissolved/ml solution). For all experiments, D3 mESC were plated on 0.2% gelatin. Images of cells were taken, and the number of attached cells was evaluated at 6 and 24 hours using a hemacytometer. Both MS and SS smoke from traditional and harm reduction cigarettes inhibited cell attachment, survival and proliferation dose dependently and induced apoptosis through the activation of caspases 3&7. The effect of SS smoke was generally more detrimental than MS smoke for all brands, and SS smoke from harm reduction cigarettes was more inhibitory than Marlboro Red SS smoke. We then tested cigarette smoke on EB formation and differentiation. EBs, which are proxies for early embryos, were formed using hanging drops and incubated in medium containing various doses of MS or SS smoke. SS smoke from Advance Lights completely inhibited EB formation at the high dose (1PE) whereas MS smoke did not. At low doses, neither MS nor SS smoke affected EB formation. Preformed EBs were then plated on 0.2% gelatin coated plates to examine the effects of cigarette smoke on the rate of EB attachment and spreading. Both Marlboro Red and Advance Light SS smoke retarded EB attachment rate and spreading at low doses, and Advance Light SS smoke was more inhibitory than Marlboro Red SS smoke. EBs treated with Advance Lights were compared to untreated controls using RT-PCR. In both MS and SS smoke treated EBs, expression of endodermal (GATA-4 and α-fetoprotein) and mesodermal (T-gene) markers increased. These embryonic stem cell toxicity assays showed that both MS and SS smoke negatively impact embryonic stem cell attachment, survival, growth, and differentiation. Moreover, smoke from harm reduction cigarettes was generally more potent than smoke from a traditional brand. Together, our data indicate that smoke from commercial cigarettes could interfere with maintenance and differentiation of stem cell populations in a growing embryo. Finally these data help establish that embryonic stem cells and embryoid bodies are valuable proxies for testing toxicants on early stages of development.

No: 72

Tracking stem cells with a hybrid fluorescence tomography and anatomical imaging system

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Stem cells hold promise in treating many severe injuries and diseases such as spinal cord injury, diabetes, and myocardial infarction. A missing link between studying stem cells in vitro and using them in living subjects is the molecular imaging techniques that are needed for localizing and quantifying the injected stem cells in vivo. Recently, fluorescence imaging has been developed as a commonly used tool for small animal imaging. Stem cells can be labeled by fluorophores, thus the injected stem cells can be tracked and monitored in vivo with a fluorescence imaging system. Conventional fluorescence imaging systems create a projectional 2D fluorescence distribution map at the object surface. However, due to the highly diffusive nature of the photon propagation in tissue, it is difficult to recover the depth, size and fluorophore concentration information accurately from a projection image. On the other hand, fluorescence tomography (FT) can provide cross-sectional or full three-dimensional (3D) images using a mathematical approach. However, its low accuracy in estimating the true fluorophore concentration in the presence of heterogeneous optical background limits its application for quantitative imaging. Our previous simulation studies have shown that the knowledge of the background optical property and the anatomical information of the object are essential for quantitative fluorescence imaging. The background optical property of the object can be obtained from a diffuse optical tomography (DOT) technique, while the structural information of the object can be acquired from a high resolution imaging modality such as magnetic resonance imaging (MRI) or X-ray CT. In this study, we built a CCD based non contact hybrid FT/DOT system which could take measurements at multiple views. Multi-modality phantoms with multiple compartments were constructed and used in the experiments to mimic a heterogeneous optical background. When the optical background property is not provided from DOT, the fluorophore cannot be localized. On the other hand, when the background optical property is obtained from the DOT, the fluorophore can be located accurately, but the calculated signal strength from the fluorophore is not accurate compared to its original strength. Above all, when the structure of the phantom is obtained from the MR image and also applied to guide the FT, the fluorophore can be recovered with high quantitative accuracy. The results demonstrate that additional DOT and structural information is pivotal to recover fluorescence concentration embedded in a heterogeneous background. Currently, we are working on transferring the bench-top set-up onto a gantry that will rotate around the sample and combine this system with a low radiation dose micro-CT for animal imaging.

No: 713

Co-dependence of autonomic neuronal and vascular fates during early human embryogenesis as modeled by pluripotent stem cells

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Throughout the vertebrate body the vascular network aligns closely with the neural network, and involuntary control of vascular function is mediated by the neural crest-derived autonomic nervous system, while the neural tube-derived central nervous system (CNS) controls higher order functions. Human embryonic stem cells (hESCs) provide an opportunity to model the earliest stages of human development. We employed hESCs to analyze vascular-neuronal interactions during the spontaneous emergence of the three embryonic germ layers in culture. We observe patterning and maturation of endothelial cells (ECs) and two distinct neuronal populations. One population represents the autonomic nervous system, which closely associates with the developing vasculature. The other population represents early CNS. We demonstrate a specific co-dependence between vascular and autonomic neural patterning, as selective inhibition of neovascularization disrupts the autonomic, but not CNS, neurons. Conversely, disruption of the autonomic neural network results in vascular patterning defects. When exogenous human neural crest cells are added to hESC cultures those neural crest cells that contact and co-align with the vasculature are able to differentiate into peripheral neurons. Evidence is provided that T-cadherin, expressed both neural crest and early vascular cells, is highly localized at the neuro-vascular junction. Moreover, addition of soluble recombinant T-cadherin extracellular domain to human stem cell cultures disrupts neural crest cell alignment with the growing vasculature. These studies provide a mechanism accounting for the co-alignment and maturation of the peripheral nervous system in concert with the developing vasculature leading to the functional innervation of human blood vessels.

No: 74

Engineering surfaces for stem cells through the high throughput identification of novel peptide ligands

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Synthetic materials have been broadly utilized platforms to support the survival and proliferation of cells in tissue engineering applications, including more recently for stem cells. For example, interpenetrating network (IPN) surfaces consist of networks of acrylamide and polyethylene glycol (PEG), and linear PEG chains grafted onto these networks allow for bioactive ligand attachment. The arginine-glycine-aspartic acid (RGD) motif is a ubiquitous integrin-binding motif that is used to mediate cell attachment. However, highly complex cellular functions, such as stem cell self-renewal and differentiation, can require more complex sets of signals from the extracellular matrix (ECM) that likely engage with multiple cell adhesion receptors. However, many such ECM motifs remain unidentified, and even for the ones identified it is entirely unclear that short peptides that exactly match the natural ECM protein are necessarily the best ligands for grafting to materials. We have developed a novel method utilizing bacterial display to select novel candidate peptides that can subsequently be chemically synthesized and grafted to IPNs. Using adult neural stem cells as a model system, we have shown that this method is very effective at identifying novel candidate peptides that can be utilized to engineer new bioactive materials to control cell and stem cell function.

No: 75

Identifying and quantitating signaling modules and networks in mESC differentiation to cardiomyocytes

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Cardiomyocyte differentiation from ESCs is inefficient using current protocols. To a large part, this is due to incomplete knowledge of the signaling pathways that control many critical steps in the differentiation program. Cardiac differentiation can be considered to proceed in a stepwise program from uncommitted mesoderm, to cardiogenic anterior mesoderm, to cardiac mesoderm and, finally to cardiomyocyte. In this project, we are taking a phospho-proteomics approach to identify signaling modules and networks integral to early ESC differentiation. The model for early differentiation signaling will facilitate protocol development by suggesting potential new stimuli and by providing insights into existing methods. To develop this platform, our experiments focus on days 0-4 of differentiation, as mesoderm is patterned to form cardiogenic mesoderm. The experiments will provide baseline information on differentiation initiated in response to native inducers, such as Nodal signaling, in serum free medium. Secondly, we will also evaluate the signaling that is initiated in response to small molecule inducers discovered as part of the laboratories small molecule screening studies (see companion posters by Bushway et al., and Okolotowicz et al.). Specifically, we are generating lysates in mouse ESCs at closely spaced time points between days 0 and 4 using a serum-free media differentiation protocol, which includes the addition of the cardiomyocyte-inducing compounds at day 2. We will process these lysates using Western blotting, mass spectrometry, and ELISA-based techniques to identify and quantify phosphoproteins. Signaling networks will be formed from this proteomic data by using statistical and inference tools and by exhaustive mining of legacy knowledge.

No: 76

The role of Bmi-1 in prostate stem cell maintenance and carcinogenesis

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Increasing evidence suggests a strong connection between cancer and stem cells. Cancer may arise from normal somatic stem cells, or a more differentiated cell which regained 'stem-like' capabilities - such as self-renewal. Normal self-renewal, proliferation and differentiation of stem cells are tightly regulated by numerous mechanisms and signaling pathways. Bmi-1 is one such gene that plays a key role in the self-renewal of several types of stem cells. The expression of Bmi-1 is significantly up-regulated in many cancers, including malignant human prostate cancer. This study investigates the role of Bmi-1 in stem cell self-renewal and cancer propagation in the murine prostate. To assess self-renewal, we have utilized a prostate sphere assay in which the stem/progenitor population of murine prostate cells can give rise to clonogenic spheres that can be serially passaged. We show that shRNA driven inhibition of Bmi-1 significantly decreases the prostate stem cell's capability to give rise to spheres, and the sphere cells' ability to passage and proliferate. Conversely, over-expression of the protein in sphere cells increases the size and number of secondary and tertiary spheres produced. Evidence suggests that cancer cells exploit the self-renewal pathways used by the normal tissue stem cells. We designed experiments to test the role of Bmi-1 in the maintenance of prostate cancer. Inhibition of Bmi-1 in prostate cancer cell lines results in decreased growth in vitro and a significant attenuation in their tumor forming capabilities in vivo. Our data shows that Bmi-1 plays a crucial role in both normal prostate stem cell maintenance as well as cancer propagation.

No: 77

The role of LSD1 in neural development

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The regulation of global and local chromatin structure via histone tail modifications is likely to play an important role in cell lineage progression and pluripotency restriction in the central nervous system during embryonic development. We began searching for expression of histone modifying enzymes that may regulate the transition from dividing neuronal progenitor to post-mitotic neuron in the spinal cord, and found that the lysine specific demethylase 1 (LSD1) is up-regulated in newborn neurons after cell cycle exit. To examine the function of LSD1 during neurogenesis, we generated an LSD1 gene trap (LSD1-GT) mouse line that has a beta-geo fusion to exon 8 of LSD1, causing a truncation of the conserved catalytic amine oxidase domain. However, at E10.5-E11.5 when neurogenesis is occurring, no homozygous LSD1-GT embryos could be recovered due to an earlier failure to complete gastrulation. To circumvent the early embryonic lethality in LSD1-GT embryos, we derived LSD1-GT ES cells containing an HB9::GFP reporter that selectively labels motor neurons (MNs). Surprisingly, LSD1-GT ES cells proliferate normally and differentiate into MNs in vitro and contribute to MNs in vivo in chimeric mice, indicating that LSD1 is not required for the acquisition of neural fate. However, gene expression profiles show that there are large numbers of genes that are mis-regulated in LSD1-GT ES cells and ES derived MNs, indicating LSD1 plays complex roles in regulating gene expression during mammalian development.

No: 78

Recreating a human hematopoietic stem cell niche in vitro by unique stroma cells from the first trimester human placenta and fetal liver

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Expansion of human hematopoietic stem cells (HSCs) ex vivo has been difficult due to limited understanding of their growth requirements and the molecular complexity of their natural microenvironments. To mimic the niches in which human HSCs normally develop and expand during ontogeny, we have derived two unique types of stromal niche cells from the first trimester human placenta and the fetal liver. These lines either support maintenance of multipotential progenitors in culture, or promote differentiation into macrophages. Impressively, the supportive lines facilitate over 50,000-fold expansion of the most immature human HSCs/progenitors (CD34+CD38-Thy1+) during 8-week culture, whereas the cells cultured on non-supportive stroma or without stroma differentiated within 2 weeks. As the supportive stroma lines also facilitate differentiation of human hematopoietic progenitors into myeloid, erythroid and B-lymphoid lineages, we were able to show that the expanded progenitors preserved full multipotentiality during long-term culture. Furthermore, our findings indicate that the supportive stroma lines also direct differentiation of human embryonic stem cells (hESC) into hematopoietic progenitor cells (CD45+CD34+) that generate multiple types of myeloerythroid colonies. These data imply that the unique supportive niche cells can both support hematopoietic specification and sustain a multilineage hematopoietic hierarchy in culture over several weeks. Strikingly, the supportive effect of the unique stromal cells from was dominant over the differentiation effect from the non-supportive lines. Even supernatant from the supportive lines was able to partially protect the progenitors that were cultured on the non-supportive lines, whereas mixing of the two types of stroma resulted in sustained preservation of the multipotential progenitors. These results indicate that the supportive stroma cells possess both secreted and surface bound molecules that protect multipotentiality of HSCs. Global gene expression analysis revealed that the supportive stroma lines from both the placenta and the fetal liver were almost identical (r=0.99) and very different from the non-supportive lines that promote differentiation (r=0.34), implying that they represent two distinct niche cell types. In summary, we have identified unique human stromal niche cells that may be critical components of the HSC niches in the placenta and the fetal liver. Molecular characterization of these stroma lines may enable us to define key mechanisms that govern the multipotentiality of HSCs.

No: 79

Human embryonic stem cells as a model to study Trophoblast differentiation

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Human Embryonic Stem Cells (hESCs) are unique tools for studying early development and differentiation. Previous studies have reported that hESCs can differentiate to trophoblast-like cells, either by spontaneous differentiation or by BMP4 induction. However, it is unclear whether this differentiation represents a truly trophectoderm/trophoblast commitment. The aim of our study was to investigate the molecular events of the cell transitions that occur during differentiation of hESCs to trophoblast in order to better understand this process. For this purpose, H7 and H9 hESC lines were induced to differentiate by BMP4. A time course was performed over 12 days in three independent replicates (n=3). Cells and culture supernatants were collected every two days, and characterization of trophoblast differentiation was assessed by using: (i) quantitative real-time RT-PCR to analyze expression of markers of pluripotency (Oct3/4, Sox2, Nanog), trophectoderm (Cdx2, Eomes), and trophoblast (CK7, HLA-G, ID2 and CGB); (ii) double immunofluorescence to detect co-expression of Cdx2/CK7; and (iii) immunoassays to measure hCG secretion. We observed the rapid onset of hESC differentiation suggested by downregulation of pluripotency genes, closely followed in a second stage by induction of gene expression of the trophectoderm (precursor of the trophoblast). Trophectoderm precursor cells have been also characterized by co-localization of cdx2 and CK7 expression within differentiated cells. The differentiation window from day 2 to day 4 of differentiation showed an interesting switch between pluripotency and trophectoderm gene expression. The expression of trophoblast markers and hCG secretion were also induced and maintained throughout the differentiation process. The sequence of gene expression changes observed in our experiments suggests the emergence of trophectoderm and the differentiation of trophoblast-like cells. Further ongoing investigations aim to characterize trophoblast cell types and identify key genes and miRNA involved in the trophoblast cell fate decision. This research was supported by CIRM grant RS1-00207-1

No: 80

Modeling for Amyotrophic Lateral Sclerosis (ALS) using human embryonic stem cells

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Amyotrophic lateral sclerosis (ALS) is a prominent neurodegenerative adult disease characterized by death of motor neurons. ALS can be induced by inherited mutations in the gene encoding superoxide dismutase 1 (SOD1). Evidence for the non-cell-autonomous nature of ALS emerged from the observation that wild type glial cells extended the survival of SOD1-mutant motor neurons in chimeric mice. To uncover the contribution of astrocytes to human motor neuron degeneration, we co-cultured HESC-derived motor neurons with human primary astrocytes expressing the mutated form of SOD1 (SOD1G37R). In this condition, we detected a selective decrease in motor neuron numbers. Here we show that motor neuron toxicity is correlated with increased inflammatory response and production of reactive species of oxygen in SOD1-mutated astrocytes, adding critical information to the non-cell-autonomous component of ALS pathology. Our data open up new possibilities for drug-screening experiments using human ALS in vitro model, as well as clinical intervention using astrocyte-based cell therapies.

No: 781

In vitro maturation and embryonic stem cell derivation techniques for human nuclear transfer

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Somatic cell nuclear transfer holds great promise for basic studies of reprogramming by oocytes and for potential development of novel diagnostics and therapeutics. However, there is limited availability of mature oocytes for research. Thus, we are exploring the feasibility of using several different sources of oocytes/embryos, including immature oocytes (germinal vesicle: GV and metaphase I: MI) and failed/abnormally fertilized oocytes/embryos, for research purposes. Previous studies, from our lab and others, showed that embryos, obtained from nuclear transfer using in vitro matured oocytes, arrested in early development (2-cell to morula stage). The aim of this study was to modify experimental aspects of human nuclear transfer including in vitro maturation (IVM) of oocytes, techniques of nuclear transfer (NT) and derivation of embryonic stem cells, via use of an abundant source of oocytes/embryos that are routinely discarded from assisted reproduction clinics. We observed several findings: First, customized, in vitro maturation medium with supplemented growth factors improved nuclear maturation of oocytes and embryo development after parthengenetic activation compared to commercial medium. Using customized medium, a blastocyst was produced after parthenogentic activation. In order to improve further IVM oocyte quality, growth factors characterized by cognate receptors that are highly expressed during oocyte maturation, will be screened for activity by single cell gene expression profiling and supplemented in in vitro maturation medium. Second, the use of a subset of oocytes for nuclear transfer resulted in cleaved embryos that expressed green fluorescent protein from a transgene in donor nuclei from human embryonic stem cells. However, the majority of nuclear transferred embryos were arrested on day 3 of in vitro culture. Finally, in order to use early stage nuclear transferred embryos to establish embryonic stem cell lines, the techniques to derive lines from single blstomeres were established by using failed/abnormally fertilized embryos based on the previous studies. A total of 67 blastomeres were isolated from 9 embryos on either day 2 or 3 of culture. Ten were divided among the isolated blastomeres, and 8 outgrowths were observed on day 8 after blastomere isolation. Our results suggest that the discarded oocytes/embryos can be used to investigate new human nuclear transfer and line derivation protocols for exploration of biological mechanisms of germ cell maturation and early embryonic development.

No: 82

Cell fate specification in the developing mammalian brain

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One of the most exciting possibilities in stem cell biology is the potential to replace damaged or diseased cortical neurons. Stem cell-derived neurons provide a potentially limitless supply for cell replacement, if we could guide cultured stem cells toward specific differentiation pathways to produce specific cortical neuronal types. During development, projection neurons in the cerebral cortex are generated from a seemingly homogeneous pool of progenitors or neural stem cells. The goal of our research is to understand the molecular mechanisms that regulate the ability of these cells to generate different projection neuronal types during development. We will present our work on identifying genes and genetic pathways that regulate cell fate specification in the developing mouse brains.

No: 83

Analysis of Notch Signaling Pathway Genes In Purified Mouse Thymic Epithelial Cell Subsets

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T lymphocytes are important regulators of the immune response. These cells mature in the thymus, a dynamic organ composed of several stromal and lymphocyte components. Stromal cells, such as fibroblasts, mesenchymal cells, endothelial cells, and thymic epithelial cells, provide the three-dimensional microenvironment through which immature thymocytes migrate during their development, as well as critical cell-to-cell interactions that result in the education of thymocytes to distinguish between self and non-self antigens. In particular, thymic epithelial cells (TECs) provide developmental cues to thymocytes, such as signaling via Notch ligands, and migration cues via production of chemokines and integrins. To date, TECs have been divided into two general classes: cortical (cTEC) and medullary (mTEC). The cTECs are critical for positive selection of developing thymocytes and the mTECs are essential for negative selection, the process by which auto-reactive thymocytes are eliminated. In healthy individuals, thymic "output" diminishes with age, and experimental evidence supports the hypothesis that this may be due in part to reduced functionality of aged TECs. To better understand the basic developmental biology of TECs and the TEC "niche", we have improved methods to isolate TECs from the mouse thymus. We have observed four types of TECs based on combinatorial expression of EpCAM, CDR1, and UEA1 cell surface markers. In initial studies, we have purified the four TEC subpopulations using fluorescence activated cell sorting and examined the expression of an array of Notch pathway genes by quantitative PCR. Gene expression profiles will be correlated with TEC subset development as well as with the ability of isolated TEC subsets to support T cell development in vitro. These studies will help to elucidate Notch-mediated mechanisms that influence TEC development. In addition, this work could have implications on the development of T-lymphocyte based therapies for autoimmune diseases and immunological tolerance to embryonic stem cell-derived grafts.

No: 84

Comparative analysis of the hematopoietic potential of human embryonic stem cell lines

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One goal of our lab is the generation of mature T lymphocyte subsets from human embryonic stem cells (hES) in vitro. T cells derived from hES cells have the potential to treat a number of diseases, ranging from autoimmune disorders to cancer. hES cells have nearly limitless in vitro expansion potential compared to other hematopoietic progenitor sources (i.e. cord blood and bone marrow), but the efficient generation of mature T lymphocytes from this pluripotent cell source has not yet been achieved. It is possible, however, that the limited number of independently-derived hES lines used in previous studies have restricted T cell potential. Therefore, we have undertaken a systematic and comprehensive comparison of the differentiation potential of a large cohort of independently-derived hES cell lines. To generate mature T lymphocytes, a stem cell must be directed through a number of developmental intermediates. These developmental intermediates, from hES cell to T cell, can be broadly classified as mesoderm, hemangioblast, hematopoietic stem cell, common lymphoid progenitor, and T cell precursor and can be identified by the sequential expression of identifiable cell surface markers. We have used these developmental intermediates as milestones to determine lineage bias among hES cell lines during sequential differentiation steps. Here we present data that suggests that several independently-derived hES cell lines differ in their differentiation potential at several of these discrete stages of development. Though certain lines appear to efficiently generate the early developmental intermediates (mesoderm and hemagioblast), their hematopoietic potential appears to be limited, and vice versa. Hence, these studies will help us to define hematopoietic-biased hES lines. In addition, we will take advantage of the inherent differences in differentiation potential between the independently-derived hES lines at various stages of development to uncover the genetic determinants of lineage commitment from stem cell to mature T lymphocyte.

No: 85

Germ cell migration in zebrafish is Smoothened-independent but cyclopamine-sensitive

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Primordial germ cells (PGCs) are the progenitors of reproductive cells in metazoans, and are an important model system for the study of cell migration in vivo. Previous reports have delineated a role for the Hedgehog signaling pathway in the control of PGC migration in the Drosophila embryo. Here we interrogate whether Hedgehog signaling is a conserved requirement for PGC migration in vertebrates. We find that cyclopamine, an inhibitor of Hedgehog signaling, causes strong defects in the migration of PGCs in the zebrafish embryo. However, analysis of two independent lines of embryos lacking maternal and zygotic expression of Smoothened indicates that cyclopamine's defects are not due to inhibition of Smoothened. Rather we find that Smoothened and thus Hedgehog signaling are not required for PGC migration in zebrafish. Cyclopamine, by contrast, acts independent of Smoothened to decrease the motility of zebrafish PGCs, in part, through dysregulation of cell adhesion. These results suggest that any role for Hedgehog signaling in the regulation of PGC migration may be restricted to the invertebrate clade, and underscore the importance of properly regulated cell-cell adhesion to permit cell migration in vivo.

No: 86

Targeting the fetal epithelium for the treatment of Cystic Fibrosis

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Cystic Fibrosis (CF) is the most common genetic disease in Caucasian's. It is a multiorgan disease affecting lung, liver and intestines among other organs, with mortality due to the pulmonary complications of the disease. The disease results from a mutation in the Cystic Fibrosis Transmembrane Regulator, an ion channel regulator, which results in an imbalance in the movement of ions and water across epithelial cells. It is predicted that CFTR expression in as few as 1-5% of the cells would provide a therapeutic benefit in CF. In the current study, we report on the feasibility of in utero delivery of recombinant lentivirus as a gene therapy method for correcting the disease phenotype in all affected organs. The first studies evaluated the persistence and organ distribution of transgene expression following in utero delivery of luciferase containing lentivirus to the amniotic fluid of E16.5 murine fetuses. These studies demonstrated the presence and persistence of transgene expression by bioluminescent imaging through P28, the last time point tested. Furthermore, the strongest signals were present in lungs, stomach and intestine, organs affected by CF. In the next series of experiments, we evaluated the cell types and proportion of epithelial cells expressing the transgene following in utero delivery of an EGFP lentivirus to E14.5 and E16.5 fetuses. EGFP transgene expression was observed in the epithelia of the trachea and airways at P7 and P28. We determine that up to 14% of the airway epithelial cells were transgene positive - a level that is predicted to be clinically relevant for CF. Proviral DNA analysis confirmed the integration of provirus into these same organs. Future studies will aim to deliver the CFTR gene with regulated expression specific to the epithelium of CF affected organs.

No: 87

DNA repair capacity of hematopoietic stem cells and myeloid progenitors

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DNA damage can be caused by intrinsic and extrinsic sources and has serious repercussions for cell and tissue function. Our goal is to understand how hematopoietic stem cells (HSC) and myeloid progenitors (MP) respond to genotoxic stress and to establish how these populations recognize and repair damaged DNA. By following their ability to form colonies in methylcellulose and to grow in liquid culture, we found that HSC are more capable of withstanding increasing doses of ionizing radiation (IR) than MP. BrdU incorporation pulses revealed that irradiated HSC have an initial and transient pause in proliferation, while irradiated MP cycled faster than untreated MP. By monitoring apoptosis with AnnexinV/7AAD and cleaved caspase 3 staining, we showed that both irradiated HSC and MP have an immediate apoptotic response but that HSC quickly recover and restore low baseline levels of apoptosis while MP maintain high levels of apoptosis. These results suggest that HSC may pause cycling to repair damaged DNA while MP increase cycling to replenish cells cleared by apoptosis. At the molecular level, irradiated HSC displayed a strong and transient induction of several pro-apoptotic genes (bax, puma, noxa) but minimal change in expression of pro-survival genes, which are already high in those cells. In contrast, irradiated MP displayed minimal induction of pro-apoptotic genes (except for puma) but decreased expression of pro-survival genes, which are already low in those cells. Taken together, these results suggest that HSC can withstand genotoxic stress better than MP due to differences in the regulation of their apoptotic machinery leading to protection of HSC and elimination of MP. To monitor the ability of HSC and MP to recognize and repair damaged DNA, we used immunofluorescence techniques to study IR-induced DNA damage foci. Irradiated HSC and MP displayed similar kinetics of DNA damage recognition as monitored by the formation and resolution of gamma-H2AX and 53BP1 foci. In contrast, the kinetics of Rad51 foci - which signal the initiation of homologous recombination (HR) DNA repair - significantly differed between HSC and MP. While Rad51 foci were immediately induced and quickly resolved in MP, very few Rad51 foci were formed in HSC up to 12 hours post-IR. Since HR only occurs during S/G2/M phases of the cell cycle, it is possible that the largely quiescent HSC can not utilize HR and instead use the more error prone DNA repair mechanism non-homologous end joining (NHEJ). At the molecular level, both HSC and MP displayed similar levels of genes associated with DNA damage recognition and repair (atm, rad50) and specific to HR (brca1), while MP display significantly lower levels of genes specifically involved in NHEJ (ku80) compared to HSC. Taken together, these results indicate that HSC and MP can recognize damaged DNA but might preferentially use different repair mechanisms. They also suggest that to become transformed and drive leukemia development, HSC might only require DNA damage, while MP might also need deregulation affecting both DNA repair mechanisms and apoptosis machinery.

No: 88

Mechanism of self-renewal of brain tumor stem cells

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Despite the dramatic improvement in the outcome of other cancers in the recent decades, brain tumors remain one of the most devastating diseases. Malignant brain tumors are composed of heterogeneous cell populations. Various investigations, including our own, have identified a stem cell population in malignant brain tumors, called 'brain tumor stem cells (BTSC)'. These malignant stem cells are capable of self-renewing and recapitulating the entire tumor mass, and represent the target of therapy. However, the mechanism of self-renewal of BTSC is poorly understood. A cell surface glycoprotein CD44 is known as the marker for various cancer stem cells including leukemia stem cells and breast cancer stem cells. Recent investigations have suggested that targeting CD44 inhibits leukemia formation by eradicating their cancer stem cells in immunodeficient mice. Here, we will present our preliminary data suggesting that CD44, and its variant form 6, are highly expressed in BTSC and regulate their proliferation in culture.

No: 89

Using engineered G-protein coupled receptors to control embryonic stem cell differentiation

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There is great growing interest in using G-protein coupled receptor (GPCR)-based drugs for the development of stem cell therapies for diseases. Unfortunately, the hormonal pathways controlling cell differentiation are poorly understood. While it has been shown that G-protein signaling is involved in diverse cellular functions such as contraction, migration, and growth, very little is known about its role in ESC stem cell differentiation. To better study the precise function of G-protein signaling in tissue formation, we are developing a series of embryonic stem (ES) cells that express designer G-protein coupled receptors that can be activated by small-molecule drugs. We have a synthetic biology approach to this problem that utilizing a series of engineered receptors (RASSLs), based on endogenous receptors that no longer respond to hormone, but can be activated by synthetic drug. To evaluate the effects of both basal and receptor activated signaling, we are employing a novel single vector tetracycline-transactivator system to gain temporal control of receptor expression in mouse ES cells. This modular expression system will allow us to investigate the roles of Gs, Gi, and Gq signaling on ES cell differentiation. Thus far, we have generated a doxycycline-responsive mouse ES cell line that expresses an engineered Gs-coupled receptor RASSL under the control of a ubiquitously expressed promoter. In response to drug, these cells show a significant increase in cAMP accumulation. In addition, we detect an increase in proliferation of differentiating cell types during teratoma formation in SCID mice. Using in vitro differentiation models, we are examining whether activated Gs signaling can direct cells to the mesodermal lineage. In addition to our synthetic biology approach, we are also using gene profiling to identify key GPCRs that are expressed in human ES cells and iPS (induced pluripotent stem) cells. Our RASSL studies will help us focus on key endogenous GPCR signaling pathways controlling stem cell differentiation. These studies will improve our understanding of G-protein signaling in tissue formation, and will assist in the development of ES cell-based therapies useful in regenerative medicine.

No: 90

Axonal transport defects lead to altered APP metabolism

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Alzheimer's Disease (AD) is a neurodegenerative disorder characterized pathologically by 1) extracellular senile plaques (SP) principally composed of amyloid-beta peptide, 2) intracellular neurofibrillary tangles (NFT), 3) synapse loss and 4) cell death. It is commonly thought that increased steady state levels of amyloid-beta peptides produced by the proteolytic processing of the amyloid precursor protein (APP) lead to this classic AD pathology. However, recent examination of early stage AD brains suggests that axonal transport defects may precede both SPs and NFTs. Similar study of brains from APP transgenic mice supports this latter finding. In addition, induction of axonal transport defects via reduction of the kinesin light chain 1 (KLC1) subunit of the axonal anterograde motor kinesin-1 in this APP transgenic mouse model leads to earlier and accentuated amyloid plaque deposition, suggesting that axonal transport defects may lead to altered APP metabolism. We hypothesize that inducing axonal transport defects in human neurons (expressing endogenous levels of APP) will alter APP metabolism. To test this idea, we are using human embryonic stem cells (hES) as a source of human neurons. To induce axonal transport defects we have made hES lines with reduced levels of KLC1. In the undifferentiated state, cells from these lines form characteristic bordered colonies and express pluripotency markers similar to the parental line. hES from these lines differentiate to neuron-like progeny expressing neural precursor marker nestin and the neuron-specific beta-III-tubulin isoform. Neuron-differentiated cells with reduced levels of KLC1 exhibit abnormal neurofilament immunoreactivity and elevated activated stress-associated protein kinase (SAPK) levels compared to neurons differentiated from control hES, suggesting that KLC1 reduction leads to axonal defects. Further, the pattern of amyloid-beta peptide secretion during differentiation is altered in cells with reduced levels of KLC1, suggesting that KLC1 reduction leads to altered proteolytic processing and/or secretion of amyloid-beta. These preliminary results support both the hypothesis that axonal transport defects can lead to altered APP metabolism in human neurons and the possibility that axonal transport defects may be an early event in the pathogenesis of AD.

No: 91

Proteomics identifies post-transcriptional regulation protein levels during differentiation of pluripotent cells

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Much work has gone into understanding the transcriptional regulation of the pluripotent state in cells such as embryonic stem cells (ES cells) and embryonal carcinoma cells (EC cells). These studies have shed much light on how cells are maintained in the pluripotent state, by networks of pro-pluripotency transcription factors, several of which have been shown to be necessary for maintaining pluripotency and sufficient to reprogram somatic cells into induced pluripotent stem cells (iPS cells). An emerging question is how the pluripotent state is destabilized, allowing cells to exit it and begin to differentiate. Since changes to the stem cell environment (niche) lead to differentiation, it is likely that signaling, a combination of post-transcriptional events, is a key factor in destabilizing the pluripotent state. We set out to study the post-transcriptional state of ES and EC cells before and after Retinoic Acid (RA) mediated differentiation, in order to identify post-transcriptional regulation of gene products, such as phosphorylation or changes to protein levels not visible at the transcript level. To measure protein levels of pluripotent cells before and after differentiation, we employed a mass spectrometry (MS/MS) approach. Innovations in peptide separation by liquid chromatography, MS/MS and isobaric tagging of peptides have made deep, semi-quantitative analysis of the proteome possible. We identified more than 6000 and 4000 phosphopeptides in undifferentiated and differentiated ES and EC cells respectively. The observed phosphorylation events included phosphorylation of two reprogramming factors, Sox2 and Lin28, as well as phosphorylation of the ES cell specific transcription factor Utf1. Further work on the role of phosphorylation of Utf1 is ongoing. We used the iTRAQ (applied biosystems) mass tagging reagent to modify peptides produced by trypsinization. The iTRAQ mass tagging reagent allows for relative quantitation of pooled peptides from pluripotent cells before and after differentiation. We identified and relatively quantified more than 4000 proteins in both ES and EC cells before and after differentiation. Relative protein levels before and after differentiation were compared to relative mRNA levels of the same cell lines grown under the same conditions reported by Aiba et al. in Stem Cells 2006; 24:889-895. These comparisons identified more than 100 cases where proteins were enriched before or after differentiation with no corresponding enrichment of mRNAs. These putative cases of post-transcriptional regulation of protein levels in differentiating pluripotent cells must be confirmed by RT-PCR and western blot. Putative targets of post-transcriptional regulation of protein levels include Racgap1, a GTPase activating protein, implicated in cell division of the preimplantation embryo and hematopoetic stem cells. Pdlim7, a signaling molecule that stabilizes the Smad family of transcription factors by sequestering Smurf1 and preventing their proteolysis. Igfbpl1 which stabilizes the insulin-like growth factors, increasing their half-life. And Surf6, which is implicated in ribosome biogenesis and is essential for preimplantation development. Conclusion: Mass-spectrometry based proteomics allows identification of post-transcriptional regulation of proteins. The functional significance of these events remains to be evaluated.

No: 92

Guided osteogenic differentiation of human mesenchymal stem cells by titanium oxide nanotubes

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Stem cells offer exciting possibilities of treating a wide range of diseases. The promising applications of stem cell therapies rely on two key areas, i.e., directed differentiation into well-known cell lineage and cell enrichment. At the moment, the necessary control of such processes, not to mention a clear understanding of the phenomena themselves, is lacking. It would be highly desirable to be able to control the cell differentiation so that only those relevant cells are preferentially produced from the embryonic or adult stem cells while a differentiation into other cells is prohibited. We have developed titanium dioxide (TiO2) nanotubes with various tip diameter and length, and researched the effect of these nanotubes on the behavior of osteoblasts, endothelial cells, and hepatocytes. Our research indicates that TiO2 nanotubes improved the (1) proliferation and mineralization of osteoblasts, (2) mobility of endothelial cells and (3) functional properties of hepatocytes because of the unique nanostructure and good biocompatibility of TiO2 nanotubes. In this research, we examine the effect of various nanostructures of TiO2 nanotubes on the guided osteogenic differentiation of human mesenchymal stem cells.

No: 93

Transcriptional regulatory activity of the ultraconserved elements during embryonic stem cell differentiation

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The ultraconserved elements (UCEs) are defined as syntenic regions at least 200 base pairs long that are completely conserved in the human, mouse, and rat genomes. The extreme conservation seen in the UCEs has not yet been explained, but implies the UCEs may perform some fundamental role in mammalian biology. The UCEs are frequently located in or near developmental regulatory (transDev) genes, and it has been hypothesized the UCEs may be critical in controlling the spatial and temporal expression of these genes. Strengthening this hypothesis, several studies have demonstrated the UCEs can function as cis-acting DNA elements promoting tissue-specific expression of reporter genes in embryo-based assays. Given that UCEs may control the expression of transDev genes, it is likely they may play important roles in undifferentiated embryonic stem (ES) cells and ES cell differentiation. ES cells show regulated transcription of transDev genes upon differentiation, and in particular neural differentiation of mouse ES cells shows differential expression of many putative UCE targets, indicating this pathway is useful for studying transDev gene regulation. I am using neural differentiation of mouse ES cells as a model system to study the effects of the UCEs on the transcription of transDev genes. I will describe an assay to measure transcriptional enhancer or repressor activities of the UCEs along the neural differentiation pathway in mouse ES cells, and I will present results demonstrating the UCEs can indeed function in this pathway. I will also describe efforts we have initiated to test the UCEs for function as non-coding RNA regulators of transcription. By testing UCEs for activity at both the DNA and RNA levels, I may uncover superposed functions which would impose multiple levels of sequence constraints on these elements. This work will provide valuable insight into the conservation behind the UCEs as well as the regulatory mechanisms underlying mammalian development and embryonic stem cell differentiation.

No: 94

Unraveling the role of cholesterol in neurodegeneration: a human stem cell derived model of Niemann Pick Type C disease

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Niemann Pick Type C (NPC) is a rare but lethal autosomal recessive disease caused by a mutation in NPC1, a housekeeping protein residing in the late endosomal compartment with a putative role in cholesterol transport. The result is a severe lipidosis characterized by massive accumulation of sterols and other lipids in the late endosome/lysosome that ultimately cause cell dysfunction and death. The particular sensitivity of neurons to the effects of abnormal NPC1 function despite an obvious absence of cholesterol accumulation is a puzzling feature of NPC. An important clue may come from the extreme polarity and size of neurons, which thus rely on efficient transport of molecules essential to their survival and function over the long distances separating the cell body from the synapse. Furthermore, the disease shares features with other neurodegenerative conditions such as Alzheimer's disease (AD) in which deficient axonal transport has been postulated to play a crucial role. Animal models of NPC are available but they fail to accurately mimic human pathology, and obtaining viable brain tissue to study live human neurons is obviously difficult. A new and powerful tool to tackle these issues is provided by human embryonic stem cells (hESC). These remarkable cells have the potential to differentiate into tissues of all three germ layers and can be manipulated genetically to create stable and proliferative models of human disease. Based on reports that loss of normal NPC1 function exerts its effects by interfering with physiological vesicular trafficking we propose to test the hypothesis that neuronal death and/or failure in NPC is caused by disrupted axonal trafficking of cholesterol and other key synaptic proteins to target sites at the synapse. We postulate that NPC1 directly or indirectly mediates this movement through its effects on targeting and/or docking of vesicular bodies. To test our hypothesis on live human neurons we have generated and are currently testing a set of genetically engineered hESC into which a lentivirus encoding a cre-inducible or cre-repressible NPC1 shRNA has been inserted. We will induce differentiation of these NPC hESC lines towards the neuronal lineage and use human NPC and control neurons grown in compartmented cultures to measure uptake and transport of fluorescent cholesterol analogs and fluorescent apolipoprotein E (apoE), the major cholesterol carrier in the brain. Because NPC and AD exhibit some phenotypic similarities, we will expand the array of useful assays by measuring neuronal phenotypes of AD such as APP processing, tau phosphorylation and conformation, and JNK activation. Corroborating the presence of features typical of axonal trafficking deficiencies in human NPC neurons will be crucial in directing future research aimed at exploring the pathogenesis of NPC and discovering new therapies that target components of movement systems such as motor proteins known to be involved in vesicular trafficking.

No: 95

Shp2 promotes human and mouse embryonic stem cell differentiation by modulating common and distinct signaling pathways

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Comparative analyses between human and mouse embryonic stem cells (hESCs and mESCs) by several groups have suggested common and distinct biological properties and molecular signaling mechanisms. In this study, we report that Shp2, an intracellular tyrosine phosphatase with two SH2 domains, has a conserved role in orchestration of signals resulting in initiation of differentiation in both hESCs and mESCs. Homozygous deletion of Shp2 in mESCs inhibited differentiation into all three germ layers, and siRNA-mediated knockdown of Shp2 expression in hESCs led to a similar phenotype of impaired differentiation. A specific Shp2 enzyme inhibitor that we isolated by screening a chemical library suppressed both hESC and mESC differentiation capacity. Shp2 modulates Erk, Stat3 and Smad pathways in ES cells and, in particular, Shp2 regulates BMP4-Smad pathway bi-directionally in mESCs and hESCs. Together, our results indicate a common signaling mechanism shared by hESCs and mESCs via Shp2 modulation of overlapping and distinctive pathways. Furthermore, we demonstrate that use of cocktail inhibitors suppressing multiple paths may be a practical means for maintenance and amplification of hESCs in culture.

No: 96

Protective effect of human amniotic fluid stem cells in acute tubular necrosis

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Acute Tubular Necrosis (ATN) causes severe damage to the epithelial tubular cells of the proximal and distal tubules that are destroyed altering all the physiological properties of a functional kidney. These alterations can lead to End Stage Renal Disease (ESRD) that has reached epidemic proportion in recent years all over the world. Within the last decade stem cells have emerged as a promising therapeutic tool. Recently, a potential role for stem cells has emerged, in particular, mesenchymal stem cells derived from bone marrow (MSC), which when injected into mouse models of kidney failure may contribute to kidney repair through a paracrine system or/and integration into damaged structures. We have previously shown that a clonal c-kit+ stem cell population derived from human amniotic fluid (AFS) can be induced to a renal fate in an ex-vivo system and was capable of contributing to the development of primordial kidney structures including renal vesicles, C- and S-shaped bodies. In this study we show the successful therapeutic application of hAFS cells in an in vivo mouse model with glycerol-induced ATN. When injected into the damaged organ, hAFS cells can be tracked using bioluminescence, can survive, can integrate into renal structures, and differentiate into tubular cells expressing proximal and distal epithelial tubular markers such as Aquaporin 2, Peanut Agglutinin and Dolichous Biflorus Agglutinin after 3 weeks. In some rare case they expressed Glial Derived Neurotrophic Factor (indicating their ability to express early glomeruli marker differentiation) and it was confirmed by RT-PCR the expression of markers such as Nephrin, Aquaporin 1, Pax-2 and Occludin. We show that hAFS cells can ameliorate the glycerol-induced ATN injury in the early phase of the acute damage as reflected by decreased creatinine and BUN levels in the blood. In addition, our preliminary data suggests that in mice with induced ATN, hAFS cells present a protective effect after injection into damaged kidney by release of various cytokines that can attenuate the acute immune response. We have observed that, in mice with induced ATN, hAFS cells provide a protective effect when injected into the damaged kidney before the severe acute phase, whereas later on they contribute to functional morphological restoration of the damaged kidney through integration and differentiation into renal epithelial tubular cells. We therefore speculate that AFS cells could represent a limitless source of ethically neutral, unmodified stem cells that may prove useful as a novel alternative for cell therapeutic techniques in kidney regenerative medicine.

No: 97

Regenerative hair waves and macro-environmental regulation of ectodermal organ stem cells

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The regenerative behavior of a single organ has been studied. However, the collective behavior of a population of regenerative organs has not been studied. Here we use hair regeneration as a paradigm to decipher the principles behind this fundamental phenomenon. A single hair follicle is known to go through regenerative cycling continuously during the adult life, but whether the thousands of hair follicles on one individual cycle randomly, simultaneously, or in coordination is not known. Starting from mice, we did year long follow up to show that the hair cycle domains are the manifestation of regenerative hair waves. These domains form because hair regeneration propagates in waves; boundaries form because there are refractory regions where the wave can not pass through. By analyzing the dynamics of hair growth, time required for regeneration after plucking, in situ hybridization and reporter activity, we showed there is oscillation of intra-follicular WNT signaling which is synchronous with hair cycling, and there is oscillation of dermal BMP signaling which is asynchronous with hair cycling. The interactions of these two rhythms lead to the recognition of refractory and competent phases in the telogen, and autonomous and propagating phases in the anagen. Boundaries form when propagating anagen waves reach follicles which are in refractory telogen. Further, we found hair waves are reset during pregnancy, implying a systemic level of regulation. We further analyze the regenerative hair waves on rabbit and other mammals. A spectrum of wave patterns ranging from rapid fractal-like spreading, traversing waves, fragmented hair cycle domains, or apparent lack of interactions were observed in different animals and /or under different physiological conditions. Distinct attributes of hair cycling and dermal BMP cycling are identified at the cellular and molecular level. A multi-scaled mathematical model based on distinct units is developed to simulate wave propagations. By establishing simple initial parameters, various complex wave patterns can be generated. Xeno-specific transplantation demonstrates the interactions among domains with distinct intrinsic properties. Thus regenerative hair waves present a visible-in-real-time and experimentally perturbable living model for studying the stochastic behavior of a population or regenerative organs. The unexpected links with Bmp2 expression in subcutaneous adipocytes give implications in system biology and Evo-Devo. The work also has practical implication for those using the mouse skin as a model for carcinogenesis study, drug delivery or stem cell research. Publications: Plikus MV, Mayer JA, de la Cruz D, Baker RE, Maini PK, Maxson R and Chuong CM. 2008. Cyclic dermal BMP signaling regulates stem cell activation during hair regeneration. Nature. 451:340-344. Plikus MV and Chuong CM. 2008. Complex hair cycle domain patterns and regenerative hair waves in living rodents. J. Invest Dermatol. 128:1071-1080.

No: 98

Modeling malignant differentiation of hematopoietic stem cells in a human-mouse chimeric immune system

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The differentiation of hematopoietic stem cells into the various hematopoietic lineages follows an ordered series of events that shape the function of the resulting progeny cells. Malignancies result as a consequence of somatic mutations that occur during differentiation and frequently result in malignant cells arrested at various stages of differentiation. Extending work describing transformation in murine B-cells, we will address whether oncogenic transformation with c-MYC and BCL2 occurs during human B-lymphopoiesis from hematopoietic stem cells. To model human B-cell lymphoma, we are utilizing the Rag2-/- gamma c-/- mouse model, which lacks its entire adaptive immune system. These mice, when intrahepatically injected at birth with human CD34+ cord blood cells, develop human B-cells and T-cells, which reconstitute hematolymphoid organs and are at least partially able to mount immune responses against viral challenge. Here, we demonstrate that we are able to express transgenes in a B-cell specific manner by lentiviral transgenesis. We are able to follow B-cell development in a semi-quantitative manner by bioluminescent imaging of these animals and observe a ten-fold increase in B-cells when CD34+ cells are transduced with BCL2. We are currently undertaking necropsy analyses of these mice in order to determine if there is malignant transformation, and whether the expansions of B-cells are in fact clonal. These studies promise to better model human B-cell malignancies and to better understand malignant differentiation of human hematopoietic stem cells in vivo.

No: 99

Hydrophobic substrates for improved embyroid body mediated embryonic stem cell differentiation

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Embryonic stem cells (ESCs) have tremendous potential for revolutionizing medicine based on their unique ability to proliferate indefinitely in culture and give rise to cells from every germ layer. There is an emerging requirement in the study of these specialized cells for new tools and methods that utilize synthetic materials to overcome the variability inherent in traditional culturing practices. Here we describe the influence that physical properties at material surfaces (e.g. wettability) have on the efficiency of embryoid body (EB) mediated ESC differentiation. After studying murine EBs according to their size, we determined that EBs with a diameter between 100-300 microns were the most proliferative with the greatest differentiation potential and the lowest rate of cell death. In an attempt to promote the formation of this subpopulation, we surveyed several biocompatible substrates and identified a trend between increased hydrophobicity and the formation of EBs with these characteristics. This correlation between surface wettability and EB development was modeled more closely using alkanethiolate self-assembled monolayers (SAMs) on gold coated substrates. In these experiments, hydrophobic SAMs, in particular those assembled using octadecanethiol, were similarly found to enrich for EBs within the desired size range. When applied to the human ES cell system, similar phenomena were observed. These findings demonstrate how tailoring surface properties to control cell-materials interactions can lead to significant improvements in the efficiency of ESC differentiation.

No: 100

Therapeutic applications of human embryonic stem cell-derived motor neuron progenitors

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Human embryonic stem cells are an important therapeutic tool as they can be differentiated in large quantities into specific cell types that may be lost to degenerative or disease states. The Keirstead research group has successfully devised protocols for the reproducible, scalable, high purity derivation of motor neuron progenitors from human embryonic stem cells (hESC-MNPs). This clinically relevant cell population can be used for replacement strategies when developing therapies for motor neuron degenerating diseases. We have demonstrated that hESC-MNPs express and secrete growth factors that have neurotrophic effects both in vitro and in vivo following cervical spinal cord injury. Transplanted hESC-MNPs survive up to 4 months post-injury and continue to express the motor neuron specific transcription factor, HB9. Moreover, the transplanted cells fasciculate to from ectopic motor tracts in the dorsal and ventral white matter indicating that they are responsive to the environment which, may be manipulated to promote axon growth to specific targets. Therefore, we developed a strategy to guide axons to the periphery by over-expressing GDNF in the skeletal muscle. GDNF is a potent chemoattractant for motor neurons and hESC-MNPs express the GDNF receptor GFRa1. Rodent-derived myoblasts can be cultured in high quantities and lentivirally transduced at high efficiency. GDNF transduced myoblasts secrete GDNF and enhance neurite outgrowth in vitro while readily forming myotubes and integrating into host tissue following transplantation in vivo. We intend to exploit the inherent biology of motor neuron progenitors by transplanting myoblasts over-expressing GDNF in the skeletal muscle and using this system to restore neuromuscular connectivity following spinal cord injury and spinal motor neuron disease.

No: 101

Depletion of follicle cell precursors leads to defects in Drosophila oogenesis associated with age

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Our work focuses on understanding the loss of proliferative capacity of adult stem cells associated with aging. To study the regulation of self-renewal, we use Drosophila ovaries, which contain two adult stem cell populations: the germline stem cell (GSC) and the somatic stem cell (SSC). The GSC gives rise, after multiple steps of differentiation, to the oocyte. The SSC supplies follicle cells (FCs) that form a protective sheet around each oocyte. These follicle cells are essential for oocyte viability. The Drosophila ovary is composed of several functional units called ovarioles, itself containing two regions: the germarium and the vitellarium. The germarium contains the GSC and the FSC, which divide to form a complex structure called the egg chamber where the oocyte will mature. The vitellarium contains several egg chambers at different stages of maturation. In this work, we describe a new mutation that, combined with a mutation in the grapes (grp) gene, leads to a dramatic reduction of female fertility with ageing. We will refer to the double mutant as the "grp, ssc mutant". In two days old grp, ssc mutant ovaries, the older egg chambers are normal but half of the younger egg chambers exhibit structural defects where 2 oocytes are encompassed within one layer of FCs. The occurrence of these "compound oocytes" increases dramatically with age. In the germarium, the SSC divides to produce a FC precursor and another SSC. The FC precursor divides several times and differentiates into 3 different classes, the epithelial FCs, the Polar cells and the Stalk cells. Using markers specific for the 3 categories of FCs, we observed that in two day old mutant female ovarioles, the three categories of FCs were present, however, in some ovarioles the stalk cells were missing. This phenotype was more dramatic in 4 days old grp, ssc mutant females. The majority of ovarioles did not have any stalk cells, some ovarioles were missing the polar cells and some oocytes were not completely surrounded by FCs. The fact that the three categories of FCs are progressively missing suggests a failure to produce FC precursors rather than a defect in differentiation of the FC precursors. In fact, careful analysis of germariums at different ages revealed a depletion of FC precursors over time leading to the protrusion of compound oocytes directly from the germarium. In young germarium, we observed no changes in the number of FC precursors in mitosis or apoptosis. However they fail to maintain their polarity. Extensive deficiency screens and recombination mapping had led to the conclusion that, even though grp alone is not responsible for the oogenesis defect, it is associated with it. The modifier of grp called 'ssc' has been mapped 5 centimorgans from grp. The identification of the modifier of grp responsible for the depletion of FC precursors during oogenesis will provide insight into SSC regulation in Drosophila. In the long term, it will provide a basis for understanding the cause of human female sterility.

No: 102

Gene targeting in a HUES line of human embryonic stem cells via electroporation

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Genetic modification is critical for achieving the full potential of human embryonic stem (ES) cells as a tool for therapeutic development and basic research. Targeted modifications in human ES cells have met with limited success due to the unique culture conditions for most human ES cell lines. The HUES lines of human ES cells were developed for ease of manipulation and are gaining increased utility in stem cell research. Conditions for gene targeting via electroporation were tested in the HUES-9 human ES cell line. Successful gene targeting was achieved at the Fezl locus (also known as Fezf2), a gene involved in corticospinal neuron development. These experiments demonstrate conditions developed for gene targeting in mouse ES cells can be readily adapted to HUES cells with few key modifications. Thus, human ES cell lines developed under appropriate conditions can be readily altered genetically, which will facilitate their application in basic science and clinical development.

No: 103

Isolation of Definitive Endoderm from hESC by lineage specific lentiviral vectors

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Gene modification of Human Embryonic Stem Cells (hESC) is an important technique to control differentiation and proliferation. Lentiviral vectors (LVs) are known as a powerful tool for direct genetic manipulation for hESCs, for example by expressing reporter genes under the control of lineage-specific promoters to identify cells that have undergone specific pathways of differentiation. We focused on two transcription factors, Sox17, as an Endoderm marker and Brachyury, as Mesendoderm and Mesoderm markers. We generated LVs with Sox17 (pCCLc-SOX17-eGFP) and Brachyury (pCCLc-Brachy-eGFP) promoters, as lineage specific promoters, and MNDU3 retroviral LTR (pCCLc-MNDU3-eGFP) as a constitutive promoter to isolate lineage specific cells from hESCs. HT29, human colon carcinoma cells were transduced with LVs and taqman qPCR and FACS analysis were performed for titration. hESC (H1 and H9) were transduced with the LVs on Matrigel and induced to Mesendoderm and Endoderm differentiation by adding high concentration of Activin A in the culture medium. Gene expression of differentiating hESCs was assessed by RT-PCR. In taqman titer of concentrated single-promoter LVs, the titer of pCCLc-MNDU3-eGFP was 6.1 x 108 IU/ml, that of pCCLc-SOX17-eGFP was 1.9 x 108 IU/ml and that of pCCLc-Brachy-eGFP was 1.0 x 109 IU/ml. In FACS titer, the titers were 1.6 x 109 IU/ml, 1.2 x 108 IU/ml and 5.0 x 108 IU/ml, respectively. By RT-PCR, Sox17 could be strongly detected during all differentiation stages but could not be detected in undifferentiated hESCs. Brachyury could be detected during all stages including undifferentiated hESCs. CXCR4 a surface marker could be detected during all stages containing undifferentiated hESCs. Studies of expression by the vectors in undifferentiated hESC and after induction of differentiation to endoderm are underway. Lineage specific LVs will be promising tools for regenerative medicine and developmental biology.

No: 104

Necessary and sufficient contributions of donor and host in the creation of tissue-engineered gastrointestinal tissues

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Objective: To produce tissue-engineered small intestine (TESI) in a murine model to identify the relative contributions of donor and host. Background: Short bowel syndrome is associated with significant morbidity and mortality. Current therapies do not offer adequate palliation. In previous work, we successfully used TESI to replace lost gastrointestinal tissues in the Lewis rat. These TESI had identical architecture to native structures including mucosa, muscularis and ganglion cells. The molecular and cellular mechanisms underlying the formation of TESI have not been determined. Methods: Organoid units (OU), multicellular units primarily containing epithelial cells, were isolated either from Rosa26 (constitutively expressing LacZ) or female (XX) wild type P4 mice. OU were implanted on a biodegradable polymer scaffold in the omentum of male (XY) wild type mice. TESI were harvested after 4 weeks for immunohistochemistry, X-gal staining and fluorescent in situ hybridization (FISH) for the Y chromosome. Results: TESI was successfully generated in a murine model. All four differentiated epithelial cell types, muscularis and intestinal subepithelial myofibroblasts, key components of the intestinal stem cell niche, were identified in the TESI. Using a Rosa26 donor, we detected beta-galactosidase staining primarily in the epithelium with a few isolated cells in the mesenchyme. Host cells (XY) stained by FISH demonstrated that the entire epithelium and only part of the mesenchyme derive from donor cells (XX). Conclusion: We report here that TESI generated in the murine model recapitulates the normal architecture of the native small intestine. Implanted OU mainly provide the epithelial layer while the host cells supply a majority of the mesenchymal component. Identifying the molecular and cellular mechanisms of TESI formation is a necessary step towards eventual use of engineered tissue as potential human therapy.

No: 105

Regulation of SIRT1 by miRNAs during mESC Differentiation

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SIRT1 is one of seven mammalian sirtuins (SIRTs) or class III histone deacetylases (HDACs), which deacetylates histones and non-histone proteins. SIRT1 is a NAD+-dependent enzyme regulated by metabolic and dietary changes in cellular NAD+ levels. In response to oxidative stress and DNA damage, SIRT1 promotes cellular survival through deacetylation of p53, Ku70, and Foxo proteins. We have found that SIRT1 is highly expressed in mouse embryonic stem cells (mESCs) and that protein levels are dramatically decreased during differentiation. However, SIRT1 mRNA levels do not change during differentiation, indicating a post-transcriptional regulation of SIRT1 expression. Likewise, SIRT1 protein levels are low in differentiated adult mouse tissues, while mRNA levels are equivalent to the levels in mESCs. To determine if miRNAs play a role in the post-transcriptional regulation of SIRT1, we examined SIRT1 expression in liver after acute knockdown of Dicer, an enzyme involved in the processing of miRNAs. Compared to control liver samples, SIRT1 protein levels were induced after Dicer knockdown. In contrast, there was no change in SIRT1 mRNA levels after Dicer knockdown, indicating that miRNAs play a role in regulating expression of SIRT1. To identify miRNAs that downregulate SIRT1 during stem cell differentiation, we profiled expression of conserved miRNAs with potential binding sites in the 1.6kb 3'UTR of SIRT1 mRNA. We identified 8 miRNA families that are induced during mESC differentiation at the time point when SIRT1 protein levels decrease. Cotransfection in mESCs of individual miRNAs with a construct containing the 3'UTR of mSIRT1 behind luciferase identified miRNAs that repress translation of the 3'UTR of mSIRT1. Mutation of 4bp in the 3'UTR corresponding to the seed-binding site of each miRNA released the repressive effect of the corresponsing miRNA. We are now confirming the role of miRNAs in regulating SIRT1 expression both during stem cell differentiation as well as in differentiated tissues where miRNAs may play an important role in the rapid induction of SIRT1 in response to cellular stress.

No: 106

Multiple early commitment pathways of hematopoietic stem cells

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Lineage commitment is a process of stepwise restrictions from the multi-potency of hematopoietic stem cells (HSC) to more differentiated progeny. The prospective isolation of both common lymphoid progenitors (CLP), and common myeloid progenitors (CMP) has provided a basic model of lineage commitment in which all lymphoid and myeloid effector cells are ultimately derived from these upstream oligopotent progenitors. However, several recent reports have challenged this model by suggesting that some aspects of lymphoid and myeloid lineage commitment may occur upstream of the CLP and CMP bifurcation. These findings suggest that population between HSC and oligopotent progenitors is still heterogeneous. Based on differential expression of three positive cell surface markers for HSC, Slam-f1, Tie-2, and Vcam-1, we subfractionated the multipotent progenitor (MPP) subset of the primitive hematopoietic compartment (CD34+c-kit+Sca-1+Lin-) into four distinct subpopulations (Slam+Tie-2+Vcam-1+, Slam-Tie-2+Vcam-1+, Slam-Tie2-Vcam-1, and Slam-Tie-2-Vcam-1-). We also found that these same markers were capable of subfractionating the original CMP population into four other distinct subpopulations. These 8 early progenitor populations were functionally characterized for lineage potential both in vitro and in vivo including platelet reconstitution capacity. These assays revealed that two of the populations have multipotency, two populations have granulo/macrophage+lymphoid potential, one population has megakaryo/erythloid+granulo/macrophage potential, one population is restricted to megakaryo/erythloid potential, and two populations are restricted to granulo/macrophage potential. In vitro tracking assays revealed a hierarchical relationship between these populations. Some HSC immediately lost lymphoid potential, and some HSC gradually lost megakaryo/erythloid potential. These data suggests lineage commitment from HSC to divergent fates can occur by multiple distinct commitment pathways,and the first restriction of lineage potentials of HSC is more complicated that previously thought. This study provides up-dated comprehensive and precise route map of early hematopoiesis for understanding molecular mechanisms regulating lineage commitment.

No: 107

Identification of modifiers of survival in human embryonic stem cells

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Human embryonic stem cells (hESCs) are pluripotent cells with the capability to differentiate into cells derived from all three embryonic germ layers. As a result, hESCs have enormous potential for use in cell replacement therapies. Before this potential can be realized, however, there remain many questions regarding the mechanisms involved in hESC growth and fate decisions. While they can be maintained indefinitely in culture, hESCs exhibit poor survival upon dissociation, which limits our ability to manipulate and then select for homogenous populations of cells. Understanding and identification of signals involved in hESC survival is hampered by the lack of a reliable method for identifying modulators of survival in pluripotent cells. To address this, we have developed a high content screening (HCS) assay for use in studying hESC survival after treatment with small molecules. These screens identified novel small molecules that improve survival of hESCs, including inhibitors of Rho kinase (ROCK) and protein kinase C (PKC). Short-hairpin RNA (shRNA) was used to decrease expression of genes corresponding to small molecule targets identified in HCS assays. Generation of a stable hESC line using shRNA validates the high-content approach for use in hESCs and also verifies the specificity of compounds identified in the HCS assays. Rescreening using the shRNA cell line allowed us to identify novel small molecules that decrease hESC survival. Currently we are evaluating the mechanisms by which survival is controlled in hESCs by investigating the role of the niche, downstream signaling, and cell polarity in hESC survival. Understanding how cell fate is regulated in hESCs could accelerate their use in regenerative therapies by improving expansion, modification, and production of cells and tissues for therapeutic uses.

No: 108

Mechanistic studies of ntl-dependent mesoderm formation using caged morpholinos

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The mechanisms by which no-tail (ntl), the zebrafish ortholog of brachyury, regulates zebrafish mesoderm development are pleiotropic, with suggested roles in both morphogenetic movements and cell fate choice. Amidst this molecular and cellular complexity, fundamental questions remain unanswered. Does ntl have distinct functions in different regions of the developing mesoderm and what are the transcriptional targets of ntl in different tissue contexts? Ntl is thought to act cell-autonomously within the axial mesoderm to promote notochord cell fates. It has been further proposed that the notochord/floor plate cell fate choice occurs within the dorsal margin during gastrulation, with the floor plate being the default fate in the absence of ntl. Using caged morpholinos (cMOs), we have found that ntl function is required for the migration of notochord progenitor cells to the axial mesoderm during gastrulation and for proper vacuolization of notochord cells during early somitogenesis. To elucidate the spatiotemporal parameters of notochord fate determination, we are investigating the consequences of ntl silencing in specific regions of the developing chordamesoderm. For example, localized ntl cMO activation 150 µm anterior to the tailbud in 1-2 somite embryos leads to depletion of Ntl protein in the targeted cells within five hours and subsequent disorganization of the chordamesoderm tissue. We are also combining the spatiotemporal precision of cMO technology with photoactivatable fluorophores, FACS, and microarray analysis to identify ntl-dependent, tissue-specific effectors of mesoderm patterning.

No: 109

Genome-wide methylation profiling in human pluripotent stem cells and somatic cells

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DNA methylation plays multiple roles in cell physiology including developmental gene regulation, genome stability, genomic imprinting and X-inactivation. Methylation patterns are dynamically regulated during embryonic development and cell differentiation or during somatic cell reprogramming to induce pluripotent (iPS) stem cells. Several recent studies have consistently identified abnormal changes in methylation patterns during long-term cultures or upon in vitro differentiation of human embryonic stem cells (hESCs). Aberrant DNA methylation patterns are associated with many human diseases including cancer, Fragile-X, ICF, and ATRX syndromes. Therefore, it is essential to monitor methylation patterns in hESCs, iPS cells, or their derivatives when they are generated in vitro for use in regenerative medicine. Here we present our data of genome-wide methylation profiling in gene promoter regions including several lines of undifferentiated hESCs and iPS cells as well as somatic cells such as fibroblasts, neural precursor cells, and adult leukocytes. Using the MeDIP-chip approach or Illumina Infinium methylation assay system, we showed that methylation in somatic cells is distinctly different from that in hESCs and iPS cells. In contrast, hESCs and iPS cells exhibit strikingly similar methylation patterns, consistent with the notion that induction of human iPS cells requires extensive reprogramming of somatic methylation patterns to achieve a hESC-like state. Moreover, upon in vitro differentiation of hESCs into somatic ells such as neural precursor cell lineages, we observed both dramatic increases and decreases of promoter methylation in specific sets of gene promoters. Finally, when we associate methylation profiles with gene expression profiles in human pluripotent stem cells and terminal differentiated somatic cells such as leukocytes, we found that the differential methylation at promoter CGIs are tightly associated with differential gene expression between stem cells and somatic cells. Our data support the notion that DNA methylation patterns are dynamically regulated in development and reprogramming. We suggest that proper regulation of DNA methylation is essential for the efficient differentiation of human pluripotent hESCs and iPS cells into somatic cells or when converting somatic cells into multipotent iPS cells.

No: 110

A requirement for serum-free, hormone-supplemented medium in the differentiation of mouse embryonic stem cells into the airway epithelial cell lineage

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RATIONALE: The self-renewability and pluripotency of embryonic stem (ES) cells hold great potential for regenerative medicine, including treatments for dysfunctional airway epithelia. For maximal treatment efficacy and minimal risk of teratoma formation, precise culture conditions are needed to guide ES cell differentiation. The objective of our study is to investigate the effects of cell density, media composition, and culture systems on the well-defined, serum-free differentiation of the D3 mouse embryonic stem cell line into the airway epithelial cell lineage. METHODS: The D3 mouse ES line was maintained pluripotently in culture medium supplemented with leukemia inhibitory factor (LIF). A serum-free hormone-supplemented differentiation medium was developed to initiate expression of airway epithelial lineage gene products, as measured by quantitative real-time PCR. The effects of individual hormone and growth factor supplements, cell seeding density, and standard culture systems on the differentiation of mouse ES cells into the airway epithelial lineage were studied. RESULTS: D3 mouse ES cells differentiated with our serum-free differentiation protocol expressed multiple airway epithelial genes, including Clara cell secretory protein (CC10), mucins (MUC5AC, MUC5B), and surfactant associated proteins C and D (SPC, SPD). Histological staining revealed cells with a columnar epithelial morphology that stained positive for mucins. Removing individual hormones and growth factors from the differentiation medium had variable effects on gene expression, but decreased expression of airway epithelial genes in the majority of cases. Varying cell density had a significant effect on several genes, with a lower cell density leading to increased expression of CC10, MUC5AC, and SPD. Transwell chambers promoted airway epithelial cell differentiation over tissue culture chambers. CONCLUSIONS: We have demonstrated the differentiation of airway epithelial lineage cells from the D3 mouse embryonic stem cell line via use of a serum-free hormone-supplemented medium. Soluble factors, cell density, and culture conditions play diverse regulatory functions in the differentiation of ES cells. Further studies on their effects may lead to cell therapy applications as well as an understanding of the airway epithelial cell lineage and stem cell niche.

No: 111

Aging and cancer resistance in lymphoid progenitors are conferred by p16Ink4a and Arf

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Aging of tissue specific stem and progenitor cells has been proposed to be the central cause of altered tissue function and diminished regenerative capacity in the elderly. Within the hematopoietic system, the consequences of aging are unbalanced within disparate lineages and their progenitors. In particular, the production of B lymphocytes in the bone marrow is markedly reduced in the elderly, while the myeloid compartment is thought to be relatively unperturbed. We determined that these dichotomous effects of aging are due in part to the preferential expression of two potent tumor suppressors, p16Ink4a and Arf, in aged lymphoid progenitors. Increased expression of p16Ink4a and Arf in aged lymphoid progenitors contributes significantly to their reduced proliferative potential and survival, while concurrently making them refractory to malignant transformation. Furthermore, down-regulation of p16Ink4a and Arf in aged lymphoid progenitors reverted their senescent phenotype and restored their susceptibility to transformation. These data provide a molecular explanation for the preferential effects of aging on lymphopoiesis and the reduced incidence of lymphoid leukemia in adults. They also indicate that aging of distinct hematopoietic stem and progenitor cell populations are differentially regulated. Finally, our data suggest that inhibiting expression of p16Ink4a and Arf can rejuvenate aged hematopoietic progenitors and depressed immune cell production in the elderly, and provide direct evidence that aging and cancer resistance are mechanistically linked processes.

No: 112

Genetic analysis of ATM in maintaining genetic stability in human ES cells

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Genetic manipulation through homologous recombination in mouse ES cells has become an invaluable tool in elucidating gene function and creating mouse models for human diseases. It is critical to develop these genetic approaches in human ES cells (hESCs), which have remained highly inefficient. We have optimized the conditions to improve the efficiency of homologous recombination in hESCs. This has allowed us to investigate the physiological function and regulation of ATM in DNA damage responses in human ES cells. In this context, we have established ATM-deficient hESCs and shown that ATM is critical for the cellular responses to DNA double-stranded break damage in hESCs. In addition, by establishing the ATMSer1981Ala knock-in mutant hESCs, we demonstrate that DNA damage induced Ser1981 phopshorylation of ATM is important to activate ATM function in hESCs.

No: 113

Transplanted interneurons follow endogenous developmental programs and stably integrate into the recipient neocortex

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The mammalian neocortex contains a diversity of inhibitory interneurons, a cell type which sculpts the oscillatory patterns, output, and developmental plasticity of the cortical circuitry. Cortical interneurons arise from the ganglionic eminences of the embryonic ventral forebrain, from which they undergo a dramatic tangential migration into the developing cortex. Remarkably, transplanted interneuron precursors migrate, differentiate and survive in the host brain, where they increase the frequency of inhibitory signaling events. In order to develop the therapeutic potential of interneuron transplantation, we have examined mechanisms that regulate the integration of interneuron precursors into the cortex. Developmental cell death eliminates large fractions of nascent neural cell types in many regions of the nervous system. In the rodent olfactory bulb, for example, nearly forty to fifty percent of newborn interneurons die once they migrate and reach morphological maturity in the granule cell layer. We are particularly interested in whether developmental cell death likewise regulates the integration of inhibitory interneurons into the laminar neocortex. We have defined the timing and extent of interneuron cell death, both in normal development and following interneuron precursor transplantation. Our results show that endogenous cortical interneurons undergo a period of apoptosis that spans the first two postnatal weeks of mouse life. During this time, over forty percent of young GABAergic neurons are eliminated from all regions of the neocortex. Following transplantation, around forty percent of heterochronically grafted interneuron precursors also die in the host cortex. Curiously, transplanted interneurons die after host interneurons, implying that cell-intrinsic programs determine the timing of interneuron cell death. Additionally, transplant cell death is constant over a range of transplant sizes, regardless of whether hundreds or millions of cells are initially transplanted. Transplantation can add at least fifty percent additional interneurons to the cortical population, without affecting the number of endogenous interneurons. These results suggest that interneuron cell death does not follow from intercellular competitive interactions. Rather, the host cortex can support large numbers of supernumerary interneurons, a feature that makes it particularly amenable to regenerative, cell-based therapies.

No: 114

Differential contribution of reprogramming factors to the induction of pluripotency

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Induced pluripotent stem (iPS) cells that functionally resemble embryonic stem (ES) cells can be obtained from fibroblasts by expression of the four transcription factors (TFs) Oct4, Sox2, Klf4 and cMyc. To understand how these factors control this dramatic change in cell identity, we have carried out genome-wide promoter analysis of their binding in murine iPS cells and partially reprogrammed cells. iPS cells are characterized by an ES-like binding pattern indicating a common TF binding signature of pluripotency, with minor disparities that might reflect the diverse origin of these cells. The four factors extensively co-occupy target promoters that are transcriptionally activated during reprogramming. In partially reprogrammed cells, binding differs broadly and many genes that fail to become reactivated are bound by fewer factors than in ES/iPS cells indicating that TF co-occupancy of target promoter regions is required for transcriptional activation of these genes. In particular transcriptional regulators of the pluripotent state that become highly upregulated during reprogramming are targets of Oct4, Sox2, and Klf4 in ES/iPS cells and often unbound, and hence not reactivated, in partially reprogrammed cells. Their reactivation during reprogramming is associated with a distinctive change in histone H3 lysine 4 and 27 trimethylation within their promoter regions. Our genome-wide analysis demonstrates a separable contribution of the reprogramming TFs to the induction of pluripotency and defines a temporal order of binding events during reprogramming.

No: 115

Role of adhesion molecules in hematopoietic and endothelial commitment of murine embryonic stem cells

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Mouse embryonic stem cells (ESC) collect information from their environment and make cell fate decisions based on intrinsic and extrinsic factors. Embryoid body (EB) formation induces the differentiation of ESC to multiple tissue lineages. Cell-cell contact or cell-environment interactions influence EB formation and ESC fate decisions within EB. However, the molecular mechanisms underlying modulation of ESC fate decisions by cell-environmental interactions are incompletely understood. Adhesion molecules influence proliferation and differentiation in multiple developing and adult tissues. We hypothesize that adhesion molecule interactions have a critical role in guiding ESC commitment to hematopoietic and endothelial lineages. Quantitative RT-PCR was used to establish the relative levels of adhesion molecule expression during EB formation and early stages of hematopoietic differentiation. Relative expression profiles were generated for 32 adhesion molecules at discrete stages of early hematopoietic and endothelial development. Adhesion molecules differentially expressed under these conditions were primarily representative of adherens junction, tight junction and gap junction pathways. These genes include E-cadherin, Claudin-4, Connexin-43, Connexin-45, Zona Cccludens-1 (ZO-1) and Zona Occludens-2 (ZO-2). Differential regulation of molecules in the junction pathways hematopoietic and endothelial development supports the hypothesis that cell-cell interactions are important for ESC fate decisions. Stable ESC lines constitutively knocking down expression of E-cadherin, Connexin-43, Claudin-4, ZO-1 and ZO-2 were generated using lentiviral transductions of shRNA constructs. Expression of E-cadherin, Connexin-43, Claudin-4 and ZO-1 does not effect the formation of early hematopoietic and endothelial cells which express Fetal Liver Kinase-1 (Flk-1). However, it does effect the expression of CD45, an extracellular protein found on most hematopoietic cells, which is decreased in cell lines with decreased expression in each of these genes. A parallel increase in endothelial differentiation is observed in these knockdown lines as indicated by VE-cadherin expression and functional assays of endothelial sprouting EB. Functional and molecular assays for hematopoietic and endothelial commitment are ongoing to determine the consequence of manipulation of adhesion molecule expression levels on transitional states downstream of Flk-1 expression.

No: 116

Cell-specific gene delivery into epidermal stem cells

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Epidermal stem cells are a well understood type of stem cells. Stem cells of the skin are located at the bulge of the hair follicle and these 'bulge' stem cells function to maintain skin integrity, regenerate hair, and repair the skin following injury. In addition to its critical function in the skin, epidermal stem cells are also attractive targets for gene therapy for various skin diseases (eg. pachyonychia congenital and epidermolysis bullosa), wound healing, and even systemic disorders. In contrast to differentiated keratinocytes, epidermal stem cells are long lived and continuously renew many components of the epidermis. However, these stem cells do not cycle rapidly, making them less attractive for traditional methods of gene transfer. Many studies have attempted to characterize markers for identifying and even isolating epidermal stem cells. Recently, CD34 was identified as a specific cell surface marker for epidermal stem cells. Here, we propose a novel method to specifically deliver genes to epidermal stem cells. We will use a lentiviral based vector (lentivector) which is efficient at infecting and transducing both dividing and non-dividing cells. For cell specific targeting of the lentivector to epidermal stem cells, we will generate a modified lentiviral envelope containing an anti-CD34 immunoglobulin gene, enabling the lentivector to specifically bind and transduce CD34+ cells. The initial CD34-specific targeting lentivectors will be tested in in vitro. Following selection of the most optimal construct, we will test this targeted gene delivery system in vivo. If successful, this targeted gene delivery approach will provide a powerful tool for understanding skin biology. In addition, the targeted lentivector system can be adopted for cell specific gene therapy to treat a variety of cutaneous and systemic diseases.

No: 117

Macrophage differentiation from embryoid bodies derived from human embryonic stem cells

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Human embryonic stem cells can differentiate into CD34+ hematopoietic progenitors by co-culture on murine feeders such as OP9 and S17. These CD34+ progenitors can be further differentiated into several cells of the hematopoietic lineage including macrophages. However, co-culture on murine feeders is time consuming and involves extensive manipulations. Furthermore, CD45 expression is low on hematopoietic cultures derived from stromal co-cultures. In this study we show for the first time a highly efficient system of generating differentiated macrophages from embryoid body cultures of human embryonic stem cells. The hematopoietic progenitors generated from these embryoid bodies express higher numbers of CD45+ cells and are able to differentiate to macrophages when cultured in presence of cytokines. Using this system we were able to generate 5 fold higher yields of CD14+ macrophages compared to traditional stromal cell culture methods. The embryoid body derived macrophages are phagocytic, respond to Toll-like receptor stimulation and express phenotypic markers of mature macrophages. Importantly, the embryoid body system generates hematopoietic progenitors suitable for clinical use by eliminating the need for murine feeder cells. Furthermore, macrophages derived by this method are amenable to genetic manipulation and may thus be used to study important mechanisms of macrophage differentiation and function.

No: 118

Multipotent somatic stem cells contribute to the stem cell niche in the Drosophila testis

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Adult stem cells reside in specialized microenvironments, or niches, that play an important role in regulating stem cell behavior1. Therefore, tight control of niche number, size and function is necessary to ensure the proper balance between stem cells and progenitor cells available for tissue homeostasis and wound repair. The stem cell niche in the Drosophila male gonad is located at the tip of the testis where germline and somatic stem cells surround the apical hub, a cluster of approximately 10-15 somatic cells that is required for stem cell self-renewal and maintenance2-4. Here we show that somatic stem cells (SSCs) in the Drosophila testis contribute to both the apical hub and the somatic cyst cell lineage. The Drosophila ortholog of epithelial cadherin (DE-cadherin) is required for SSC maintenance and, consequently, the apical hub. Furthermore, our data indicate that the transcriptional repressor escargot regulates the ability of somatic cells to assume and/or maintain hub cell identity. These data highlight the dynamic relationship between stem cells and the niche and provide insight into genetic programs that regulate niche size and function to support normal tissue homeostasis and organ regeneration throughout life.

No: 119

MicroRNAs regulate G1/S transition of ES cells

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Embryonic Stem (ES) cells have a rapid cell cycle with a short G1 phase. The mechanisms underlying their unique cell cycle structure remain largely unknown. Recently, we generated a Dgcr8 knockout model to globally remove microRNAs (miRNA) in mouse ES cells. These miRNA depleted ES cells showed a decreased proliferation rate with an associated accumulation of cells in the G1 phase of the cell cycle, implicating important roles of miRNAs in regulating the ES cell cycle. To identify the specific miRNAs, we designed a screening strategy in which individual miRNA mimics were reintroduced into Dgcr8 knockout ES cells and evaluated for rescue of the proliferation and cell cycle defects. A library of 266 known mouse miRNAs was screened. Fourteen of these miRNAs dramatically improved Dgcr8 knockout ES cell proliferation. Mimics for ten of these miRNAs were resynthesized and further evaluated. These ten miRNAs reduced the fraction of DGCR8 knockout cells in the G1 phase. In contrast, these same miRNAs had no effect on the proliferation or cell cycle of wild-type ES cells, suggesting they already exist at saturating levels in ES cells. Consistent with this, five of the miRNAs are highly expressed in undifferentiated ES cells. Interestingly, these miRNAs share a similar seed sequence. Therefore, they likely regulate common targets. Western analysis and luciferase reporter assays identified several key regulators of the G1/S transition targeted by these miRNAs. Our results show that miRNAs play an important role in expediting the G1/S transition. A shortened cell cycle is critical during early mammalian embryogenesis allowing the young embryo to rapidly grow prior to somatic differentiation. The large number of miRNAs found in our screen suggests a large degree of redundancy in this critical cellular function. Inappropriate expression of these miRNAs in differentiated cells could lead to abnormally rapid cell growth. Therefore, these same or related miRNAs may support cellular transformation and associated tumor growth. Our ongoing studies are aimed at testing these hypotheses as well as searching for other potential cell cycle targets of miRNAs.

No: 120

Functional switch of adult brain germinal zone cells: a strategy that may be used to replace inner ear sensory cells after damage

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Loss of auditory hair cells is the most common cause of hearing disorders. This impairment is irreversible in mammals because inner ear hair cells (HCs) have a limited capacity to regenerate. Here we report that the germinal zone of adult brain of both rodents and humans contains cells with proliferative potential, which share morphological and functional characteristics with HCs. In addition, putative neural stem cells (NSCs) from the germinal zone can differentiate into functional spiral ganglia-like neurons (SGNs). We assert that proliferative germinal zone cells may undergo an epigenetic functional switch, assuming functional characteristics of HCs and SGNs, respectively. This report suggests that uncovering the functional plasticity of renewable cells and conditions that promote functional reprogramming can be employed for cell therapy in the auditory setting.

No: 121

Maintenance of multi-potency of epicardial progenitor cells

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Coronary artery disease is a leading cause of mortality in the USA. Novel therapies to ameliorate coronary function and/or restore damaged coronary vasculature are desirable goals that could be achieved by expanding the current knowledge on the mechanisms that govern coronary progenitor cell biology. During development, progenitor cells of the coronary vasculature are recruited from a transient structure located at the septum transversum, named proepicardium. Previous work used epicardial monolayer explants as a tool to study the differentiation potential of epicardial cells, but it was not known whether epicardial cultures could be maintained and expanded as undiferentiated progenitors, thus providing a controllable system that could serve as a substrate in cell-therapy procedures. Since epicardial biopsies are a routinely performed and minor surgical procedure, manipulation of epicardial progenitor cells may be a favorable procedure to generate patient-specific cardiac cells for tissue replacement. Here, I present the generation and culture of explanted epicardial cells. We have established the conditions to make epicardial cells from explanted embryonic heart to form cardiosphere-like structures, and keep the epicardial cells in culture by alternating Poly-D-Lysine and fibronectin conditions. Epicardial cells under these conditions preserve the potential to differentiate into smooth muscle cells and cardiomyocytes. One hypothesized mechanism by which epicardial cells can maintain their multi-potency in culture is that they produce factors inhibiting differentiation. To test this model, we have co-cultured rat epicardial mesothelial cells (EMC) with mouse embryonic stem cells in differentiation media. EMCs strongly inhibit the differentiation of the stem cells while enhance the preservation of the pluripotency of the stem cells, suggesting that epicardial cells can produce factors inhibiting differentiation in a non-cell-autonomous manner. In summary, we have developed a method to culture epicardial progenitor cells from epicardial explants in vitro, in which the multi-potency to differentiate can be maintained. The maintenance of the multi-potency of the epicardial cells may be a result of non-cell-autonomous factors produced by epicardial cells.

No: 122

Stem cell replacement through reversion of differentiating progenitor cells

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The correct balance between stem cell self-renewal and differentiation of daughter cells is achieved by cues from the stem cell microenvironment, or niche. When stem cells are lost, the niche could direct stem cell replacement by promoting symmetric division of remaining stem cells or reversion of differentiating cells back to a stem cell state. We are using the Drosophila testis as a model system to study the reversion of partially differentiated cells as a possible mechanism for maintaining adequate stem cell numbers for tissue homeostasis. Germline stem cells (GSCs) surround a cluster of somatic cells called the apical hub, a critical component of the stem cell niche. GSCs divide asymmetrically to produce a daughter stem cell and another cell that initiates differentiation to produce a cyst of 16 spermatogonia that ultimately develop into mature sperm. Previously, we demonstrated that the average number of GSCs declines with age. Although the 35% decrease in the average number of GSCs is significant, even more striking is the maintenance of GSCs in older flies. We hypothesized that reversion of spermatogonia is a mechanism to maintain GSCs in older flies and have developed a permanent marking strategy to detect candidate cells used to replace lost GSCs. Preliminary data suggest that the likely candidates for reversion are spermatogonia closest to the hub. Furthermore, our data imply that reversion may be compromised in aged flies, hinting that compromised reversion of differentiating cells could contribute to the decline in GSCs in older flies.

No: 123

Novel genes involved in the maintenance of pluripotency in human embryonic stem cells

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Human embryonic stem cells (hESC) are pluripotent cells derived from the inner cell mass of human blastocysts. The transcription factors Oct4, Sox2 and Nanog are key regulatory players in the maintenance of hESC pluripotency, by activating genes that support self-renewal and repressing genes that promote differentiation. Previous studies demonstrated that Oct4, Sox2 and Nanog co-occupy a substantial portion of their target genes. Furthermore, they are able to regulate each other and effectively forming a core regulatory circuitry regulating stem cell pluripotency, where Oct4, Sox2 and Nanog are proposed to be the master regulators of this transcriptional network. However, the role of the downstream effectors of these transcription factors remains unknown. Studies that address this key question will prove helpful in understanding the molecular mechanisms governing ES cell pluripotency as well as designing assays that direct lineage-specific differentiation. This study aims to investigate the role of the novel genes involved in this core regulatory network of Oct4, Sox2 and Nanog. Previous microarray studies have identified genes that are up-regulated in undifferentiated hESC. Many of them are novel genes not previously associated with controlling stem cell pluripotency. By comparing these novel genes with the list of target genes of Oct4, Sox2 and Nanog, we are able to identify some novel genes that are potentially important in regulating hESC pluripotency. We plan to characterize their expressions in hESC and study their biological function by siRNA knockdown.

No: 124

Sonic Hedgehog signaling controls hair follicle regeneration via a novel dermal pathway

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Hair follicle (HF) regenerates in the adult mammalian body throughout life. Like other organs, HF regeneration requires precise reciprocal inductive signaling between HF stem cells of the epithelium and the stromal organizer dermal papilla (DP) cells of the dermis. However, the mechanisms of signaling cross-talk between the two cell types remain poorly understood, partly due to the lack of a reliable dermal-Cre driver. To dissect the mechanisms of these signaling cross-talk, we have developed a rapid, genetically tractable hair regeneration system, allowing gene knockdown in either the isolated newborn mouse epithelial or dermal cells that are competent to induce hair regeneration in a skin wound on nude mice. One of the key HF inductive signals, Sonic hedgehog (Shh), is expressed only in the epithelial compartment and required for HF formation. While both the HF epithelial and DP compartments show Shh target gene induction, previous studies have supported a role for Shh signaling only in the epithelial compartment for epithelial progenitors expansion. To understand Shh signaling cross-talk, specifically whether Shh signaling has a dermal role during HF induction, we knockdown the critical Shh transducer Smoothened (Smo) in the dermal cells using lentivirus expressed Smo small hairpin RNA, and perform hair regeneration assay with a cell mixture of Smo knockdown dermal cells and wild-type epithelial cells. We showed that sustained Smo knockdown reduces 90% of Shh target gene induction. Hair regeneration with Smo knockdown dermal cells resulted a dramatic reduction in the number of regenerated HFs. While growth delayed, the remnant HFs following dermal-Smo knockdown displayed normal HF differentiation markers, indicating dermal Smo function is only required for HF progenitor proliferation. Smo knockdown dermal cells expressed reduced levels of a subset of DP markers, suggesting Smo may regulate some aspects of DP differentiation. We conclude that Shh controls epithelial HF progenitor proliferation through a novel indirect signaling pathway via the DP, in addition to a direct Shh signaling within the HF epithelial progenitors. To further dissect Shh/Smo dependent downstream signaling in the DP cells that reciprocally cross-talk with the proliferating epithelial progenitors, we examined if a candidate downstream reciprocal signal Noggin rescues dermal-Smo knockdown hair regeneration defects, while in parallel we performed microarray analysis to identify DP-specific Shh induced genes that differentially expressed in Smo knockdown dermal cells.

No: 125

Developing human Familial Alzheimer's Disease model using human embryonic stem and pluripotent cells

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Alzheimer's disease (AD) is a devastating disease and the most common cause of dementia that is incurable. Despite intensive research into the disease mechanism, the pathophysiology is poorly understood. In addition, animal models of familial AD (FAD), an autosomal dominant mutation that causes early and fulminant onset of AD, do not fully recapitulate the human pathology, possibly due to the difference between the human and mouse genetic make up. Thus, the ideal model for human disease would be of human origin. We propose to build a human model system for FAD by creating a knock-in of a known FAD mutation in human embryonic stem cells (hESC) or by reprogramming FAD patient fibroblasts into induced pluripotent stem (iPS) cells. We are developing methods to enrich for specific cell types from heterogeneous differentiation cultures by fluorescent activated cell sorting. We will be able to use our system to test the hypothesis that engineered hESC and iPS cells can serve as a human AD model by showing AD associated disease pathologies both biochemically and at the cellular level.

No: 126

Analysis of the initial lineage decisions during human preimplantation development

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Introduction. In mammals, the first few days of development prepare the embryo for implantation, which in humans optimally occurs ~six days post-fertilization. During this time, the mature blastocyst forms-a hollow structure consisting of an outer layer of polarized trophectoderm (TE) cells and the inner cell mass (ICM), an eccentric aggregate of cells in its fluid-filled interior. The TE, which connects the blastocyst to the uterus, is absolutely essential for the subsequent development of the ICM, which gives rise to the embryo proper. In this context, the first major fate decision, segregation of the TE and ICM lineages, has been extensively studied in the mouse. Four models have been proposed: 1) pre-patterning-allocation of cells to either the TE or ICM lineage is determined at the egg stage; 2) regulative-divergence, which occurs after compaction, is determined by whether a blastomere resides on the surface or interior of the embryo; 3) cryptic pre-formation-cleavage patterns influence allocation such that progenitors and their descendants occupy either an outer or inner position; 4) stochastic-cells become randomly distributed. In humans, the timing and regulation of the first major fate decision is poorly understood. Aims. We tested the hypothesis that in humans allocation of the TE and ICM lineages is the result of asymmetric cell division, which determines, by the blastocyst stage, whether a cell is exteriorized. To test this theory, we characterized the expression, during the first five days of development, of markers that are associated with asymmetric cell division (Numb) or apical-basal polarity (ZO-1, Occludin 4 and 6 and the PKC/PAR complex). hCG expression was used to identify components of the TE. Methods. Human embryos were cultured for 3-5 days before the molecules of interest were immunolocalized and the staining patterns imaged by using conventional and confocal microscopy. In total, we analyzed the embryos at the following stages in the numbers indicated: five 8-10 cell embryos; three early morula-stage embryos; five early-stage blastocysts; and two late-stage blastocysts. Results. At the 8-10 cell stage, Numb expression was associated with the basolateral surface of blastomere plasma membranes and was absent from the apical portions of cells that were in contact with the zona pellucida. Patchy ZO-1 expression was associated with the basolateral portions of the plasma membranes of a subset of the blastomeres at the embryo surface. By the blastocyst stage, Numb expression, which was absent in the ICM, was restricted to some, but not all TE cells. ZO-1 localized to the TE, which also expressed hCG. Conclusions. Evidence of segregation of the TE and ICM lineages was evident at the 8-10 cell stage. The Numb and ZO-1 expression patterns were consistent with cell specification as a consequence of asymmetric division, which leads to the formation of an outer layer of polarized cells. Blastocyst-stage embryos had a fully developed TE that was characterized by ZO-1 and hCG expression.

No: 127

Self-renewal of mammary stem cells

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A stem cell's decision to self-renew or differentiate is influenced by signals from their environment, which constitute a niche. It is postulated that stem cells compete for limiting concentrations of local growth factors in the niche, thereby maintaining a balance between the numbers of self-renewing and differentiated cells. We have tested how stem cells from the mammary gland respond to self-renewing signals, by changing either the availability of factors or the sensitivity of the cells to the factors. We found that addition of Wnt3A protein in vitro increases the clonogenicity of the cells and suggests that Wnt signaling is an important regulator of mammary stem cell self-renewal. These cells retain their full developmental potential, generating mature and functional mammary glands when transplanted in vivo. We also found that stem cells that are intrinsically more sensitive to Wnt signaling have a competitive advantage to populate the mammary gland in transplantation assays. Our data directly show that Wnts are important niche signals influencing normal mammary stem cell self-renewal. We also demonstrate, for the first time, that these adult stem cells can be clonally expanded in cell culture for several generations, with full retention of their competence to regenerate a functional organ.

No: 128

Derivation of functionally polarized retinal pigment epithelial cells from human embryonic stem cells

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The retinal pigment epithelium (RPE) is required for support and maintenance of the outer neural retina including photoreceptor cells. Dysfunction and/or death of RPE plays a critical role in the pathogenesis of at least two major groups of blinding diseases: age-related macular degeneration (AMD) and some types of retinitis pigmentosa (RP).The emerging strategy of cell replacement therapy provides a new approach for the treatment of AMD and RP. Although RPE cells derived from human embryonic stem cells (hES-RPE) are a potentially unlimited resource for this medical therapy, the production of clinically usable amount of pure and functionally competent hES-RPE cells remains a major technical challenge at present. Based on the fact that RPE cells derive from neural ectoderm and share common characteristics with neural retinal cells or neurons, we experimented with a novel two-stage induction procedure to produce functionally polarized hES-RPE cell monolayers from hES-3 cell line in vitro, and showed that this two-stage approach was technically feasible and more efficient for the production of hES-derived RPE. The hES-3 cell aggregates were first kept in suspension culture in neural differentiation medium supplemented with DKK-1, Noggin and IGF to undergo stage-one induction into neural precursors. The neural precursors were then transferred to RPE differentiation medium for further differentiation into putative RPE-like cells. After these stages of induction and differentiation, the highly pigmented hexagonal RPE-like cell colonies were mechanically picked, trypsinized and subcultured on gelatin- or laminin-coated plates, or on laminin-coated transwells to form polarized RPE cell sheets. The putative pigmented hexagonal RPE cells appeared as early as 4 weeks in our culture conditions and reached high enough cell numbers for subculture at about 8 weeks. The hES-RPE cells could be subcultured for several passages and kept their pigmented hexagonal morphology. RT-PCR and Western blot analysis confirmed that these hES-RPE cells expressed high levels of RPE-specific gene products including RPE65, VMD2, PEDF and CRELBP. The hES-RPE cells were highly polarized, with the formation of tight junctions revealed by immuno-fluorescent staining of ZO-1 protein, and the formation of microvilli on cell surfaces shown by scanning electron microscopy. Phagocytosis analysis using FITC-labeled rod outer segments (ROS) demonstrated positive phagosomes in hES-RPE cells, indicating that these polarized hES-RPE cells had similar phagocytotic function as RPE cells found in vivo. Normal RPE cells form a quiescent, polarized epithelial monolayer with distinct apical/basal characteristics that are essential for their physiologic functions. The proper orientation of hES-derived RPE cells before transplantation is considered to be one of the critical factors for a successful graft. In this study, we successfully induced the differentiation of hES-3 cells into large numbers of functionally polarized hES-RPE cells that are suitable for subretinal transplantation experiments in animal models. We believe that the direct graft of a sheet of an in vitro differentiated and polarized hES-RPE cell monolayer will provide the recipients with a replacement RPE layer that is morphologically and physiologically similar to normal RPE in vivo, and is more likely to exhibit normal function and survive longer after transplantation.

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Fate tracing reveals the endothelial origin of hematopoietic stem cells

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Hematopoietic stem cells (HSCs) originate within the aortic-gonado-mesonephros (AGM) region of the midgestation embryo, but the cell type responsible for their emergence is unknown since critical hematopoietic factors are expressed in both the AGM endothelium and its underlying mesenchyme. Here we employ a temporally restricted genetic tracing strategy to selectively label the endothelium, and separately its underlying mesenchyme, during AGM development. Lineage tracing endothelium, via an inducible VE-cadherin Cre line, reveals that the endothelium is capable of HSC emergence. The endothelial progeny migrate to the fetal liver, and later to the bone marrow, are capable of expansion, self-renewal, and multi-lineage hematopoietic differentiation. HSC capacity is exclusively endothelial as ex vivo analyses demonstrate lack of VE-cadherin Cre induction in circulating and fetal liver hematopoietic populations. Moreover, AGM mesenchyme, as selectively traced via a myocardin Cre line, is incapable of hematopoiesis. Our genetic tracing strategy therefore reveals an endothelial origin of HSCs.

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