2007 ANNUAL CIRM SCHOLARS MEETING

CALIFORNIA INSTITUTE FOR

CIRM

REGENERATIVE MEDICINE

Annual CIRM Scholars Meeting

The California Institute for Regenerative Medicine (CIRM) is pleased to hold the first Annual CIRM Scholars Meeting that brings together CIRM Scholars, mentors, and program directors from each of the 16 training institutions. The purpose of this meeting is to offer CIRM Scholars the opportunity to present their preliminary data, engage in scientific discussion and exchange ideas. This is also an occasion to meet with peers and mentors that share similar interests in stem cell research as well as to discover what others are working on. For the CIRM, it is an opportunity to see first hand the product of our first Request for Applications (RFA) that funded the training grant awards.

For the convenience of attendees and to minimize travel, two meetings were planned. Northern California training programs from The Gladstone Institutes, Stanford University, UC Berkeley, UC Davis, UC Santa Cruz, and UC San Francisco will meet in San Francisco at the UCSF Mission Bay Campus. Southern California programs from the Burnham Institute, California Institute of Technology, Children's Hospital of Los Angeles, the Salk Institute, Scripps Research Institute, UC Irvine, UC Los Angeles, UC San Diego, UC Santa Barbara and the University of Southern California will meet in Irvine at the University Club of the UC Irvine campus.

Northern California Training Programs

Mission Bay Campus, University of California, San Francisco
Tuesday September 11, 2007
8:00am to 5:00pm

Southern California Training Programs

University Club, University of California, Irvine
Friday, September 28, 2007
8:30am to 5:30pm

Attendance is limited to program directors, CIRM Scholars, and their mentors to promote community-building among the developing scientists and to encourage the presentation and frank discussion of preliminary, unpublished findings. Each meeting will include 3 basic elements.

1. Short oral presentations of their research given by one CIRM Scholar selected from each institution. Program directors have selected one trainee from their institution to make a presentation.
2. A two-hour poster session will allow CIRM Scholars to display and discuss their research with others on a one-on-one basis. A total of 115 abstracts for posters and oral presentations have been submitted for both meetings.
3. CIRM Scholars will lead and participate in discussion groups on selected topics of interest such as Stem Cell Self-Renewal and Pluripotency, Translational Challenges, and Career Transitions.

About CIRM

Governed by the Independent Citizens Oversight Committee (ICOC), the California Institute for Regenerative Medicine (CIRM) was established in 2004 with the passage of Proposition 71, the California Stem Cell Research and Cures Initiative. The statewide ballot measure, which provided $3 billion in funding for stem cell research at California universities and research institutions, was approved by California voters, and called for the establishment of an entity to make grants and provide loans for stem cell research, research facilities, and other vital research opportunities. The CIRM is the largest source of funding for human embryonic stem cell research in the world. To date, grants totaling more than $208.5 million have been approved by the ICOC. For more information, please contact us at info@cirm.ca.gov or visit our website at www.cirm.ca.gov.

Table of Contents

CIRM TRAINING PROGRAM

The CIRM Training Program was developed to train a cadre of basic and clinical scientists who will contribute to the expansion of stem cell research in California. The growth of stem cell research in California will require the scientific training of individuals at all levels to provide a continuing supply of well-trained scientists with the knowledge and skill to lead effective research programs.

In April of 2006, CIRM awarded three-year training grants to 16 California non-profit, academic institutions to foster training of pre-doctoral students, post-doctoral fellows and clinical fellows. Each grantee institution brings unique strengths and areas of specialization in their stem cell research programs. Accordingly, the level of training offered by each institution differs. Three types of training programs were available to accommodate the varied institutional capabilities.

Type I - Comprehensive Training Programs: Comprehensive programs offer training at all three educational levels: pre-doctoral, post-doctoral and clinical. Each institutional grant may support up to 16 trainees, with a total (direct and indirect) cost/budget of up to $1.25 M per year. Type I grantees include universities with medical schools that have large stem cell research programs that span basic to clinical research and well-established programs of graduate training.

Type II - Intermediate Training Programs: Intermediate programs offer training at two of the three levels of education (e.g., pre-doctoral and post-doctoral; post-doctoral and clinical; or pre-doctoral and clinical). Each award may support up to 10 trainees, with a total (direct and indirect costs) budget of up to $800,000 per year. Type II grantees include institutions that may have less extensive stem cell research programs than Type I programs, but with strong training opportunities.

Type III - Specialized Training Programs: Specialized programs offer training at one or two levels of education. Each grant may support up to 6 trainees, with a total (direct and indirect costs) budget of no more than $500,000 per year. Grantees include smaller institutions with relatively small but developing stem cell research programs.

All training programs, regardless of type, offer one or more classes in stem cell biology and its application to health and disease, and also a course in the social, legal and ethical implications of stem cell research. Moreover, all programs offer opportunities for laboratory work under the direction of a mentor in stem cell biology or clinical training that is closely related to stem cell research. During the first year, approximately 160 trainee appointments were made under the CIRM Training Program. These trainees are engaged in research training in a variety of fields including developmental biology, cell biology, neurobiology, molecular biology, cardiology, bioengineering, as well as ethics and law. The selection of CIRM Scholars has become quite competitive among the different institutional programs, which suggests that our goal to educate and prepare exceptional trainees for careers in stem cell research is being realized through this initiative.

2007 Annual CIRM Scholars Meeting
Mission Bay Conference Center
University of California, San Francisco
San Francisco, CA

September 11, 2007

NORTHERN CALIFORNIA SPEAKERS

Christopher Arnold
Stanford University
Title*: MiRNA Mediated Stem Cell Self-Renewal*

Megan Hall
University of California, Santa Cruz
Title*: Regulated Alternative Splicing During Stem Cell Differentiation*

Kathy Ivey
The J. Gladstone Institutes
Title*: MicroRNA Regulation of Cell Lineages in Mouse and Human Embryonic Stem Cells*

Gregory Potter
University of California, San Francisco
Title*: D1x1 and D1x2 Control Neuron/Glia Fate Acquisition in the Developing Forebrain*

Sue Sohn
University of California, Berkeley
Title*: Coaxing Hematopoietic and Lymphoid Potential out of hESCs*

Basha Stankovich
University of California, Merced
Title*: Adhesion Molecule Expression in Early Hematopoietic Commitment of Mouse Embryonic Stem Cells*

2007 Annual CIRM Scholars Meeting
University Club
University of California, Irvine
Irvine, California

September 28, 2007

SOUTHERN CALIFORNIA SPEAKERS

Anthony Boitano
Scripps Research Institute
Title: *Human Hematopoietic Stem Cell Screens*

Weiwei Fan
University of California, Irvine
Title*: Introduction of a Joint Missense and Frameshift Mutation into the Mouse Germline Resulted in the Loss of the Frameshift Mutation and Created the First Mouse Model of Cardiomyopathy Caused by a MtDNA Mutation*

Jeff Lindquist
Burnham Institute
Title*: Selective Patterning of Peripheral Neurons with the Vasculature During Stem Cell Differentiation*

Agnes Lukaszewicz
California Institute of Technology
Title*: Control of Neural Stem-To-Progenitor Transitions by Cyclin D Family Members in ES Cells*

Todd MacFarlan
The Salk Institute for Biological Studies
Title*: Modeling Motor Neuron Development with Stem Cells*

Laura Perin
Childrens Hospital Los Angeles
Title*: Amniotic Fluid Stem Cells and their Application for Kidney Regeneration*

JingJing Sun
University of Southern California
Title*: Msx Genes Function Downstream of BMP Signaling Pathway in Regulating the Production and Migration of Primordial Germ Cells*

Hao Wu
University of California, Los Angeles
Title*: Integrative Genomic and Functional Analyses Reveal Neuronal Subtype Differentiation Bias in Human Embryonic Stem Cell Lines*

Na Xu
University of California, Santa Barbara
Title*: Human Embryonic Stem Cells: From Epigenetics to MicroRNAs*

Samantha Zeitlin
University of California, San Diego
Title*: Role of ATM-Directed Chromatin Remodeling During Neural Lineage Differentiation*

Northern California Meeting Attendees
Mission Bay Conference Center
University of California, San Francisco
September 11, 2007

Angeli, Franca	Mentor	University of California, San Francisco
Ares, Manny	Mentor	University of California, Santa Cruz
Arnold, Christopher	CIRM Scholar	Stanford University
Batchelder, Cynthia	CIRM Scholar	University of California, Davis
Benjamin, Ruha	CIRM Scholar	University of California, Berkeley
Blelloch, Robert	Mentor	University of California, San Francisco
Bruneau, Benoit	Mentor	J. David Gladstone Institutes
Cano, David	CIRM Scholar	University of California, San Francisco
Chen, James	Mentor	Stanford University
Cheyette, Benjamin	Mentor	University of California, San Francisco
Chung, Won-Suk	CIRM Scholar	University of California, San Francisco
Conklin, Bruce	Mentor	J. David Gladstone Institutes
Cord, Branden	CIRM Scholar	Stanford University
Dadi, Tedla	CIRM Scholar	University of California, Davis
Deane, S.	CIRM Scholar	University of California, Davis
Delgado, Paul	CIRM Scholar	J. David Gladstone Institutes
Dhahbi, Joseph	CIRM Scholar	University of California, Berkeley
Dorighi, Kristel	CIRM Scholar	University of California, Santa Cruz
Feldheim, David	Mentor	University of California, Santa Cruz
Fisher, Susan	Program Director	University of California, San Francisco
Fong, Yick	CIRM Scholar	University of California, Berkeley
Fu, Jidong	CIRM Scholar	University of California, Davis
Fuller, Margaret	Mentor	Stanford University
Gaur, Meenakshi	CIRM Scholar	University of California, San Francisco
Gip, Phung	CIRM Scholar	University of California, Berkeley
Giudice, Linda	Mentor	University of California, San Francisco
Grskovic, Marica	CIRM Scholar	University of California, San Francisco
Gupta, Naveen	CIRM Scholar	University of California, San Francisco
Hall, Megan	CIRM Scholar	University of California, Santa Cruz
Haussler, David	Program Director	University of California, Santa Cruz
Hebrok, Matthias	Mentor	University of California, San Francisco
Hsiao, Edward	CIRM Scholar	University of California, San Francisco

Northern California Meeting Attendees
Mission Bay Conference Center
University of California, San Francisco
September 11, 2007

Insco, Megan	CIRM Scholar	Stanford University
Ivey, Kathryn	CIRM Scholar	J. David Gladstone Institutes
Jang, Jae-Hyung	CIRM Scholar	University of California, Berkeley
Kareta, Michael	CIRM Scholar	University of California, Davis
Koeva, Martina	CIRM Scholar	University of California, Santa Cruz
Kogan, Scott	Mentor	University of California, San Francisco
Kosinski, Cindy	CIRM Scholar	University of California, San Francisco
Kumar, Atul	CIRM Scholar	Stanford University
Lee, Jin-A	CIRM Scholar	J. David Gladstone Institutes
Li, Gang	CIRM Scholar	J. David Gladstone Institutes
Li, Ronald	Mentor	University of California, Davis
Liu, Cheng	CIRM Scholar	University of California, San Francisco
Liu, Jennifer	CIRM Scholar	University of California, Berkeley
Lloyd, Kent	Mentor	University of California, Davis
Longaker, Michael	Program Director	Stanford University
Ma, Joyce	CIRM Scholar	University of California, Davis
Maltepe, Emin	CIRM Scholar	University of California, San Francisco
Martin, David	Mentor	University of California, Berkeley
Masaki, Kinuko	CIRM Scholar	Stanford University
Matthay, Michael	Mentor	University of California, San Francisco
Melichar, Heather	CIRM Scholar	University of California, Berkeley
Meyers, Frederick	Program Director	University of California, Davis
Mich, John	CIRM Scholar	Stanford University
Mizokami, Myra	CIRM Scholar	University of California, Berkeley
Onodera, Courtney	CIRM Scholar	University of California, Santa Cruz
Ootani, Akifumi	CIRM Scholar	Stanford University
Oshima, Kazuo	Mentor	Stanford University
Peltier, Joe	CIRM Scholar	University of California, Berkeley
Potter, Gregory	CIRM Scholar	University of California, San Francisco
Reijo Pera, Renee	Program Director	Stanford University
Rosen, Galit	CIRM Scholar	University of California, San Francisco

Northern California Meeting Attendees
Mission Bay Conference Center
University of California, San Francisco
September 11, 2007

Royou, Anne	CIRM Scholar	University of California, Santa Cruz
Rubenstein, John	Mentor	University of California, San Francisco
Saunders, Laura	CIRM Scholar	J. David Gladstone Institutes
Schaffer, David	Mentor	University of California, Berkeley
Scott, Matthew	Mentor	Stanford University
Shih, Laura	CIRM Scholar	University of California, Davis
Sohn, Sue	CIRM Scholar	University of California, Berkeley
Southwell, Derek	CIRM Scholar	University of California, San Francisco
Stankovich, Basha	CIRM Scholar	University of California, Davis/Merced
Stuart, Joshua	Mentor	University of California, Santa Cruz
Sullivan, William	Mentor	University of California, Santa Cruz
Suriben, Rowena	CIRM Scholar	University of California, San Francisco
Tarantal, Alice	Mentor	University of California, Davis
Thompson, Charis	Mentor	University of California, Berkeley
Vaidyanathan, Hema	CIRM Scholar	University of California, Santa Cruz
Verdin, Eric	Mentor	J. David Gladstone Institutes
Wang, Liping	CIRM Scholar	Stanford University
Wang, Yangming	CIRM Scholar	University of California, San Francisco
Wei, Dongguang	CIRM Scholar	University of California, Davis
Woo, Wei-Meng	CIRM Scholar	Stanford University
Wright, Alyssa	CIRM Scholar	Stanford University
Wu, Reen	Mentor	University of California, Davis
Xin, Chen	Mentor	University of California, San Francisco
Yamoah, Ebenezer	Mentor	University of California, Davis

Southern California Meeting Attendees
The University Club
University of California, Irvine
September 28, 2007

Anderson, Aileen	Mentor	University of California,	Irvine
Bellusci, Saverio	Mentor	Children's Hospital of LA	
Blanchi, Bruno	CIRM Scholar	University of California,	Los Angeles
Blurton-Jones, Mathew	CIRM Scholar	University of California,	Irvine
Boitano, Anthony	CIRM Scholar	Scripps Research Institute	
Braswell, Jennifer	Mentor	University of California,	San Diego
Bryant, Peter	Program Director	University of California,	Irvine
Buchholz, David	CIRM Scholar	University of California,	Santa Barbara
Chang, David	CIRM Scholar	Children's Hospital of LA	
Clegg, Dennis	Program Director	University of California,	Santa Barbara
Crooks, Gay	Mentor	Children's Hospital of LA	
Dao, Kim-Hien	CIRM Scholar	University of California,	San Diego
De Filippo, Roger	Mentor	Children's Hospital of LA	
Demeterco-Berggren, Carla	CIRM Scholar	University of California,	San Diego
Deverman, Ben	CIRM Scholar	California Institute of Technology	
Ding, Sheng	Mentor	Scripps Research Institute	
Djabrayan, Nareg	CIRM Scholar	University of California,	Santa Barbara
Donovan, Peter	Mentor	University of California,	Irvine
Dorrell, Michael	CIRM Scholar	Scripps Research Institute	
Dorshkind, Ken	Program Director	University of California,	Los Angeles
Douglas, Dorothea	CIRM Scholar	Children's Hospital of LA	
Dravid, Gautam	CIRM Scholar	Children's Hospital of LA	
Dubois, Claire	CIRM Scholar	University of California,	Irvine
Earthman, James	Mentor	University of California,	Irvine
Fan, Guoping	Mentor	University of California,	Los Angeles
Fan, Weiwei	CIRM Scholar	University of California,	Irvine
Fong, Helen	CIRM Scholar	University of California,	Irvine
Frank, Matthew	CIRM Scholar	University of California,	Los Angeles
Freude, Kristine	CIRM Scholar	University of California,	Irvine
Gleason, Darius	CIRM Scholar	University of California,	Irvine
Goldstein, Larry	Program Director	University of California,	San Diego
Golub, Sidney	Mentor	University of California,	Irvine
Guan, Zhibin	Mentor	University of California,	Irvine
Guangshuo, Ou	CIRM Scholar	University of California,	San Francisco
Gulsen, Gultekin	Mentor	University of California,	Irvine
Guo, Wei	CIRM Scholar	University of California,	Los Angeles
Havton, Leif A.	Mentor	University of California,	Los Angeles
Hikita, Sherry	Mentor	University of California,	Santa Barbara
Hochgreb, Tatiana	CIRM Scholar	California Institute of Technology	

Southern California Meeting Attendees
The University Club
University of California, Irvine
September 28, 2007

Hong, Young	Mentor	University of Southern California
Hsu, H.R. Jessie	CIRM Scholar	University of Southern California
Hu, Cory	CIRM Scholar	California Institute of Technology
Ichiro, Nakano	CIRM Scholar	University of California, Los Angeles
Itkin-Ansari, Pamela	Mentor	University of California, San Diego
Jaksch, Marie	CIRM Scholar	Burnham Institute
Jiang, Xiaohua	CIRM Scholar	Children's Hospital of LA
Kavalerchik, Edward	CIRM Scholar	University of California, San Diego
Kearns, Mary	Mentor	Children's Hospital of LA
Kim, Youngjun	CIRM Scholar	University of California, San Diego
Kohn, Donald	Program Director	Children's Hospital of LA
Kye, Min-Jeong	CIRM Scholar	University of California, Santa Barbara
LaFerla, Frank	Mentor	University of California, Irvine
Lane, Thomas	Mentor	University of California, Irvine
Lawlor, Elizabeth	Mentor	Children's Hospital of LA
Lee, Eva	Mentor	University of California, Irvine
Lee, Seung-Hee	CIRM Scholar	Burnham Institute
Lee, Sunju	CIRM Scholar	University of Southern California
Leung, Joanne	CIRM Scholar	University of California, Los Angeles
Li, Chihui	CIRM Scholar	University of California, Irvine
Li, Long	CIRM Scholar	California Institute of Technology
Liao, Chun-Peng	CIRM Scholar	University of Southern California
Liao, Sophia	CIRM Scholar	University of California, Irvine
Lin, Yuting	CIRM Scholar	University of California, Irvine
Lindquist, Jeff	CIRM Scholar	Burnham Institute
Lo, Frederick	CIRM Scholar	University of California, San Diego
Lo, Roger	CIRM Scholar	University of California, Los Angeles
Lock, Leslie	Mentor	University of California, Irvine
Lou, Huiqiang	CIRM Scholar	California Institute of Technology
Lu, Haihui	CIRM Scholar	University of Southern California
Lukaszewicz, Agnes	CIRM Scholar	California Institute of Technology
Lutzko, Carolyn	Mentor	Children's Hospital of LA
MacFarlan, Todd	CIRM Scholar	Salk Institute
Maxson, Robert	Program Director	University of Southern California
Mishra, Suparna	CIRM Scholar	University of Southern California
Mueller, Ulrich	Mentor	Scripps Research Institute
Müschen, Markus	Mentor	Children's Hospital of LA
Nolan, Rhiannon	CIRM Scholar	University of California, San Diego
O'Brien, Robert	CIRM Scholar	University of California, San Diego

Southern California Meeting Attendees
The University Club
University of California, Irvine
September 28, 2007

Oh, Seunghan	CIRM Scholar	University of California, San Diego
Ohlsson, Marcus	CIRM Scholar	University of California, Los Angeles
Ordonez, Maria	CIRM Scholar	University of California, San Diego
Pais, Eszter	CIRM Scholar	University of Southern California
Pallavicini, Maria	Mentor	University of California, Merced
Pang, Yuhong	CIRM Scholar	Burnham Institute
Pao, Gerald	CIRM Scholar	Salk Institute
Patel, Jigar	CIRM Scholar	University of California, San Diego
Patterson, Paul	Program Director	California Institute of Technology
Perin, Laura	CIRM Scholar	Children's Hospital of LA
Radakovits, Randor	CIRM Scholar	Scripps Research Institute
Rossi, Sharyn	CIRM Scholar	University of California, Irvine
Rothenberg, Ellen	Mentor	California Institute of Technology
Ruby, Katherine	CIRM Scholar	University of California, San Diego
Sadeghi, Angizeh	CIRM Scholar	University of Southern California
Sakurai, Ken	CIRM Scholar	Children's Hospital of LA
Salazar, Desiree	CIRM Scholar	University of California, Irvine
Sander, Maike	Mentor	University of California, Irvine
Sauka-Spengler, Tatjana	CIRM Scholar	California Institute of Technology
Schaumburg, Chris	CIRM Scholar	University of California, Irvine
Scolnick, Jonathan	CIRM Scholar	Salk Institute
Shauna, Yuan	CIRM Scholar	University of California, San Diego
Shen, Yin	CIRM Scholar	University of California, Los Angeles
Signer, Robert	CIRM Scholar	University of California, Los Angeles
Singer, Oded	CIRM Scholar	Salk Institute
Smith, Joel	CIRM Scholar	California Institute of Technology
Subramanian, Aparna	CIRM Scholar	University of California, Los Angeles
Sun, Jingjing	CIRM Scholar	University of Southern California
Sun, Yi	Mentor	University of California, Los Angeles
Tai, Cindy	CIRM Scholar	Children's Hospital of LA
Tsai, Steven	CIRM Scholar	University of Southern California
Tuszynski, Mark	Mentor	University of California, San Diego
Voog, Justin	CIRM Scholar	University of California, San Diego
Wallace, Douglas	Mentor	University of California, Irvine
Wang, Miao	CIRM Scholar	University of Southern California
Wu, Hao	CIRM Scholar	University of California, Los Angeles
Xu, Na	CIRM Scholar	University of California, Santa Barbara
Yue, Zhicao	CIRM Scholar	University of California, Los Angeles
Zeitlin, Samantha	CIRM Scholar	University of California, San Diego
Zovein, Ann	CIRM Scholar	University of California, Los Angeles

ABSTRACTS
NORTHERN CALIFORNIA

STANFORD UNIVERSITY
THE J. DAVID GLADSTONE INSTITUTES
UNIVERSITY OF CALIFORNIA, BERKELEY
UNIVERSITY OF CALIFORNIA, DAVIS/MERCED
UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
UNIVERSITY OF CALIFORNIA, SANTA CRUZ

STANFORD UNIVERSITY N 001

CIRM SCHOLAR: CHRISTOPHER ARNOLD

TITLE: **MiRNA mediated stem cell self-renewal**

AUTHORS: **Christopher Arnold and Chang-Zheng Chen**

MiRNAs are ~22-nt small regulatory RNAs that can repress translation and initiate transcript degradation via imperfect base pairing with cognate target mRNAs. They represent at least 1-5% of the predicted genes in worms, flies, mice, and humans. Previous research has already shown that these small non-coding RNAs have a role in cellular processes such as proliferation, morphogenesis, apoptosis, and differentiation. Intriguingly, several lines of evidence have implicated miRNAs in the developmental regulatory decisions of stem cell maintenance and differentiation in flies and mice. Since the miRNA pathway has been shown to control stem cell division and the kinetics of lineage differentiation in several species, I hypothesize that miRNAs play a conserved role in the quantitative control of the number of cell divisions of the self-renewing stem cell and the transit-amplifying cells. To this end we have constructed miRNA libraries from several adult stem cell populations including rat and mouse neural stem cells, and mouse long-term hematopoietic stem cells (LT-HSCs), short term HSCs (ST-HSCs), common myeloid progenitor cells (CMPs), and common lymphoid progenitor cells (CLPs). Furthermore, we have also conducted QPCR profiling of various mouse stem cell populations including both PTEN mutant and wildtype LT-HSCs, muscle satellite/stem cells, and neural stem cells. Preliminary sequencing of these stem cell miRNA libraries in addition to QPCR profile analysis has already uncovered several miRNAs that are shared by multiple adult stem cell populations, and appear to be involved in cell cycle regulation and self-renewal decisions. To explore the roles of these candidate stem cell miRNAs we have setup cell culture assays to investigate their capacity to influence embryonic stem cell proliferation and self-renewal.

STANFORD UNIVERSITY N 002

CIRM SCHOLAR: BRANDEN CORD

TITLE: **Guiding movement in Parkinson's Disease, human embryonic stem cell derived dopamine neurons can respond to exogenous axon guidance cues**

AUTHORS: **Branden J. Cord, Jie Li, Susan K. McConnell, Theo Palmer and Mary A. Hynes**

Embryonic stem cell technologies are optimally positioned to provide a reproducibly consistent, customizable, high-quality source of material for cell replacement therapies. Neurodegenerative diseases, which affect a large portion of the population, are characterized by the loss of very specific populations of neurons. Victims of Parkinson's Disease (PD) suffer from debilitating tremors and have difficulty in initiating even basic movements because of the loss of a very defined population of dopaminergic (DA) neurons located in the substantia nigra. These cells make up the nigrostriatal circuit which begins in the midbrain, and sends projections to innervate the striatum located in the forebrain. Previous clinical trials in which fetal midbrain was injected directly into the striatum of PD patients failed to show any therapeutic benefits, likely related to the variable quality of tissue source, a lack of cell survival, and improper integration of neurons into the brain. Ideally, therapeutic transplants would fully recapitulate the nigrostriatal circuit by injected ES-derived DA neurons into the substantia nigra, and guiding there axons to grow out and innervate their correct targets in the striatum.

During development, the outgrowth and targeting of axons and dendrites in the CNS are guided by distinct families of guidance molecules. Previous work has established the role of netrin and slit in guiding the axonal outgrowth of DA neurons from primary explant cultures. However, the ability of ES-derived DA neurons to respond in a similar fashion has been questioned. Here we demonstrate that human ES-derived DA neuron cultures are attracted to sources of netrin-1 and repelled by sources of slit-2, as predicted by similar results using primary rat midbrain explants. Additionally, the magnitude of this response varied depending on when in the differentiation protocol human ES-derived DA neurons were assayed, suggesting that implanting cells at 42 days in-vitro may provide the greatest opportunity for persuading neurons to integrate into the brain correctly. This model provides more evidence that ES-derived DA neurons accurately reflect midbrain DA neurons found in vivo, and should serve to accelerate the discovery of other axon guidance factors involved in DA neuron development. Furthermore, this model will be essential to predict how neuroinflammatory factors, likely to be present as a result of disease and transplant related damage, will affect the ability of these cells to wire correctly into the nigrostriatal circuit in a clinical setting, and what strategies must be employed to provide the best therapeutic outcome.

STANFORD UNIVERSITY N 003

CIRM SCHOLAR: MEGAN INSCO

TITLE: **Investigating the regulation of transit amplifying divisions and the switch to terminal differentiation in cells descending from an adult stem cell lineage**

AUTHORS: **Megan Insco and Margaret Fuller**

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 undergo terminal differentiation 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 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 involved in translational control.

STANFORD UNIVERSITY N 004

CIRM SCHOLAR: KINUKO MASAKI

TITLE: **Initial steps toward differentiating hESCs into inner ear cell types**

AUTHORS: **Kinuko Masaki, Veronika Starlinger and Stefan Heller**

Debilitating hearing loss is estimated to affect more than 250 million people worldwide. Degeneration and death of cochlear hair cells and their associated spiral ganglion neurons is causal in >80% of individuals with hearing loss. In mammals, cochlear hair cells cannot be replaced by natural regeneration. Consequently, the functional replacement of hair cells represents the ultimate treatment modality for deafness. Different strategies have been used to stimulate regeneration of hair cells in the adult mammalian cochlea. These approaches include treatment of the damaged cochlea with various growth factors and transfer of key developmental genes into cochlear non-sensory cells. Nevertheless, none of these strategies have led to efficient cochlear hair cell regeneration in animal models of hearing loss.

Recently, stem cells have been introduced as an alternative approach for regenerating cochlear hair cells in vitro and in vivo. A major advance in this quest came with the discovery of a selection/induction protocol to generate murine hair cells. This protocol led to proliferating inner ear progenitor cells that were capable of differentiating into hair cells in vitro and after transplantation into the developing inner ear.

The goal of my project is to develop a stepwise guidance protocol for differentiating human embryonic stem cells (hESCs) into human inner ear progenitor cell populations. I plan to use these progenitor cells for cell grafting experiments into the murine or chicken cochlea.

In this poster, I am presenting preliminary results. First, I will compare two methods of differentiating hESCs into primitive ectoderm. This method involves using a conditioned media from HepG2 cells called MedII, which has been shown to differentiate mouse ESCs into primitive ectoderm. The alternative method is using combinations of different growth factors to promote primitive ectoderm differentiation. I will further test whether the use of primitive ectoderm provides an advantage over using embryoid bodies as starting cell population for guidance toward inner ear progenitors. In a second series of experiments, I am developing assays for testing the competence of ESC-derived cell populations to respond to otic placode-inducing signals. One preliminary result of these experiments is that upregulation of early inner ear markers in human cells happens within 48-72 hours, which is not considerably slower than otic induction using murine cells. The results presented in this poster are the initial steps toward establishing a robust stepwise protocol to generate inner ear cell types from hESCs.

STANFORD UNIVERSITY N 005

CIRM SCHOLAR: JOHN MICH

TITLE: **Role for Hedgehog signaling components in vertebrate germ cell migration**

AUTHORS: **John K. Mich and James K. Chen**

Many progenitor cell types undergo active migration to find their proper locations where they can give rise to the tissues of the body. This migration is not only essential to normal organogenesis, but can cause cancer metastasis when dysregulated. To more fully understand the signaling mechanisms that govern this cell migration, we are investigating a possible role for Hedgehog signaling components in the migration of primordial germ cells in the zebrafish embryo, an easily observed and manipulated in vivo cell migration system. We have found that treatment with cyclopamine, a Hedgehog antagonist, and misexpression of Hedgehog signaling components can lead to defects in cell migration. To confirm these findings, we are creating a genetic deficiency for Hedgehog signaling in the zebrafish to assay cell migration.

STANFORD UNIVERSITY N 006

CIRM SCHOLAR: LIPING WANG

TITLE: **Channelrhodopsin-2: a temporally precise genetic tool for the optical control of neuronal activity in intact brain tissue**

AUTHORS: **Li-Ping Wang, Feng Zhang, and Karl Deisseroth**

Channelrhodopsin-2 (ChR2), a light-activated cation channel from Chlamydomonas reinhardtii, has been shown to provide for reliable optical control of neuronal activity on the millisecond timescale. We have developed high titer lentiviral vector methodology to achieve strong, widespread expression and functional activity of ChR2 in the dentate gyrus (DG) of the rodent hippocampal formation in vivo, without application of any exogenous cofactors. All-trans retinal did not need to be added or supplemented at any point in these intact slice experiments. Whole-cell recordings in acute brain slices showed that illumination using 470 nm blue light evoked rapid inward currents and drove reliable action potential trains (up to 50 Hz) in ChR2- expressing cells. Light-evoked excitatory synaptic transmission also could be elicited readily. To integrate optical stimulation and imaging together in the same experiment in intact brain tissue, we delivered the calcium-indicator dye Fura-2 into the ChR2-expressing hippocampus in vivo, and prepared acute brain slices to image light-triggered neuronal activity assayed by imaging calcium transients. This complete system for the noninvasive modulation and assaying of neural activity in intact brain tissue provides a reliable, high-speed, optical technology for interrogating mammalian neural circuits.

STANFORD UNIVERSITY N 007

CIRM SCHOLAR: WEI-MENG WOO

TITLE: **Hedgehog controls hair follicle regeneration through a novel dermal signaling pathway**

AUTHORS: **Wei-Meng Woo, John Van Arnam, Hanson H. Zhen, and Anthony E. Oro**

To understand the set of inductive signals stem cells receive from and deliver to the surrounding stroma during adult organ regeneration, we have developed a rapid, genetic system using hair follicle (HF) regeneration as a model. Like other organs, HF regeneration requires precise reciprocal signaling between HF epithelial stem cells and the adjacent mesenchymal dermal papilla (DP) cells. Purified HF epithelial stem cells can regenerate all cell types of the HF mini-organ and the skin epidermis on a wound only when placed together with functional DP cells, suggesting DP cells provide specific stromal factor(s) necessary to induce stem cellls to undergo organ regeneration.

Although we know a number of signaling molecules play roles in the reciprocal interaction, the mechanisms of signaling cross talk between HF epithelial stem cells and DP cells remain poorly understood. While gene functional studies have been limited by the speed of generating genetic mouse models, using a rapid, genetically tractable hair regeneration system to perform gene knockdown with lentiviral based small hairpin RNA in either the epithelial or DP cell types, can speed up gene functional analysis to within a couple of months.

One of the key inductive signals in HF regeneration is Sonic hedgehog (Shh), which is normally expressed only in the epithelial compartment and required for HF morphogenesis. While both HF epithelial and DP compartments express Shh target genes, previous studies have supported a role for Shh only on epithelial cells, in which aberrant Shh target gene induction is sufficient to drive epithelial progenitor cell proliferation and results in basal cell carcinoma. To better understand a potential role for Shh in the DP cell type, we use the rapid hair regeneration system to knockdown the critical Shh signal transducer Smoothened (Smo) in the DP cell pupulation. We showed that sustained knockdown of Smo in the DP cell type resulted in greater than 90% reduction in Shh target gene induction. Hair regeneration with Smo knockdown DP cells and wild type epithelial cells resulted in dramatic reduction in HF number and length. While growth delayed, these remnant mutant HFs displayed normal HF differentiation markers, suggesting Smo function in the DP cells may be to regulate the proliferation of the HF epithelial progenitors and thus affecting HF growth but not differentiation. Smo mutant DP cells expressed reduced levels of a subset of DP markers, suggesting Smo may also regulate some aspects of DP differentiation. We conclude that Shh controls epithelial HF progenitor proliferation indirectly through a novel DP signaling pathway, in addition to a direct Shh signaling within the HF progenitors.

STANFORD UNIVERSITY N 008

CIRM SCHOLAR: ALYSSA WRIGHT

TITLE: **The chromatin remodeling factor Chd1l in embryonic stem cells**

AUTHORS: **Alyssa Wright, Tian Wang, and Matthew Scott**

Embryonic stem cells (ESCs) are esteemed for their therapeutic potential and their ability to generate all the tissue types of an adult organism. They are a fascinating model system to study the many events that occur during embryonic development. Energy-dependent chromatin remodeling provides one explanation for how a cell alters gene expression in order to differentiate. One chromatin remodeling factor, called Chd1l, is highly enriched in the inner cell mass (ICM) of the blastocyst and is also expressed in ESCs. Preliminary data demonstrate that disregulating the expression of Chd1l induces apoptosis in ESCs but not in differentiating cells derived from the same ESCs. Interestingly, disregulation of Chd1l even provides resistance to the transient apoptotic crisis that afflicts ESCs upon in vitro differentiation. Apoptosis is a critical process during early development for organogenesis and preventing excessive growth. Given the proliferative capacity of ESCs and the pluripotency of ESCs and the ICM, additional apoptotic requirements are likely at play in the pre-implantation embryo. Biased apoptosis can also skew differentiation choices toward a particular lineage. An apoptotic process occurring uniquely in undifferentiated ESCs has not been described, and we are interested in exploring whether this death can selectively promote lineage choice.

J. DAVID GLADSTONE INSTITUTES N 009

CIRM SCHOLAR: PAUL DELGADO

TITLE: **Histone methylation in myocardial cell differentiation**

AUTHORS: **Paul Delgado and Benoit Bruneau**

Cell differentiation is regulated by coordinated gene expression, which is controlled by general as well as tissue-specific transcription factors. In order to access their corresponding gene targets, transcription factors need to overcome physical restrictions imposed by compact chromatin structure or repressor complexes which recruitment is favored by the presence of posttranslational modifications in histones. A series of recent studies revealed the genomewide histone methylation pattern in ES cells; they shown that such epigenetic pattern is dynamic under differentiation stimuli, and led to the proposal that tissue-specific genes are repressed in ES cells, becoming remodeled and activated during differentiation. Accordingly, in ES cells, members of the polycomb group of transcriptional repressors contact genes regulating important aspects of cell differentiation and development like Gata, Tbx, and Nkx families, between others. These facts predict a central role of chromatin modifiers like histone methyltransferases and demethylases in cell differentiation and development; however, the function of such factors in specific differentiation programs needs to be addressed. This issue gains relevance if the fact that directed ES cell fate acquisition and differentiation holds promise for future therapeutic protocols, is considered. In this work, we propose a strategy to uncover the histone methylation pattern underlying cardiac cell differentiation. Notch, Wnt and Bmp signaling pathways participate in the establishment of cardiac precursors, which differentiate into beating cardiomyocytes by the coordinated action of cardiac-specific transcription factors, however, the epigenetic processes favoring or impeding their action remain to be explored. To address this issue, we set two main goals. 1) To uncover the histone methylation pattern in ES cells-derived cardiomyocytes, and 2) identify histone modifiers favoring cardiomyocyte differentiation in ES-cells. To reach the first goal we will employ a new technology termed ChIP-seq. First, we will induce chemical ES cell differentiation to obtain cardiomyocytes, from which mononucleosomes will be obtained by MNase digestion. The chromatin will be immunoprecipitated with antibodies recognizing the mono-, di-, and tri-methylated lysines 27 and 4 of histone H3, associated generally with gene repression and activation, respectively. Adaptors will be ligated to the obtained chromatin, which will be further PCR amplified and sequenced. The estimation of the number of sequence tags will reflect the proportion of nucleosomes containing particular histone modifications. The second goal will involve the screen for histone methyltransferases and known, as well as putative, histone demethylases potentially affecting cardiac cells differentiation from ES cells. To this end, we will stably transfect mouse ES cells, expressing EGFP under control of the beta MHC or Nkx2-5 promoter, with vectors expressing histone methyltransferases and demethylases. The stably transfected pools will be induced to differentiation in embryoid bodies. The levels of EGFP expression in differentiating embryoid bodies, as analyzed by quantitative real time PCR, will permit us address the capacity of the tested epigenetic modifiers to activate or repress the cardiogenic program. The identification of potential histone modifiers affecting the cardiogenic cell differentiation in ES cell will open the possibility to explore the epigenetic mechanisms leading to cardiac-specific gene expression, establishment of cardiac progenitor cells and cardiogenesis.

J. DAVID GLADSTONE INSTITUTES N 010

CIRM SCHOLAR: KATHRYN IVEY

TITLE: **MicroRNA regulation of cell lineages in mouse and human embryonic stem cells**

AUTHORS: **Kathryn N. Ivey, Alecia Muth, Ru-Fang Yeh, Yong Zhao, Frank King, Joshua Arnold, Nathan Salomonis, Bruce Conklin, Harold Bernstein, and Deepak Srivastava**

Cell fate decisions of pluripotent embryonic stem (ES) cells are dictated by differential activation and repression of lineage-specific genes. Numerous signaling and transcriptional networks serve to progressively narrow and specify the potency of ES cells. Whether microRNAs, which regulate post-transcriptional events, could be used to manipulate ES cell differentiation has largely been unexplored. Here, we show that two muscle-specific microRNAs, miR-1 and miR-133, are enriched during ES cell-derived cardiomyocyte differentiation and that both can positively affect mesoderm formation but have opposing effects on muscle differentiation. More strikingly, miR-1 and miR-133 are potent repressors of non-muscle gene expression and cell fate during mouse and human ES cell differentiation, even in the presence of the neuroectoderm (retinoic acid) or endoderm (nodal) inducing agents. This repression was also observed in vivo, as hearts of embryonic miR-1-2 knockout mice aberrantly expressed non-mesodermal genes. miR-1 misexpression in ES cells resulted in translational repression of the Notch ligand, Delta-like 1 (Dll-1), and knockdown of Dll-1 in ES cells was sufficient to recapitulate many of the cell fate effects of miR-1. Our results indicate that manipulation of miRNAs may be useful in regulating cell fate decisions from pluripotent ES cells and suggest a role for muscle-specific miRNAs in reinforcement of silencing of non-muscle gene expression during differentiation.

CIRM SCHOLAR: JIN-A LEE

TITLE: **The role of ESCRT-III in neurodegeneration**

AUTHORS: **Jin-A Lee and Fen-Biao Gao**

Frontotemporal dementia (FTD) is a common cause of presenile dementia. FTD differs from Alzheimer's disease (AD) in several aspects, including more rapid neuronal degeneration in FTD (Roberson et al., 2005). FTD is genetically heterogeneous. Dominant mutations in CHMP2B, a subunit of the endosomal sorting complex required for transport (ESCRT)-III, cause FTD3 (Skibinski et al., 2005). However, the molecular mechanisms by which dysfunction of ESCRT-III leads to neurodegeneration are largely unknown. Moreover, therapeutic interventions for FTD3 or other neurodegenerative disorders are still unavailable despite many years of research. Human stem cells hold great promise, both for regenerating damaged neuronal circuits in the brain and for probing the molecular mechanisms of neurodegenerative disease. Snf7 is an evolutionarily conserved key subunit of ESCRT-III in flies and in mice (Sweeney et al., 2006, Lee et al., 2007). Snf7-1 was highly expressed in neuronal stem cells in developing mouse brains, in postmitotic neurons in adult brains, and in human H9 embryonic stem cells. Snf7-1 was predominantly localized in the cytoplasm in these cells but was found in both the nucleus and the cytoplasm in postmitotic neurons. Moreover, in mature cortical neurons, expression of mutant CHMP2B or loss of mSnf7-1 reduced dendritic arborization and caused cell death (Lee et al., 2007). We are trying to establish a human neuronal cell culture model of FTD3 to understand the role of ESCRT-III in stem cell differentiation. These works will provide novel insight into the molecular mechanisms underlying FTD3 and suggest that dysfunction of ESCRT-III in the endosomal-lysosomal pathway causes neurodegeneration in human.

CIRM SCHOLAR: GANG LI

TITLE: **Isoform-specific effect of Apolipoprotein E on adult hippocampal neurogenesis in mice**

AUTHORS: **Gang Li, Qin Xu, Aubrey Bernardo, Walter J. Brecht, Robert W. Mahley, and Yadong Huang**

Apolipoprotein E (apoE) is a major risk factor for Alzheimer's disease (AD). AD patients with apoE4 have greater hippocampal atrophy than those without apoE4, and even normal middle-aged subjects with apoE4 have a smaller hippocampus. To determine if apoE4 causes hippocampal atrophy by impairing hippocampal neurogenesis, we analyzed human apoE3 and apoE4 knock-in (KI) mice. The hippocampus of apoE4-KI mice was 8% smaller at 6-7 months. To study hippocampal neurogenesis, 6-7-month-old mice received an intraperitoneal injection of bromodeoxyuridine (BrdU) to label proliferating cells. The brains were examined by immunostaining for neuronal and glial differentiation markers, immunofluorescence, and confocal microscopy at 1 and 3 days, 4 and 10 weeks after BrdU injection. In the subgranular zone of the hippocampus, BrdU-positive cells in apoE4-KI mice were twofold more abundant at 1 day than in apoE3-KI mice but showed reduced survival at 4 weeks (22% vs. 30%). Among surviving BrdU-positive cells, mature neurons were more numerous in apoE3-KI mice (38% vs. 16%), and immature neurons were more numerous in apoE4-KI mice (40% vs. 21%). At 4 weeks, BrdU-positive cells in apoE knockout mice survived at a rate similar to that in apoE3-KI mice but most were astrocytes (45%) rather than mature (16%) or immature (18%) neurons. These findings suggest that apoE4 reduces the number of mature neurons, leading to a feedback increase in the number of proliferating cells. Thus, during adult hippocampal neurogenesis, apoE plays a crucial role in cell-fate determination toward neuronal development, and apoE4 inhibits neurogenesis by impairing neuronal maturation.

CIRM SCHOLAR: LAURA SAUNDERS

TITLE: **HDAC expression in mouse embryonic stem cells**

AUTHORS: **Laura R. Saunders and Eric Verdin**

Epigenetic modifications of histone proteins (methylation, acetylation) and DNA (methylation of CpG dinucleotides) ensure that a specific transcriptional program is established during development and differentiation into various cell lineages. Lineage-specific transcription factors recruit large chromatin remodeling complexes to confer cell identity through chromatin modification. There are three classes of HDACs that catalyze the removal of acetyl groups from histone and non-histone proteins to modify chromatin and regulate gene expression. Class I HDACs (HDACs 1-3, 8, 11) are ubiquitously expressed regulators of gene expression. Class II HDACs (HDACs 4-7, 9-10) are tissue specific regulators of differentiation during thymic, skeletal, cardiac, bone and neuronal development. Class III HDACs or sirtuins (SIRTs 1-7) are NAD+-dependent deacetylases that regulate metabolism and aging. We evaluated the expression level of various HDACs/SIRTs in undifferentiated mouse embryonic stem cells (mESCs) and evaluated changes in their expression following differentiation. We also compared the expression level of HDACs/SIRTs in mESCs to differentiated mouse cell lines of various tissue types. We found several HDACs/SIRTs that are highly expressed in undifferentiated mESCs. We further identified HDACs/SIRTs whose expression changes during differentiation, indicating that the expression profile of HDACs/SIRTs may play an important role in the differentiation of stem cells into specific tissue types. Manipulation of expression levels of specific HDACs/SIRTs may aid in the production of exclusive populations of specific cell types to advance cell-replacement therapies.

UNIVERSITY OF CALIFORNIA, BERKELEY N 014

CIRM SCHOLAR: RUHA BENJAMIN

TITLE: **Culturing Consent: Producing novel cells, publics, and interests in the stem cell state**

AUTHORS: **Ruha Benjamin**

In this study I chronicle the relationship between what are commonly thought of as two distinct spheres- the public sphere of politics and values and the scientific sphere of truth and facts. The passage of the California Stem Cell Research and Cures Act provides a fertile case in which to explore the coming together of these two spheres thought to be soo distinct.

I find that the implementation of Prop. 71 is not simply emblematic of what we might consider the politicization of science. It is also indicative of an inverse process, the scientization of politics, evident in elections and governance whenever a calculated and standardizing account (e.g. polling) of the public churns out categories of people and interests. This creation of citizen-types is indicative of a phenomenon science studies analysts have described as the 'tandem processes of knowledge-making and people-making' such that one rarely takes form without the other.

The scientization of politics is part of the more general process of what I refer to as 'culturing consent', which in the specific context of Prop.71, involves generating and maintaining public trust in a highly contentious enterprise. The connection between culturing (i.e. replicating) stem cells in the lab and culturing (i.e. producing values) assent for this novel scientific endeavor is what this study tracks. 'Culture' here also refers to the different rationalities (knowledge-generation, patient advocacy, for-profit, participatory democracy, etc) that social actors invoke in a struggle to define what should be the driving engine and aims of stem cell research.

Fieldwork for this project is comprised of three phases which together map the varying perspectives on and practices around stem cell research in the state of California: Phase I occurred in two biomedical settings- a cord blood banking program and a clinic for inherited blood disorders. Phase II focused on the main state agency responsible for implementing Prop.71 as well as lobbyists and activists whose activities were geared towards influencing this agency. Phase III turned the ethnographic lens onto the symbolic sphere of stem cell-related political debate.

The methods I employ across these three phases of fieldwork include analysis of national and CA-specific survey data on public views of SCR, semi-structured interviews with key actors as well as patient families who are potentially impacted by developments in this field, participant observation at relevant meetings and conferences, and content analysis of televised and internet-based commercials & commentary. My investigation is multi-sited in so far as the people, practices, and relationships that I chronicle are not found in a single location, but are spread across institutional, geographic, and even virtual space.

UNIVERSITY OF CALIFORNIA, BERKELEY N 015

CIRM SCHOLAR: JOSEPH DHAHBI

TITLE: **Screening for agents that alter the epigenetic program of hematopoeitic stem cells**

AUTHORS: **Joseph Dhahbi, Wendy Magis, and David Martin**

Cell differentiation is an epigenetic process in which some genes are silenced while others remain active; the phenotype of cell is determined by the set of genes that are expressed. During differentiation of adult-stage erythrocytes in higher primates, the γ-globin gene is epigenetically silenced; this "switch" occurs at the stem cell stage. ß-thalassemia and sickle cell disease, in both of which the ß-globin gene is defective, are the world's most common single-gene disorders; worldwide most people born with either disease die in early childhood. They can be ameliorated if the silencing of γ-globin is prevented; a small molecule that could accomplish this might have a dramatic impact on the lives of those born with the diseases, and health care budgets in many countries. This project aims to develop a cell-based screen for small molecules that can disrupt epigenetic silencing: these molecules will be candidate agents for treatment of ß-hemoglobinopathies, but they will also be candidates for manipulation of other gene expression pathways in cell differentiation. The molecules may thus be useful in channeling differentiation or gene expression in a wide variety of stem-cell-derived cell types.

UNIVERSITY OF CALIFORNIA, BERKELEY N 016

CIRM SCHOLAR: YICK FONG

TITLE: **Isolation of a human embryonic stem cell coactivator complex**

AUTHORS: **Yick W. Fong, Carla Inouye, and Robert Tjian**

The transcriptional activators Oct4, Sox2, and Nanog are key regulators essential for self-renewal of pluripotent embryonic stem (ES) cells. Oct4, Sox2 and Nanog target genes were recently identified and they overlap substantially, suggesting they collaborate to regulate a common set of genes governing pluripotency, self-renewal, and cell fate determination. Chromatin immunoprecipitations and gene expression microarrays also reveal that Oct4, Sox2 and Nanog co-occupy the cis-regulatory elements of their own genes, thus forming an interconnected autoregulatory loop, which is thought to confer upon ES cells the ability to maintain their stem cell identity yet permit rapid responses to various developmental cues.

Oct4 and Sox2 activate Nanog transcription by binding to the Octamer and Sox elements upstream of the putative transcription start site. However, Oct4 and Sox2 are not sufficient to activate Nanog transcription in differentiated cells like 293, suggesting the additional requirement of stem cell-specific cofactor(s). Here we describe, using an unbiased biochemical complementation assay coupled with reconstituted transcription reactions, the identification and isolation of a new human cofactor, SCC (Stem Cell-Coactivator), that is required for the transcriptional activation of Nanog by Oct4 and Sox2. SCC is distinct from previously identified cofactors such as CBP/p300, Mediator/CRSP, or TFIID and related complexes. SCC is also highly enriched in human ES cells. Purification of SCC identifies a multisubunit complex with a relative molecular mass of ~600K.

SCC may therefore represent a specialized coactivator complex that selectively potentiates the transcription of target genes required for stem cells to renew and remain pluripotent, and may potentially provide a novel mechanism to reprogram cells back to a multipotent state.

UNIVERSITY OF CALIFORNIA, BERKELEY N 017

CIRM SCHOLAR: PHUNG GIP

TITLE: **Profiling glycans on stem cells**

AUTHORS: **Phung Gip, David Schaffer, and Carolyn Bertozzi**

The goal of my research proposal is to elucidate patterns of glycoprotein expression and biosynthesis toward identification of stage-specific markers during differentiation. Glycans decorate eukaryotic cell surfaces where they are poised to mediate a variety of molecular recognition events and are ideal candidates for new biomarkers. Such glycomic "fingerprints" will provide a platform for specific selection and enrichment of human embryonic stem cells (hESCs) of various stages of differentiation, and also provide structural information to define the functional roles of glycans in hESC development. Human embryonic stem cells will be differentiated into various cell types using established protocols. To identify stage-specific markers associated with glycoprotein expression and biosynthesis I will use innovative and state-of-the-art technology from our lab and collaborators to achieve three specific aims:

(1) Establish a global glycan "fingerprint" of defined stage-specific changes upon differentiation;
(2) Perform glycoproteomics analysis using mass spectrophotometry; and
(3) Characterize the genomic transcripts associated with glycoproteins.

UNIVERSITY OF CALIFORNIA, BERKELEY N 018

CIRM SCHOLAR: JAE-HYUNG JANG

TITLE: **Adeno-associated virus-mediated gene delivery to stem cells**

AUTHORS: **Jae-Hyung Jang, James T. Koerber, and David V. Schaffer**

Stem cells have significant promise for regenerative medicine, but they first require an understanding of molecular or environmental cues that can regulate their proliferation and differentiation, as well as means to manipulate these signals to control cell behavior. Delivery of genes encoding molecules capable of regulating stem cell function can serve as an effective means to both investigate stem cell biology and to control cell fate for therapeutic applications. Additionally, gene delivery coupled with gene targeting has the potential to introduce mutations, and thereby generate disease models, as well as to correct deleterious genetic mutations in stem cell populations. However, a major obstacle to such applications continues to be the development of efficient and safe gene delivery vectors. Adeno-associated viral (AAV) vectors, which are being broadly explored in clinical trials, have significant promise as therapeutic vectors due to their safety and delivery efficiency, as well as their potential for gene targeting. Unfortunately, no natural AAV variants have been found with optimal properties for infecting stem cells. Due to the significant advantages of the vector, however, engineering AAV vectors to overcome rate limiting steps (i.e., cellular binding, intracellular trafficking, viral unpackaging, etc.) in stem cell transduction may have a high impact for stem cell investigations. Current approaches to design custom AAV vectors are limited to rational peptide insertion into or chemical modifications of the viral capsid structure. However, since the structure-function relationships of the complex AAV capsids are not fully understood, rationally designing and modifying the AAV capsid to meet specific needs are still challenges.

To overcome these challenges, we have developed directed evolution approaches, composed of mutant viral library generation and selection, to engineer enhanced vehicles. Specifically, we created large and highly diverse (~10^7) libraries of AAV variants using three different technologies: i) DNA shuffling, ii) error-prone PCR followed by the Staggered Extension Process (StEP), and iii) inserting random 7 amino acid peptides into the AAV cap loop domains. The resulting libraries were added to both murine embryonic stem cells (mESC) and rat neural progenitor cells (NPC), and the "fittest" AAV variants were rescued at each selection step. Importantly, the selected variants were subjected to additional rounds of evolution (i.e., mutagenesis by error prone PCR plus selection) in order to further enhance the infectivity of the AAV pool. As a result, the newly evolved AAV variants exhibited progressively improved capacities for both mESC and NPC infection as compared with both wild type AAV2 and the variants that emerged from the previous round of evolution. These new viral variants or "serotypes" will provide powerful tools that may aid numerous investigations of stem cell biology and therapy. Finally, we will explore potential applications enabled by the newly developed AAV vectors in this study (e.g., gene targeting in stem cells).

CIRM SCHOLAR: JENNIFER LIU

TITLE: **Bioethics and culture: a view from Taiwan**

AUTHORS: **Jennifer A. Liu**

This study is an examination of the making of stem cell research regulatory policy in Taiwan. It considers the different parties whose interests and expertise are at stake in policy-making while also considering the diversity of opinions and concerns expressed by members of various social groups. In some ways the public debates on stem cell ethics and policy are uniquely configured in Taiwan, while in other ways they appear to be quite similar to international regulations from other countries and bodies including the UK, the US, and the ISSCR.

This study examines the process of policy-making in the specific case of Taiwan and links it to broader transnational movements and standardizations in ethical regulations regarding stem cell research. Taiwanese representatives negotiate making autonomous policy appropriate to their specific polity and circumstances, while simultaneously taking seriously the island's own role in and reliance on international practices and policies in this arena.

Below, I address specific concerns to Taiwan including the question of how stem cell policy-making articulates with democratic processes more generally and how Confucian and Buddhist perspectives weigh-in. I consider further what Taiwan might teach us about the possibility of a global bioethics when considerations of cultural difference are taken seriously. This is a view of policy-making from multiple scales -- from large scale transnational considerations of the place of Taiwan in stem cell research to the very personal considerations that render a human embryonic stem cell researcher reluctant to work on human embryos.

Furthermore, I suggest that the lack of clear ethical policy regarding the use of human embryos may have, as an unanticipated consequence, the effect of delaying biotechnological progress. In at least some cases, researchers proceed more slowly as they feel that they must do the ethical work as well as the scientific work in the absence of "expert" ethical guidance. This contradicts the hyped fears expressed in the US in the early years of the new century that Asian countries would race ahead in stem cell research and therapeutics because of a lack of ethical regulatory restrictions.

UNIVERSITY OF CALIFORNIA, BERKELEY N 020

CIRM SCHOLAR: HEATHER MELICHAR

TITLE: **Comparative analysis of mesoderm lineage development in human embryonic stem cell lines**

AUTHORS: **Heather Melichar, Ou Li, Hilary Haber, Dragona Cado, and Ellen Robey**

Less than a decade after their isolation, human embryonic stem (HuES) cells are now thought to be a potential cure for any number of diseases. One goal of our lab is the generation of mature T lymphocytes from this pluripotent cell source. However, for stem cell-based therapy to be a clinical reality, the molecular determinants of stem cell differentiation and the normalcy of stem cell-derived tissue are of supreme interest. Hence, the identification of markers of stem cell potential is paramount in realizing the full potential of HuES cell lines. With the assumption that differences in the potential of distinct stem cell lines to generate mesoderm affects the development of down-stream derivatives, including the blood lineages, we propose to comprehensively describe the potentiality of a cohort of HuES cell lines toward mesoderm differentiation. Here we present preliminary data that suggests that several independently derived HuES cell lines differ in their developmental and differentiation potential. We note that several of these cell lines vary in their ability (or kinetics) to form cystic embryoid bodies. Additionally, analysis of mesoderm and hematopoietic differentiation markers in embryoid bodies suggests that different proportions of differentiating cells become restricted to these lineages. Finally, we present proposed studies that aim to uncover genetic determinants of mesoderm formation with the ultimate goal of describing a molecular signature in HuES cells that can be used to distinguish their developmental potential into the three germ layers and beyond.

CIRM SCHOLAR: JOSEPH PELTIER

TITLE: **Molecular mechanisms of adult neural progenitor proliferation and self-renewal**

AUTHORS: **Joseph Peltier and David V. Schaffer**

Adult hippocampal neural progenitor cells (AHNPCs) can generate nearly all major cell types within the mammalian brain, including neurons, astrocytes, oligodendrocytes, and endothelial cells, which makes them promising candidates for the treatment of neurological injuries and diseases. Additionally, adult neurogenesis may play roles in learning and memory, stress and depression, response to injury, and aging. Because the processes of AHNPC proliferation and self-renewal appear to be significant regulatory points for adult neurogenesis, the purpose of this work is to determine 1) how extracellular mitogens activate intracellular signaling networks mediating AHNPC proliferation, and 2) whether mechanisms that promote proliferation also promote self-renewal. We have found that the PI3K/Akt pathway, which is broadly important for self-renewal of multiple stem cell types, is vital in mitogen-induced AHNPC proliferation. Specifically, Akt stimulation is sufficient to induce cell proliferation and inhibit differentiation. However, PI3K stimulation is not sufficient to induce proliferation, implying that mechanisms other than PI3K are necessary for Akt activation. Furthermore, the transcription factor CREB is a downstream mediator of Akt signaling and promotes AHNPC proliferation. These results provide insight into the biochemical mechanisms that regulate adult neural progenitor cell proliferation and self-renewal, eventually leading to stem-cell based therapies.

UNIVERSITY OF CALIFORNIA, BERKELEY N 022

CIRM SCHOLAR: SUE SOHN

TITLE: **Coaxing hematopoietic and lymphoid potential out of hescs.**

AUTHORS: **Sue J. Sohn and Asrar Winoto**

Regulatory T cells (Tregs) possess the ability to suppress inflammatory responses and thus, the potential for their use toward anti-inflammatory therapies for human diseases, including autoimmune diseases, is significant. My aim is to induce differentiation of Treg cells from undifferentiated human embryonic stem (huES) cells. Since all lineages of blood cells, including T cells, are derived from hematopoietic stem cells (HSCs), I have focused on first inducing HSCs and committed T cell precursors in vitro from pluripotent huES cells. Several published studies have shown that pluripotent ES cells require cell-cell contact to acquire characteristics of more differentiated HSCs. Typically embryoid body (EB) culture or stromal cell co-culture system has been utilized to show that huES-to-HSC commitment is possible in these systems, albeit with severely limited efficiencies. I am employing a combination approach wherein initial differentiation of HSCs is established in the context of EBs, followed by extended culture on stromal cell monolayers. My preliminary results suggest that the stromal cells play important roles in survival, expansion, and/or differentiation of HSCs subsequent to their initial commitment. Detailed characterization of hematopoiesis in these systems at the cellular and molecular levels will define key parameters that support T cell differentiation in vitro.

CIRM SCHOLAR: CYNTHIA BATCHELDER

TITLE: **Human embryonic stem cells differentiated towards renal precursors for kidney repair**

AUTHORS: **Cynthia A. Batchelder, C. Chang I. Lee, Jennifer M. Bolin, Daniel F. Jimenez, and A. F. Tarantal**

Embryonic stem cells (ESC) can be differentiated into cells of all three germ layers and may be useful for kidney repair and treatment of renal disease if efficient differentiation strategies can be developed. We hypothesize that molecular signals important in early gestation metanephric kidney development will be essential in directing human ESC (hESC) differentiation in vitro toward renal precursors. Our approach to the development of such a strategy is two-fold: (1) To understand kidney development at the structural and molecular level during early gestation using a model of human development, and (2) To mimic this pattern of development in hESC by manipulation of cell culture conditions. For the first aim, we have focused on the study of sequential stages of embryonic and fetal development using immunohistochemical and morphological techniques. This information, coupled with a review of the literature, was incorporated to develop a theoretical model of expected gene expression patterns for directed differentiation of hESC (UC06) when cultured as embryoid bodies (hEBs) and using qRT-PCR over an 18-day culture period. Prior to initiation of experiments, a well-characterized cell bank was established using standardized techniques (karyotype; immunohistochemical markers such as Tra-1-81, SSEA3, SSEA4, Oct4; and quantitative RT-PCR) and cryopreserved. Undifferentiated hESC were maintained on mouse embryonic fibroblast feeder layers until dissociated, then cells were plated on ultra low binding plates with or without the presence of nephrogenic factors (0.1 μM retinoic acid, 10 ng/mL Activin A, 50 ng/mL BMP-7; designated RA7) in the culture medium. The hEBs were harvested at 2-3 day intervals and RNA expression quantified relative to the housekeeping gene, HPRT1, by real-time RT-PCR. OCT4, a marker of undifferentiated hESC, declined over time while markers of intermediate mesenchyme such as Pax2 and Six2 were noted to increase over the first six days of culture in RA7 medium when compared with hESC cultured in control medium. Relevant transcription factors were upregulated after 4 days of culture while markers of mature kidney, in general, were not noted to be upregulated in RA7 vs control medium. Overall our results are similar to findings in murine ESC (Kim and Dressler, J Am Soc Nephrol 16:3527-3534, 2005) and suggest that RA7 nephrogenic treatment is effective for differentiation of hESC toward intermediate mesenchymal phenotypes. Future experiments are aimed at additional manipulation of culture conditions to improve kidney-specific markers and cell differentiation.

UNIVERSITY OF CALIFORNIA, DAVIS N 024

CIRM SCHOLAR: TEDLA DADI

TITLE: **Assessment of pluripotency and multilineage differentiation potential of Apolipoprotein-E deficient (ApoE-/-) mice-derived embryonic stem cells**

AUTHORS: **Tedla D. Dadi, Ming W. Li, and K.C. Kent Lloyd**

In addition to their usefulness for studying nuclear reprogramming, embryonic stem cells (ESC) are a promising tool for cell therapy, regenerative medicine and tissue repair. Further, the possibility of manipulating somatic cell-derived ESC in vitro for the treatment of genetic diseases is intriguing. However, to do so, optimization of somatic cell reprogramming, ESC derivation and specific cell lineage differentiation remains a key goal. In this project, we sought to determine whether ESC derived from Apolipoprotein-E (ApoE) gene deficient mice can be maintained undifferentiated in culture and retain their pluripotency. In addition, we determined their capacity to undergo targeted differentiation using growth factor supplementation. For this study, we analyzed the expression of ESC pluripotency markers (Oct-4, Nanog, PECAM-1 and SSEA-1), and three germ layers markers (early endoderm marker (Nestin and Keratin-8)); mesoderm (Brachyury and BMP-4), and ectoderm (AFP and HNF-4) using fluorescence-activated cell sorting (FACS), immunocyochemistry, and immunoblotting techniques. We observed that ESC derived from ApoE deficient mice express undifferentiated pluripotent and differentiated cell markers (germ layer markers), and are capable of proliferating for extended periods of time without differentiating and maintaining pluripotency. Further, although they spontaneously differentiate in the absence of leukemia inhibitory factor (LIF), we found that supplementation of mitogenic growth factors enhance the differentiation potential of ESC and improve fate determination into desired germ layers.

UNIVERSITY OF CALIFORNIA, DAVIS N 025

CIRM SCHOLAR: JIDONG FU

TITLE: **Driven maturation of human embryonic stem cell-derived cardiomyocytes**

AUTHORS: **Jidong Fu and Ronald Li**

Background: Loss of non-regenerative, terminally differentiated cardiomyocytes (CMs) is irreversible; moreover, myocardial repair is hampered by a severe shortage of donor cells and organs. Self-renewable, pluripotent human (h) embryonic stem cells (ESCs) can provide an unlimited cell source. However, their cardiac derivatives have embryonic-like and immature properties.

Objectives: To test the hypotheses that 1) immature hESC-CMs can cause arrhythmias, and that 2) ex vivo driven maturation can improve safety and efficacy.

Results: Electrophysiological recordings of single murine (m) and human (h) ESC-CMs reveal a number of pro-arrhythmic cellular properties. However, driven maturation could be accomplished via somatic gene transfer to render the electrophysiological phenotype adult-like.

Conclusion: Our present experiments provide proof-of-concept support that driven maturation enhances safety and can greatly facilitate hESC-based heart therapies.

CIRM SCHOLAR: MICHAEL KARETA

TITLE: **Establishing a role for DNMT3L in directing DNA methylation in embryonic stem cells**

AUTHORS: **Michael S. Kareta and Frédéric Chédin**

Hypothesis: To determine if DNMT3L directly contributes to targeting DNA methylation to specific genomic loci in embryonic stem cells.

Background: 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 heritable and reversible marks dictating gene expression. DNA methyltransferases (Dnmts), which catalyze the transfer of methyl groups to cytosine ring of CG dinucleotides, are highly expressed during the blastocyst stage and are required for proper cellular differentiation of the epiblast. The Dnmt3a and Dnmt3b enzymes, in particular, are active Dnmts that are required for proper embryonic development. Here, we further address the possibility that the Dnmt3L protein, in addition to its role as a general stimulatory factor for DNA methylation by Dnmt3a and Dnmt3b, also serves to target DNA methylation to specific genomic loci through interactions with specific proteins.

Results: To characterize these interactions, we first used specific methylated DNA immunoprecipitations (MeDIP) on both wild-type and Dnmt3L deficient murine embryonic stem cell lines.

Conclusions: A subset of genes has been identified which rely on the activity of the Dnmt regulator, Dnmt3L, for proper promoter methylation.

CIRM SCHOLAR: JOYCE MA

TITLE: **Neural Stem Cell Chemokines: A red/ET recombineering approach**

AUTHORS: **Joyce H. Ma and David E. Pleasure**

Neuroinflammatory autoimmune diseases such as multiple sclerosis can be described as resulting from dysregulated communication between fully differentiated cells, neural stem cells that persist in the adult central nervous system (CNS), and cells of the immune system. Our focus is on the role played by neural precursor cells in modulating and contributing to the panoply of extracellular signaling molecules responsible for recruitment of immune cells and cells necessary for regeneration to sites of inflammatory lesions. Thus the communication between neural precursor cells (NPCs) and immune cells has become a topic of investigation in neuroinflammatory diseases. The signaling molecules that mediate this communication include chemokines induced during an inflammatory response. One such chemokine is CXCL10, which is secreted by both NPCs and reactive astrocytes in neuroinflammatory diseases, and serves to recruit T helper cells to sites of inflammation.

As one of the three IFN-gamma-inducible CXC chemokines, CXCL10 has been shown to play a key role in the feed-forward loop for the maintenance and amplification of the inflammatory reaction in organ systems other than in the brain. It remains to be elucidated whether IFN-gamma also induces the same feed-forward loop in the CNS, where local IFN-gamma-mediated recruitment of inflammatory cells bearing the receptor for CXCL10 (CXCR3) in turn produce more IFN-gamma.

In order to understand the role of CXCL10 in the communication between different cell types in an animal model of multiple sclerosis called experimental allergic encephalitis (EAE), it would be useful to be able to silence CXCL10 expression in specific cell types and to compare the differences in disease progression. One robust approach would be the construction of a conditional knockout mouse (CKO) model by flanking CXCL10 exons with loxP sites. Thus it will become possible to conditionally ablate the function of the floxed chemokine in specific cell types by crossing the conditional knockout mouse with cell-type specific inducible Cre mice.

In generating the conditional knockout mouse we initially applied the bacterial recombineering approach designed by Copeland et al. 2003 to target the specific loxP sites such that they would insert to flank the second and third exons of CXCL10. A series of technical problems led us to abandon this powerful but frequently unsuccessful approach, which lacks built-in internal controls for immediately detecting errant recombination events, in favor of the Red/ET system that provides for controls at every recombination step.

Future applications of our work include conditionally ablating CXCL10 expression in the CNS to study the progression of EAE in the absence of recruitment of CXCR3-expressing cells of the oligodendrocyte cell lineage to areas of demyelinated lesions. The floxed CXCL10 mouse will also allow us to determine the therapeutic value of CXCL10 function-blocking therapeutics in other organ systems for the treatment of hepatitis C infection, and in autoimmune endocrine diseases such as autoimmune thyroid disease, diabetes mellitus, and primary adrenal deficiency, in which CXCL10 has been implicated as a major contributor to dysfunctional immune signaling.

UNIVERSITY OF CALIFORNIA, DAVIS N 028

CIRM SCHOLAR: LAURA SHIH

TITLE: **Regeneration of airway epithelium by embryonic stem cells**

AUTHORS: **Laura B. Shih, Christy M. Kim, Pimtip Sanvarinda, Philip Thai, and Reen Wu**

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 determine the well-defined, serum-free culture conditions required for optimal 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 medium was developed to initiate differentiation into the airway epithelial lineage with expression of mouse CC10, MUC5B, and SPD gene products, as measured by quantitative real-time PCR and immunohistochemistry. The effects of individual hormone and growth factor supplements, cell seeding density, and embryoid body formation on the differentiation of mouse ES cells into the airway epithelial lineage were studied.

RESULTS: Removal of retinoic acid, cholera toxin, and dexamethasone led to decreased expression of airway epithelial genes. Time course studies demonstrated a varying time-dependence of these three factors for airway epithelial cell development. Furthermore, airway epithelial gene expression was higher at lower cell seeding densities. Embryoid body formation for up to 10 days led to increased endoderm and ectoderm and decreased mesoderm marker expression.

CONCLUSIONS: We have demonstrated the trained, time-dependent 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 seeding density, and embryoid body formation 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.

CIRM SCHOLAR: BASHA STANKOVICH

TITLE: **Adhesion molecule expression in early hematopoietic commitment of mouse embryonic stem cells**

AUTHORS: **Basha Stankovich, Esmeralda Aguayo, and Maria Pallavicini**

Mouse embryonic stem cells (ESC) collect information from their environment and make cell fate decisions based on intrinsic and extrinsic factors. ESC are maintained in an undifferentiated state in the presence of Leukemia Inhibitory Factor (LIF). The removal of LIF induces the formation of embryoid bodies (EB) which contain cells with the potential to differentiate to multiple lineages in tissues. 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 commitments to hematopoiesis. 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 during six days of ESC differentiation. EB were formed either in the absence of LIF to promote differentiation or under defined media conditions that promote hematopoietic development. The resulting expression profiles were evaluated to identify genes associated preferentially with hematopoietic differentiation compared to loss of pluripotency. Thirteen genes showed differential expression during EB formation. Among this subset, six genes are categorized as junction molecules due to their role in adherens junction, tight junction or gap junction pathways. These genes include E-cadherin, claudin-4, connexin-43, connexin-45, zona occludens-1 (ZO-1) and zona occludens-2 (ZO-2). The heterogeneity of the EB was reduced by sorting subpopulations based on expression of the markers Fetal Liver Kinase-1 (Flk-1), an extracellular receptor expressed on the surface of hematopoietic precursors; Brachyury (Bry), a transcription factor associated with mesodermal commitment; and Stem Cell Leukemia (Scl), a transcription factor necessary for hematopoietic commitment. Adhesion molecule transcript levels in sorted subpopulations were quantified and compared with expression in unsorted populations. Among the 32 targets analyzed, E-cadherin, claudin-4, ZO-1 and ZO-2 emerged once more as genes that were either upregulated or downregulated in the populations transitioning along stages of early hematopoietic development. Differential regulation of molecules in the adherens junction, tight junction and gap junction pathways during EB formation and hematopoietic development supports the hypothesis that cell-cell interactions are important for ESC fate decisions. Knockdown studies, using RNAi technology, to evaluate the functional consequence of differential expression of proteins in junction molecule pathways and their impact on hematopoietic development are in progress.

UNIVERSITY OF CALIFORNIA, DAVIS N 030

CIRM SCHOLAR: DONGGUANG WEI

TITLE: **Functional repopulation of adult mammalian inner ear sensory cells**

AUTHORS: **Dongguang Wei and Ebenezer Yamoah**

Mature mammalian inner ear is thought to be incapable of repopulating damaged hair cells and spiral ganglion neurons, thus, the sensorineural hearing loss is irreversible. We found a previously not described intrinsic population of progenitor cells may exist within the adult inner ear that could possibly repopulate the sensory cells. Meanwhile we demonstrated that exogenous stem cells could differentiate into sensory cell phenotypes and make functional synapses with adult mammalian inner ear cells, which could offer therapeutic option for the hearing impaired.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO N 031

CIRM SCHOLAR: DAVID CANO

TITLE: **Generation of mouse ES-cell derived endodermal cells**

AUTHORS: **David Cano, Sapna Puri, Grace Wei, and Matthias Hebrok**

The generation of definitive endoderm in vitro has important therapeutic implications as a step to the production of cells such as hepatocytes and insulin-producing beta-cells. We have implemented a protocol to reproducibly generate cells that express markers of definitive endoderm from murine ES cells. In vivo, micro RNAs (miRNAs) have been shown to guide the development of specific cell lineages. We propose to analyze and characterize in detail the role of miRNAs during the generation of endodermal cells from ES cells. As a first step, we plan to obtain a pure population of mouse ES-cell derived endodermal cells. Thus, we have focused in the optimization of the protocol for generation of endodermal cells from ES-cell. These experiments have determined that cell density is a key factor for efficient differentiation of endodermal cells.

CIRM SCHOLAR: WON-SUK CHUNG

TITLE: **Intra-endodermal interactions are required for pancreatic beta cell induction**

AUTHORS: **Won-Suk Chung and Didier Y. R. Stainier**

The differentiation of endodermal cells into pancreatic beta-cells is thought to be regulated by mesodermal signals. Here, while studying the cell autonomy of the pancreatic beta-cell defects in zebrafish smoothened mutants, we found that interactions between endodermal cells are in fact essential for pancreatic beta-cell induction. Cell transplantation experiments reveal that Smoothened function, which is required for the differentiation of pancreatic beta-cells, is not required in the beta-cell lineage. However, Smoothened function is required for critical cell-cell interactions within the endodermal sheet. When wildtype cells contribute to laterally located endodermal cells in the pancreatic region of smoothened mutant embryos, adjacent cells located medially differentiate into pancreatic beta-cells. We further show by lineage tracing, that the lateral cells contribute to the exocrine and intestine while the medial cells give ries to pancreatic endocrine cells. These results reveal a cell non-autonomous requirement of Hedgehog signaling as well as critical endoderm-endoderm interactions for pancreatic betea-cell induction.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO N 033

CIRM SCHOLAR: MARICA GRSKOVIC

TITLE: **Systematic identification of Cis-regulatory sequences active in mouse and human embryonic stem cells**

AUTHORS: **Marica Grskovic, Christina Chaivorapol, Alexandre Gaspar-Maia, Hao Li, and Miguel Ramalho-Santos**

Understanding the transcriptional regulation of pluripotent cells is of fundamental interest and will greatly inform efforts aimed at directing differentiation of embryonic stem (ES) cells or reprogramming somatic cells. We first analyzed the transcriptional profiles of mouse ES cells and Primordial Germ Cells (PGCs) and identified genes up-regulated in pluripotent cells both in vitro and in vivo. These genes are enriched for roles in transcription, chromatin remodeling, cell cycle and DNA repair. We developed a novel computational algorithm, CompMoby, which combines analyses of sequences both aligned and non-aligned between different genomes with a probabilistic segmentation model to systematically predict short DNA motifs that regulate gene expression. CompMoby was used to identify conserved over-represented motifs in genes up-regulated in pluripotent cells. We show that the motifs are preferentially active in undifferentiated mouse ES and Embryonic Germ cells in a sequence-specific manner, and that they can act as enhancers in the context of an endogenous promoter. Importantly, the activity of the motifs is conserved in human ES cells. We further show that the transcription factor NF-Y specifically binds to one of the motifs, is differentially expressed during ES cell differentiation and is required for ES cell proliferation. Our study provides novel insights into the transcriptional regulatory networks of pluripotent cells.

CIRM SCHOLAR: NAVEEN GUPTA

TITLE: **Intrapulmonary treatment with mesenchymal stem cells reduces endotoxin induced acute lung injury and mortality in mice**

AUTHORS: **N. Gupta, X. Su, V. Serikov, and M. A. Matthay**

Rationale: Mesenchymal stem cells (MSC) have recently been shown to have immunomodulatory properties and beneficial effects in experimental models of lung injury. We tested the potential benefit of intrapulmonary treatment with MSC in a mouse model of endotoxin-induced acute lung injury (ALI).

Methods: C57BL/6 mice were intratracheally (IT) instilled with LPS from E. coli (5mg/kg). Four hours after LPS instillation, mice received either IT MSC, fibroblasts, apoptotic MSC (750,000 cells in 30 μl) or PBS (30 μl). At specified time points (8, 24, and 48 hours after injury) samples were collected for measurement of lung water, protein and cytokine analysis. Survival over this time period in each group was also recorded.

Results: MSC treatment resulted in improved survival at 48 hours after injury compared to PBS treated mice (MSC group 80%, PBS group 42%, n = 30 per group, $p < 0.01$). There was also a significant decrease in excess lung water, a measure of pulmonary edema (145 ± 50 μl vs 87 ± 20 μl, n = 12 per group, $p < 0.01$), and bronchoalveolar lavage (BAL) protein, a measure of endothelial and alveolar epithelial permeability (3.1 ± 0.4 mg/ml vs 2.2 ± 0.8 mg/ml, n = 10-11 per group, $p < 0.01$), in the MSC treated mice. These protective effects were not replicated by fibroblasts or apoptotic MSC. MSC administration mediated a shift from a pro to an anti-inflammatory response to endotoxin, increasing the levels of IL-10 and IL-1ra.

Conclusions: In a mouse model of endotoxin-induced acute lung injury, the intra-pulmonary treatment with MSC reduces the severity of lung injury and improves survival. This benefit is mediated, at least in part, by a shift from a pro to an anti-inflammatory response to endotoxin. This protection is not replicated by fibroblasts or apoptotic MSC suggesting that the beneficial effect is specific to undifferentiated, viable MSC.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO N 035

CIRM SCHOLAR: EDWARD HSIAO

TITLE: **Regulating stem cell differentiation through expression of an engineered Gs-coupled receptor**

AUTHORS: **Edward C. Hsiao, Benjamin M. Boudignon, Wei C. Chang, Yuko Yoshinaga, Trieu Nguyen, Pieter de Jong, Bernard P. Halloran, Robert A. Nissenson, and Bruce R. Conklin**

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 complimentary 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 conception 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 at 3 weeks. Whole body bone densitometry (DEXA analysis) demonstrated a 3-fold increase in bone mineral density in 9-week-old mice over controls; microCT imaging confirmed a massive and generalized increase in mineralized bone by 9 weeks of age. The affected mice also displayed decreased muscle mass as well as altered bone marrow adiposity. These phenotypic changes were not observed in mice where Rs1 expression was suppressed from conception through the first 4 weeks of postnatal life. These results indicate that Gs-GPCR signaling in osteoblasts during the first 4 weeks of life 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 development using embryonic stem cells (ESC) 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 ESC expression of Rs1 and other signaling molecules. In order to facilitate this analysis, we are also developing a series of fluorescent BAC reporters to allow identification of specific cell types during ESC differentiation. The results of these projects will provide complimentary in vivo and in vitro models for understanding the roles of G protein signaling in stem cell differentiation.

CIRM SCHOLAR: CINDY KOSINSKI

TITLE: **The role of BMP antagonists and subepithelial myofibroblast cells in intestinal stem cell renewal and differentiation**

AUTHORS: **C. Kosinski, J. Zhang, S. Leung, and X. Chen**

Intestinal epithelial cells undergo rapid and continuous cell turnover. This self-renewal process is driven by a multipotent stem cell that resides at the base of the intestinal crypt. The intestinal stem cell (ISC) generates progenitor cells that differentiate into functional epithelial cells as they migrate upward along the intestinal crypt-villus axis. To maintain the integrity of the intestinal epithelium, an equilibrium must exist between stem cell self-renewal and differentiation. It is believed that stem cell activity is regulated by secreted factors produced by surrounding intestinal subepithelial myofibroblasts (ISEMF) within the pericryptal mesenchyme. However, little is known about the signaling molecules emanating from these ISEMF cells.

A recent study conducted in the lab used microdissection and DNA microarrays to study gene expression programs during intestinal cell differentiation. A cluster of co-regulated genes highly expressed in the intestinal crypt consisting of secretory factors and cell matrix proteins were identified. Notably, three BMP antagonists (Gremlin1/GREM1, Gremlin2/GREM2, and Chordin-like1/CHRDL1) are included in this cluster. Furthermore, initial characterization by in situ hybridization (ISH) and RT-PCR of two genes (GREM1 and GREM2) in the crypt secretory gene cluster revealed that both are expressed by ISEMFs and smooth muscle cells located at intestinal crypts. These data suggest that these genes may represent important secreted factors by ISEMFs. Additional experiments are currently underway to determine whether these genes play a role in regulating intestinal stem cell self-renewal and differentiation. These studies will provide a better definition of the ISC niche.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO N 037

CIRM SCHOLAR: CHENG LIU

TITLE: **Molecular regulation of Oct4, Nanog and Sox2 by TGF-beta family signaling in embryonic stem cells**

AUTHORS: **Cheng Liu, Masayo Sakaki-Yumoto, Miguel Ramalho-Santos, and Rik Derynck**

Members of the TGF-beta family play pivotal roles in most if not all cell differentiation systems. They signal through receptor complexes consisting of type II and type I transmembrane serine/threonine kinases. These receptors directly activate regulatory Smads that act as intracellular signal transducers and regulate the transcription of selected target genes. Previous studies have revealed that Smads act as cell-intrinsic regulators of mesenchymal differentiation, which are regulated by cell-extrinsic stimuli that are provided in an autocrine or paracrine manner. Their roles as cell-intrinsic regulators of differentiation are likely to extend to other differentiation systems including embryonic stem cells. Recent studies have shown that Oct4, Sox2 and Nanog are master transcription factors for the self-renewal and pluripotency of mouse embryonic stem cells. In the nucleus, these transcription factors activate or repress gene expression, which results in stem cell self-renewal and pluripotency and inhibit the differentiation. The identification of the genes that are targeted for direct regulation by these transcription factors is the subject of intense investigation. In addition, Oct4 and Sox2 can also regulate the expression of Nanog. Although we conceptually know how Smads function to activate or repress transcription and differentiation, the molecular mechanism underlying the regulation of self-renewal and pluripotency by members of TGF-beta family and the interplay between those signals and the master transcription factors, Oct4, Nanog and Sox2, are largely unexplored. We are studying the regulation of the expression and activities of Oct4, Sox2 and Nanog by the Smad pathways using approaches to activate or inhibit the expression and activities of individual Smads. We then examine the effects of these manipulations on the expression and transcription activities of the individual transcription factors, using a combination of RT-PCR and transcription reporter assays, and the concomitant changes in self renewal and pluripotency.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO N 038

CIRM SCHOLAR: EMIN MALTEPE

TITLE: **HIF-dependent HDAC activity determines stem cell fate in the placenta.**

AUTHORS: **E. Maltepe, J. Tsai, K. Okazaki, G. W. Krampitz, and S. J. Fisher**

The cellular response to hypoxia is mediated by the Hypoxia-inducible Factor (HIF) family of transcriptional regulators. Genetic inactivation of this response is embryonic lethal due to defects in vascular, hematopoietic, cardiac and placental development. We have discovered a novel role for the HIFs during development. Trophoblast stem (TS) cells, the extraembryonic equivalent of ES cells, give rise to the placenta. HIF-deficient TS cells exhibit profound alterations in cell fate. While wild-type TS cells give rise predominantly to spongiotrophoblasts and trophoblast giant cells, HIF-deficient TS cells form chorionic trophoblasts and syncytiotrophoblasts. HIF-null TS cells represent the first in vitro model of syncytiotrophoblast formation in the mouse. As the primary interface between the maternal and fetal circulations, these cells regulate critical transport functions and play important roles in disease processes ranging from preeclampsia and fetal programming. Interestingly, this early cell fate decision is regulated in an oxygen-independent fashion due to a differentiation-dependent HIF induction. This novel mode of HIF activation is dependent on Mek1/2 and results in alterations in cellular epigenetic states. Our results thus reveal a previously unrecognized crosstalk between the oxygen signaling and epigenetic regulatory pathways during stem cell fate determination in the placenta.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO N 039

CIRM SCHOLAR: GREGORY POTTER

TITLE: **Dlx1 and Dlx2 control neuronal versus oligodendroglial cell fate acquisition in the developing forebrain**

AUTHORS: **Gregory B. Potter, Magdalena A. Petryniak, David H. Rowitch, and John L.R. Rubenstein**

Progenitors within the ventral telencephalon generate GABAergic neurons and oligodendrocytes, but regulation of the neuron-glial switch is poorly understood. We investigated the combinatorial expression and function of Dlx1&2, Olig2, and Mash1 transcription factors in the ventral telencephalon. We show that Dlx homeobox transcription factors, required for GABAergic interneuron production, repress oligodendrocyte precursor cell (OPC) formation by acting on a common progenitor to determine neuronal versus oligodendroglial cell fate acquisition. We demonstrate that Dlx1&2 negatively regulate Olig2-dependant OPC formation and Mash1 promotes OPC formation by restricting the number of Dlx+ progenitors. Progenitors transplanted from Dlx1&2 mutant ventral telencephalon into newborn wild-type mice do not produce neurons but differentiate into myelinating oligodendrocytes that survive into adulthood. Our results identify a new role for Dlx genes as modulators of neuron versus oligodendrocyte development in the ventral embryonic forebrain.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO N 040

CIRM SCHOLAR: GALIT ROSEN

TITLE: **Identification of leukemia stem cells in a mouse model of acute promeylocytic leukemia**

AUTHORS: **Galit Rosen and Scott Kogan**

Efforts to identify the leukemic stem cell in acute myeloid leukemia (AML) have led to considerable insights, but also to a number of unresolved questions. While the LSC is believed to arise primarily from the hematopoietic stem cell, data from chronic myelogenous leukemia blast crisis and acute promyelocytic leukemia (APL) suggest that they may arise from more committed cells. In order to clarify the potential of committed progenitors to become LSCs in some human AMLs, we utilized the MRP8-PML/RARA transgenic murine model of APL to perform initial studies directed at the identification of the LSC in APL.

Unsorted spleen cells of twice-passaged MRP8-PML/RARA leukemic mice injected into cohorts of sublethally irradiated mice using limiting dilutions showed that the LSC of APL is present in 1:100 to 1:200 cells. FACS analysis of six primary and serially transplanted leukemic specimens revealed that normal hematopoetic stem cells and myeloid progenitors were rare, comprising .007% and .078% of total splenic cells, respectively. These data raised the possibility that the LSC is present at higher frequency than these normal progenitors and does not belong to any of these populations.

Given the scarcity of normal progenitors, we surmised that the LSC may be part of the leukemic population. Transplantation experiments showed that the LSC is within the myeloblast population which has the abnormal phenotype of lymph-Gr1+ckit+ CD16/32+CD34 mod/+. A smaller population of lymph-Gr1-ckitlo/mod leukemic cells did not transplant disease. Myeloblast staining with SLAM and stem cell markers CD48, CD133, CD150, CD244 did not elucidate any heterogeneity.

We infer from the dilution analysis data that the LSC in this MRP8-PML/RARA mouse model of acute promyelocytic leukemia cannot be a hematopoietic stem cell or a normal myeloid progenitor cell. Also, it is apparent that the entire blast population is not capable of transferring disease; otherwise, all the mice injected would have developed leukemia. Future work will further delineate the immunophenotype of the LSC in this model and in human leukemia, as well as molecular abnormalities that are important in differentiating from the LSC into the myeloblast phenotype.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO N 041

CIRM SCHOLAR: DEREK SOUTHWELL

TITLE: **Programmed cell death as a regulator of inhibitory circuitry development and interneuron transplantation**

AUTHORS: **Derek Southwell and Arturo Alvarez-Buylla**

The cerebral cortex is composed of two broad classes of neurons, the glutamatergic excitatory neurons and the GABAergic inhibitory interneurons. These two cell types are produced from distinct embryonic progenitors; excitatory neurons originate in a dorsal pallial region and inhibitory interneurons derive from the ventral subpallial area. While excitatory neuronal precursors migrate radially into the cortical laminae, inhibitory precursors follow a dramatic pattern of dorsally-directed tangential migration into the cortex. Interneuron precursors then undergo a protracted maturation that extends well into early postnatal life.

Our laboratory has previously shown that transplanted interneuron precursors retain their remarkable migratory capacity when introduced into postnatal recipients. In collaboration with the Baraban and Rubenstein labs, we have further shown that grafted interneuron precursors differentiate into the major subtypes of inhibitory interneurons, and increase the frequency of host inhibitory signaling events. These observations raise the exciting possibility that interneuron transplants may restore the neural circuitry in diseases such as epilepsy, Parkinson's and schizophrenia.

To better guide the therapeutic application of interneuron transplantation, I am examining normal developmental patterns of apoptosis in the cortical inhibitory population. Temporal windows of programmed cell death could critically regulate the engraftment of transplanted interneuron precursors by defining periods when the brain is more or less receptive to the engraftment of additional cells. Moreover, grafted interneuron precursors may also follow intrinsic apoptotic programs after they are transplanted into the host brain.

I have first completed a developmental series of cleaved caspase-3 staining in the postnatal cortex of GAD67-GFP knock-in mice. My results show that GABAergic neurons undergo apoptosis during the first two postnatal weeks, with maximum levels of apoptosis occurring at the end of the first postnatal week. Through stereological counting, we have measured the GABAergic population across these timepoints: surprisingly, 40% of cortical GABAergic neurons are eliminated during this period of apoptosis.

I have recently begun to analyze the survival of transplanted embryonic GABAergic precursors. Using heterochronic transplantation methods, I am determining whether grafted cells die in the recipient brain, and if so, whether this process is intrinsically timed - in other words, lagging the disappearance of endogenous cells - or whether it occurs in sync with apoptosis of host neurons. My preliminary results suggest that very few, if any, of the grafted interneurons die in the recipient cortex. This suggests that the elimination of endogenous GABAergic neurons is directed by temporally discrete environmental signals to which grafted cells are unresponsive. It remains unclear, however, if transplant engraftment occurs at the expense of endogenous cells. We are thus studying whether the number of transplanted precursors influences the survival fraction of native interneurons. Furthermore, I am beginning dose-response experiments to determine if the transplantation of larger cell numbers results in a competitive interaction within the grafted cohort.

Apoptosis plays a critical role in determining inhibitory circuit composition. By understanding the patterns and mechanisms of interneuron apoptosis, we can better devise rational and targeted approaches for the therapeutic application of interneuron transplantation.

CIRM SCHOLAR: ROWENA SURIBEN

TITLE: **Analysis of Dact1 function during early mouse development**

AUTHORS: **Rowena Suriben and Benjamin N.R. Cheyette**

Embryonic development initiates from a few dividing cells which ultimately differentiate into the many cell types of the mature organism. To do this, cell populations must be able to both proliferate and differentiate in the presence of developmental cues. One such population of cells in vertebrates is the primitive ectoderm, an epithelium which gives rise to the three germ layers during gastrulation. Cells of the primitive ectoderm delaminate from this epithelium and migrate ventrolaterally to adopt endodermal and mesodermal fates. Cells which remain in the epithelium instead differentiate into neuroectoderm which subsequently differentiates into both the nervous system and the skin. The cells of the primitive ectoderm also proliferate and self-renew contributing to the embryo's growth. Because of the ability of primitive neuroectoderm cells to both self-renew and give rise to all three germ layers, they can be viewed as a stem cell population which exists transiently during development. The Wnt family of secreted proteins is involved in differentiation of the primitive ectoderm. For example, mutations of the Wnt3a gene in mice lead to mis-specification of future mesoderm progenitors from the primitive ectoderm, leading to overspecification of neuroectoderm. I am studying the Wnt signal transduction molecule Dact1 and its function in regulating such Wnt signaling events involved in tissue differentiation and proliferation of the primitive ectoderm during gastrulation. Using gene-targeted mouse lines, I am dissecting the molecular events regulating these processes.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO N 043

CIRM SCHOLAR: YANGMING WANG

TITLE: **MicroRNA regulation of embryonic stem cell division**

AUTHORS: **Yangming Wang, Joshua Babiarz, Archana Shenoy, Lauren Baehner, and Robert Blelloch**

A remarkable feature of embryonic stem (ES) cells is their rapid division rate, accompanied with a shortened G1 phase. The molecular mechanism behind this unique cell cycle regulation is poorly understood. Previously we reported that microRNA-knocking out ES cells proliferate slower than wild-type ES cells and show G1 accumulation phenotype, suggesting regulatory roles of microRNAs in G1/S transition of ES cells. By screening a library of microRNAs, we identified several ES cell specific microRNAs that can rescue the proliferation defects. Further characterization shows these microRNAs can also rescue the G1 accumulation phenotype. Currently we are in the process of identifying mRNA targets of these microRNAs. Preliminary results indicate these microRNAs regulate some of key regulators of G1/S transition. This study presents the first direct evidence of microRNA regulation of ES cell cycle progression.

UNIVERSITY OF CALIFORNIA, SANTA CRUZ N 044

CIRM SCHOLAR: KRISTEL DORIGHI

TITLE: **Kismet recruits trxG proteins to counter PcG function during transcription and development**

AUTHORS: **Kristel Dorighi, Shrividhya Srinivasan, and John W. Tamkun**

Factors that regulate chromatin structure and transcription play important roles in development. Members of the Polycomb group (PcG) of transcriptional repressors and their antagonists, the trithorax group (trxG), 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. We are currently investigating how Kismet interacts with other trxG proteins to counteract PcG-mediated transcriptional repression. Using Drosophila melanogaster as a model organism, we found that Kismet promotes the recruitment of two histone H3 lysine 4 methyltransferases of the trxG family - Ash1 and Trithorax to chromatin. By contrast, Kismet opposes the methylation of histone H3 on lysine 27, a repressive histone modification catalyzed by PcG proteins that is required for the maintenance of stem cell pluripotency. Since the function of PcG and trxG proteins have been highly conserved during evolution, these findings suggest that the human counterpart of Kismet - CHD7 - functions as a key regulator of stem cell pluripotency and differentiation. Progress toward testing this hypothesis will be presented.

UNIVERSITY OF CALIFORNIA, SANTA CRUZ N 045

CIRM SCHOLAR: MEGAN HALL

TITLE: **Regulated alternative splicing during stem cell differentiation**

AUTHORS: **Megan P. Hall, Roland J. Nagel, and Manuel Ares Jr.**

Little is known about how alternative splicing (AS) regulation contributes to stem cell identity, pluripotency and differentiation. Recent genome-wide methods for analyzing AS, such as alternative splicing microarrays, permit identification of large numbers of AS events. Preliminary results using a mouse myoblast cell line indicate complex and distinct sets of changes in AS during differentiation. These changes are at least partially mediated by a unique branchpoint-like UACUAAC motif present in transcripts with muscle-specific splicing, including the Capzb transcript. RT-PCR of RNA isolated from C2C12 myoblast cells transiently transfected with a series of Capzb reporter constructs revealed that this motif is required for Capzb exon 7a inclusion. RNA affinity chromatography identified hnRNP K as binding specifically to the region important for exon 7a regulation. Additionally, changes in AS during stem cell differentiation are mediated in part by the activation of distinct signaling pathways. However, how these differentiation-induced signaling pathways affect AS generally, and which splicing events are regulated by which signaling pathways, remains unknown. We are investigating whether these pathways regulate AS through specific cis-acting sequence elements using our micriarray technology.

UNIVERSITY OF CALIFORNIA, SANTA CRUZ N 046

CIRM SCHOLAR: MARTINA KOEVA

TITLE: **Spatial and temporal relationships between co-expressed genes in stem cells and differentiating cells**

AUTHORS: **Martina Koeva and Josh Stuart**

As a cell differentiates we often observe some significant changes in the expression of the genes involved in the maintenance of a stem cell or in the process of differentiation. These transcriptional changes, as reflected in many microarray experiment results, are associated with the necessary change in the regulation of the activity of those genes in response to the transition from quiescence to differentiation, or from quiescence to proliferation. We are interested in understanding how genes coordinately change their expression patterns, based on their location on the chromosome and on their temporal position in the differentiation process. In particular, we want to analyze the relationship between changes in chromosomal domains of co-expressed genes and time. We develop a computational method to analyze time series gene expression data for microarray experiments in hematopoietic, embryonic and other types of stem cells. We apply this method to a publicly available genome-wide time course study of gene expression in embryonic stem cells. We look for statistically significant clusters of genes that are co-expressed within a single time point and between multiple time points and attempt to resolve the significance of the identified co-expression domains.

UNIVERSITY OF CALIFORNIA, SANTA CRUZ N 047

CIRM SCHOLAR: COURTNEY ONODERA

TITLE: **Changes in transcriptional enhancer activity of the second-fastest-evolving human genomic region**

AUTHORS: **Courtney Onodera, Sara Sowko, Bryan King, Armen Shamamian, Matt Weirauch, Sofie Salama, and David Haussler**

The recently discovered Human Accelerated Regions (HARs) are human genomic regions highly conserved in mammals but with extensive substitutions since our last common ancestor with the chimpanzee[1,2]. The functions of these elements largely await experimental validation, but are likely to provide clues towards the specifics of uniquely human evolution. We have focused our studies on the second-highest ranking HAR, HAR2, which is a 119 base-pair non-coding element containing 12 human-specific substitutions. We hypothesize HAR2 functions as a distal transcriptional regulatory element, and further that the human-specific substitutions lead to an altered regulatory capacity in human relative to other mammals. We show using a LacZ reporter assay in transgenic mice that HAR2 functions as a neural-specific transcriptional enhancer. To further investigate this finding we have developed an assay using a luciferase reporter to test the ability of HAR2 to regulate transcription throughout the differentiation of mouse embryonic stem cells (mESCs) to neurons. From preliminary experiments we demonstrate HAR2 is capable of driving expression in undifferentiated mESCs. We show in addition that the human and chimpanzee versions of HAR2 have differing activity. To assess which of the human-specific substitutions contribute to this altered activity, we analyzed the human and chimpanzee sequences for transcription factor binding sites. Results from two independent methods indicate several novel binding sites are created and two are ablated in human HAR2 as a result of the human-specific substitutions. Based on this analysis, we attempt to reconstitute human-like activity in chimpanzee HAR2 using site-directed mutagenesis; current progress with this work will be presented. Our comparative study of human and chimpanzee HAR2 activity throughout the neural differentiation process may shed light on regulatory changes that led to human-specific features of the human brain.

UNIVERSITY OF CALIFORNIA, SANTA CRUZ N 048

CIRM SCHOLAR: ANNE ROYOU

TITLE: **A somatic stem cell divisions failure phenotype associated with ageing in Drosophila ovaries**

AUTHORS: **Anne Royou and William Sullivan**

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. Each ovariole contains several oocytes at different stages of maturation. In this work, we describe a new mutation that lead to a dramatic reduction of female fertility with ageing. We observed that the mutant females lay half as many eggs as wild type, and that the fertility in the mutant dramatically decreases with age. In contrast to wild type, the female mutants are sterile by the time they reach 11 days old. In two days old females, the ovarioles look normal although some have oocytes at different stages of maturation encompassed within one layer of FCs. The occurrence of these "compound oocytes" increases dramatically with age. As the grp mutant ages, most of the oocytes from each ovariole are encompassed within only one layer of FCs. As a result, these oocytes degenerate. This lack of FC production associated with age is a novel phenotype and strongly suggests a defect in SSC division. We located the mutation on the left arm of the chromosome 2 at the region 31E7 and 31F5. This region comprises 18 genes. One has been shown to be involved in oogenesis. We are currently testing alleles of several genes comprised in that region. The identification of the gene responsible for the lack of FC production during oogenesis will provide insight on SSC regulation in Drosophila. In the long term, it will provide a basis for understanding the cause of human female sterility.

UNIVERSITY OF CALIFORNIA, SANTA CRUZ N 049

CIRM SCHOLAR: HEMA VAIDYANATHAN

TITLE: **Eph/ephrin signaling in mouse embryonic stem cells.**

AUTHORS: **Hema Vaidyanathan and David Feldheim**

Cell contact dependent signaling plays an important role in the stem cell niche by regulating the decisions of the stem cell progeny to self renew, differentiate down a variety of pathways, and migrate out of the niche into their proper destination. The stem cell niche is maintained by a network of complex signals set off by cell-cell interactions, interactions with extracellular matrix molecules, secreted growth factors, and molecules present in the surrounding vasculature. While adherens junction proteins have been shown to be important cell-cell signaling molecules in the niche, other key molecules are also likely to be used to regulate decisions in the niche.

The Eph family of receptor tyrosine kinases and their cell surface bound ligands, ephrins, form a large family of cell-contact mediated signaling molecules. They are composed of two major sub-families, the A and B type Ephs and ephrins. Eph/ephrin signaling is unique in that both the receptor and the ligand are capable of transducing the signal into the cell and thus Eph and ephrin containing cells can communicate bi-directionally. Eph/ephrin signaling is involved in diverse processes such as axon guidance, angiogenesis, proliferation and adhesion and neural stem cell proliferation.

Here, we show our work on understanding the role of Ephs and ephrins function in the differentiation of mouse embryonic stem cells. Multiple members of these large gene families are expressed in ES cells and during neural differentiation. Induction of EphA and ephrin-A signaling pathways either by addition of soluble Ephs and ephrins, or by differentiating embryoid bodies on ephrin coated surfaces influences neural differentiation. We also find that signaling through ephrin-As has different effects on stem cell differentiation than signaling through EphAs, suggesting a model whereby cells can compartmentalize differentiation decisions within a niche. Understanding the role of Eph/ephrin signaling in maintaining the self-renewing properties of these cells will help develop better strategies to improve differentiation and culture conditions for these cells.

ABSTRACTS
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CIRM SCHOLAR: BEN DEVERMAN

TITLE: **Exogenous leukemia inhibitory factor stimulates oligodendrocyte progenitor cell proliferation and the generation of oligodendrocytes following cuprizone-induced demyelination.**

AUTHORS: **B.E. Deverman, S. Bauer, and P.H. Patterson**

Multiple sclerosis (MS) is characterized by motor, sensory, and cognitive deficits that result from focal demyelinated lesions in the brain and spinal cord. These lesions are often remyelinated in the early stages of relapsing-remitting MS by newly generated oligodendrocytes derived from oligodendrocyte progenitor cells (OPCs). While remyelination can be efficient, it is often inconsistent and is largely absent in the chronic lesions that accumulate during the course of this progressive disease. Currently there is a paucity of therapies aimed at promoting remyelination. Factors that stimulate OPC proliferation and/or encourage oligodendrocyte maturation are being investigated for their potential use as treatments aimed at promoting remyelination and ameliorating clinical deficits. One factor that may play a positive role in remyelination is leukemia inhibitory factor (LIF). Based on our previously reported findings that exogenous LIF stimulates neural stem cell self-renewal and OPC proliferation in vivo, 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, we fed mice a diet containing cuprizone for 12 weeks, a course of treatment that induces chronic demyelination, and injected the mice 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 enhanced OPC response as demonstrated both by a significant increase in NG2 staining in the demyelinated medial corpus callosum (CC) and hippocampus, and by an increase in the number of proliferating OPCs in the hippocampus. By 6 weeks, the number of CC1+ oligodendrocytes in the hippocampus of LIF-treated mice is increased as compared to Ad-lacZ controls, but there is no significant change in the number of oligodendrocytes in the medial CC. Our finding that LIF can promote oligodendrocyte generation 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.

CIRM SCHOLAR: CORY (RONG-GUI) HU

TITLE: Controlled genetic ablation of arginyl-transferase (ATE1) in embryonic stem (ES) cells and its effect on differentiation of ES cells in vitro

AUTHORS: Cory Rong-Gui Hu, Christopher Brower, and Alexander Varshavsky

Many intracellular proteins are targeted for degradation through the recognition of their specific features, called degradation signals or degrons, by the ubiquitin system. An essential determinant of one class of degrons, called N-degrons, is a substrate's destabilizing N-terminal residue. The set of destabilizing residues in a given cell type yields a rule, called the N-end rule, which relates the in vivo half-life of a protein to the identity of its N-terminal residue. The N-end rule has a hierarchic structure. In particular, N-terminal cysteine (Cys) is a tertiary destabilizing residue in that it functions through its modifications, at first, by nitric oxide (NO) and oxygen, and subsequently through its conjugation, by ATE1-encoded isoforms of Arg-tRNA protein transferase (R-transferase), to Arg, one of the primary destabilizing residues. R transferase also arginylates the secondary destabilizing N-terminal residues Asp and Glu. N-terminal arginylation was previously shown to be essential for viability in mammals, in part through its role in the cardiovascular development. Our previous work identified G protein regulators RGS4, RGS5 and RGS16 as NO/arginylation-dependent N-end rule substrates (Hu et al., 2005 Nature, 473: 981-986).

In the present work, we are employing the recently constructed mouse embryonic stem (ES) cells that lack one copy of the ATE1 gene and have another copy of it "floxed", i.e., poised to be deleted through a (transient) presence of the Cre recombinase in ATE1-/flox ES cells. As expected, the expression of Cre was found to yield ES cells that lacked arginylation. This work has also involved adjustments of cell culture conditions that allowed the growth of undifferentiated ES cells without a feeder cell layer.

Using this recently developed experimental setup, we are beginning to explore perturbations in the ability of ES cells to differentiate into specific cell types in culture as a function of their containing or lacking R transferase (ATE1). Amongst several regimens that are being employed are conditions that direct wild type (ATE1 containing) ES cells to differentiate, in particular, into cardiomyocytes. The procedure involves a transient introduction of the His6-TAT-NLS-Cre protein (a regimen that bypasses DNA based transfection) into ATE1-/flox ES cells, followed by the addition of cardiogenol and incubation with this inducer of cardiomyocyte differentiation. The appearance of cardiomyocytes in culture is monitored using several criteria, including spontaneous rhythmic contractions of cardiomyocytes, as well as the presence of cardiomyocyte-specific markers, such as myosin heavy chain (MHC), GATA-4, and the cardiac muscle cell-specific transcription factors MEF2 and Nkx2.5.

CIRM SCHOLAR: LONG LI

TITLE: **Molecular analysis of T-lineage commitment: the role and regulation of Bcl11b**

AUTHORS: **Long Li and Ellen V. Rothenberg**

Hematopoietic stem cells (HSC) commit to the T-lineage by gradually losing developmental plasticity and gaining T cell identity. Hematopoietic stem cells (HSC) commit to the T-lineage by gradually losing developmental plasticity and gaining T cell identity. A recently discovered transcription factor Bcl11b is expected to be a key regulator in this process. In hematopoietic tissue, the expression of Bcl11b is restricted to the T-lineage. Its mRNA level increases dramatically once HSCs commit their fate to T-lineage. Loss-of-function studies revealed that Bcl11b was essential for T cell development. We have found its expression is opposite to that of PU.1, which represents at a later stage the potential to differentiate into B and myeloid lineages, and to other stem cell genes such as SCL, Id2 and GATA2. However, Bcl11b is apparently a repressor, not an activator, because it interacts directly with histone deacetylases. Based on this evidence, it is rational to speculate that Bcl11b could be one of the genes for which we have been looking that stimulates T cell development by inhibiting developmental plasticity of T cell precursors. The objective of this project is to address the role of Bcl11b and the mechanism that turns it on in T cell development.

Our data so far has shown that one transcript of Bcl11b: exons 1-3-4 (Bcl11b 1-3-4) may stimulate T cell development in vitro. Fetal liver HSCs infected with retroviral Bcl11b (MigR1-Bcl11b 1-3-4) were co-cultured with OP9-Delta1 (OP9-DL1) bone marrow stromal cells or OP9 control cells. OP9 control cells have been shown not to support the development of HSC to T-lineage, because the cells do not produce an essential T cell development signal Notch ligand Delta1. Our data showed that after 4 days of co-culturing HSCs with OP9 control cells, infected HSCs showed no developmental progression: almost all cells were c-kit+ CD25-; however, MigR1-Bcl11b 1-3-4 transfected cells reached the c-kit+, Thy1+ and CD25+ DN2 stage only on OP9-DL1 cells. Our data revealed that Bcl11b may stimulate T cells development and may even have a novel relationship to Notch. We are going to study functional role of all four transcripts of Bcl11b (Bcl11b 1-2-3-4,1-2-4, 1-3-4 and 1-4) in early T cell development by using retroviral gene delivery and Bcl2 transgenic mouse model.

The expression pattern of Bcl11b is unique, because it is the only gene we have found so far showed such developmental stage as well as T-lineage specific expression pattern. Understanding of how Bcl11b is regulated will dramatically increase our knowledge on T-lineage commitment. We have so far mapped DNA methylation of promoter region in Bcl11b by using bisulfite DNA sequencing. Apparently, there is an unmethylated window from -900 to -300 bp in Bcl11b expressing P2C2 cells. We are going to map chromatin markings, conserved enhancers/repressors, and transcription factors that regulate the expression of Bcl11b in T-lineage.

CIRM SCHOLAR: AGNES LUKASZEWICZ

TITLE: **Control of neural stem-to-progenitor transition by CyclinD family members.**

AUTHORS: **Agnes Lukaszewicz and David J. Anderson**

Human embryonic stem (hES) cells are believed to soon be a key tool to repair or replace diseased or damaged tissue. For instance, replacing motorneurons (MN) degenerating in the Amyotrophic Lateral Sclerosis appears to be one of the most promising treatments for this disease. Improving the knowledge of molecular mechanisms regulating the differentiation of ES cells into neurons is the first crucial step to achieve this goal.

Dr. David Anderson's lab has been interested for several years in understanding the molecular control of neural fate specification during development, using MN as a model. MNs are derived from a specific progenitor domain: the pMN domain of the spinal cord. While carrying a systematic characterization of changes in gene expression in this domain, genes coding for cell cycle regulators, the CyclinDs, have been isolated as potential candidates to regulate the homeostasis of the developing spinal cord.

CyclinD1 and D2 have been identified as specifically expressed in distinct subset of precursors, dynamically regulated during the neuronal to glia transition. This led us to hypothesize that CyclinD1 may regulate neurogenesis, whereas CyclinD2 may regulate the maintenance of the NSC. By modulating CyclinD1 level of expression, we monitored effects on the neurogenesis in accordance to our hypothesis. Furthermore, we showed that CyclinD1 re-expression is sufficient for glial-restricted progenitors to regain their neurogenic potential when transplanted into a permissive environment. Hence, we demonstrated that CyclinD1, independently of any effects on cell cycle, modulates the generation of MN. We believe this constitute an unexpected result of great importance for the field.

In parallel, CyclinD2 seemed to exert opposite effects on neurogenesis. In order to determine if this reflects a role in maintaining NSC in the developing spinal cord, the impact of CyclinD2 expression on neurosphere formation activity is now being tested. Furthermore, these exciting observations raise the question whether the modulation of CyclinD1/D2 level of expression could be a way to influence ES cells along the in vitro neuronal differentiation pathway. This possibility will be tested by modulating CyclinDs level of expression in differentiating ES cells.

CIRM SCHOLAR: JOEL SMITH

TITLE: In vivo regulation of embryonic stem cells during sea urchin development

AUTHORS: Joel Smith, Evelyn Chou, and Eric Davidson

Significant technical barriers exist in the primary established systems for embryonic stem cell research. We are seeking to take advantage of several features of the sea urchin developmental model to achieve a better functional understanding of ES cell regulation in vivo and to gain evolutionary perspective.

The chief experimental benefits are:
(1) Sea urchin embryonic stem cells can be studied within their native context for all stages of development. Mammalian and chick cell cultures require upfront, empirical identification of factors driving stem cell fate choices for each and every differentiation pathway. The ability to study ES cells within the animal instead allows systematic genetic pertubation (see below) to define the regulatory factors controlling ES cell behavior.

(2) Previous work has identified regulatory relationships governing territorial specification in the embryo, including a complete accounting of all transcription factors expressed during embryogenesis. This supplies the foundation for building related regulatory networks governing sea urchin ES cells.

(3) Genetic perturbations are straightforward by means of microinjection of nucleic acids for gene overexpression, targeted gene knockdown or reporter assays.

(4) Sea urchins are the best-studied non-chordate deuterostome. Whether ES cells in sea urchins share common ancestry with those of chordates (homology) or are the result of an independent invention (homoplasy), a study of sea urchin ES cells will inform our understanding of the evolutionary origins of ES cells.

Sea urchins possess a distinct population of embryonic cells with ES cell characteristics called the coelomic pouch. Two lineages contribute cells to the coelomic pouch, the founding group being the small micromeres, born of an unequal cleavage with the micromeres at the embryonic vegetal pole. The small micromeres appear to resist the differentiation programs of neighboring cell populations, including that of their large micromere sisters which will ingress and become the larval skeleton, the endoderm with which they are in close contact, and the secondary mesenchymal cells (SMCs) which ingress into the blastocoelar cavity at gastrulation and differentiate into a number of cell types including muscle and cells with putative immune functions.

Instead the small micromeres remain undifferentiated and slowly dividing at the site of the future coelomic pouches. Near the later stages of larval development a subset of SMC-derived cells, the second group to contribute to the coelomic pouches, migrate to the sites of the small micromeres. At this point the coelomic pouch cells undergo multiple rounds of division. By means that are still unclear but which involve patterning by Nodal and Lefty, the left coelomic pouch is subsequently specified for differentiation into adult tissue.

The transcription factor FoxY is the earliest known gene expressed exclusively in the small micromeres. The sequencing of the sea urchin genome and subsequent exhaustive search for genes coding for DNA-binding motifs has revealed a dozen or so other transcription factors expressed only slightly later in the coelomic pouch cells. Our primary experimental goal is to determine the regulatory relationships of all the genes expressed in the small micromere lineage.

CHILDREN'S HOSPITAL OF LOS ANGELES S 006

CIRM SCHOLAR: DAVID CHANG

TITLE: **Molecular dissection of the functional domains of NANOG**

AUTHORS: **David F. Chang, Dinithi Senadheera, Xingchao Wang, Xiao Jin Yu, and Carolyn Lutzko**

Background: Embryonic stem cells (ESCs), derived from the inner cell mass (ICM) of pre-implantation blastocysts, have unique capabilities for unlimited self-renewal while maintaining the ability to generate all tissue types of an embryo. The development of human ESCs (hESCs) brings hope that these cells will provide great promise for cell replacement therapy, drug discovery, as well as the study of early human development. In our present study, we focus our attention to a key transcriptional regulator, NANOG, a homeodomain protein that is necessary to maintain ESCs undifferentiated and proliferative in culture.

Hypothesis: We hypothesized that the structure and function of individual protein domains of NANOG may be involved in self-renewal and differentiation of stem cells.

Methods: Using RT-PCR, human NANOG cDNA was amplified from H1 human ESC total RNA lysate, and subsequently cloned into a mammalian expression construct, pcDNA3.1. This construct was used as template to generate a series of deletion-mutant and point-mutant constructs.

Results: Analysis of the cellular localization of these clones via heterologous transient transfection revealed putative nuclear localization signal sequence and nuclear export signal sequences within NANOG. Furthermore, functional characterization via luciferase reporter assay identified distinct protein domains that confer four or five fold more transcriptional active or repressive, respectively, of the full-length NANOG.

CIRM SCHOLAR: DOROTHEA DOUGLAS

TITLE: **The role of Bmi 1 in the Ewing sarcoma family of tumors**

AUTHORS: **Dorothea Douglas, Jessie Hao-Ru Hsu, Long Hung, Aaron Cooper, John van Doorninck, Grace Peng, and Elizabeth R. Lawlor**

BACKGROUND
The Ewing's Sarcoma Family of Tumors (ESFT) comprises a group of aggressive bone and soft tissue tumors. ESFT express chromosomal translocations that create fusion genes, most commonly EWS-FLI 1. It functions as an aberrant transcription factor but the precise mechanisms underlying malignant transformation are unknown. EWS-FLI 1 mediated transformation of primary fibroblasts requires that tumor suppressor pathways be inactivated.

The polycomb gene Bmi-1 which is critical for self renewal of normal somatic stem cells is highly expressed in many human cancers. It functions as a cooperative oncogene in murine and human leukemogenesis. In this study we investigate the role of Bmi1 as a potential cooperative oncogene in the pathogenesis of ESFT.

RESULTS
We found that all ESFT cell lines tested express Bmi-1. Among primary tumors, over two thirds display diffuse tumor cell positivity while only occasional Bmi-1 positive cells are seen in the remainder.

In contrast to reports of other human tumor cell lines, knockdown of Bmi-1 does not induce ESFT cell death. In addition, although Bmi-1 knockdown results in reduced BrdUrd uptake, this does not significantly alter the rate of culture expansion. However, our data do support a pivotal role for Bmi-1 in promoting the anchorage independent growth of ESFT cells. Overexpression of Bmi- 1 in ESFT cell lines resulted in enhanced colony formation whereas knockdown resulted in inhibition of colony formation. Animal studies to assess the effects of Bmi-1 modulation on tumorgenicity in vivo are in progress. Interestingly, Bmi-1 knockdown does not result in an increase in p16 expression and Bmi-1 overexpressing cells do not demonstrate reduced p16. This suggests that the growth promoting effects of Bmi-1 may be independent of p16 repression.

Finally, to assess if Bmi1 and EWS-FLI 1 cooperate in cellular transformation we evaluated the consequences of EWS-FLI1 expression in normal human fibroblasts expressing Bmi-1 as a result of genetic modification. In preliminary studies we have found that while expression of EWS-FLI1 in these cells induces growth arrest, this arrest response is abrogated in cells that express both EWS-FLI1 and Bmi-1.

CONCLUSION
We have shown that the polycomb gene Bmi-1 is expressed by ESFT and that it functions as a growth-promoting gene in ESFT cell lines. Furthermore, our data show that expression of Bmi-1 in normal fibroblasts may create a permissive environment for EWS-FLI1 expression. Our findings support the hypothesis that Bmi-1 functions as a crucial cooperative oncogene in the pathogenesis of ESFT.

CHILDREN'S HOSPITAL OF LOS ANGELES S 008

CIRM SCHOLAR: GAUTAM DRAVID

TITLE: **Mapping the hierarchical development of definitive hematopoietic stem cells during human embryonic stem cell differentiation.**

AUTHORS: **Gautam Dravid, Yuhua Zhu, Lora Barsky, and Gay Crooks.**

The goal of our studies is the derivation of definitive and transplantable human hematopoietic stem and progenitor cells. Differentiation of hESC using stromal co-cultures or by formation of embryoid bodies (EBs) has been demonstrated. Most of these studies use serum with or without cytokines, thus making it difficult to assess the relative importance of each component of the culture system. In this initial phase of the project, our aim was to decipher using serum-free, stroma-free conditions, specific morphogens involved in mesoderm commitment, and the timing for production of hematopoietic stem and progenitors cells during the differentiation process. hESC (H9 and H1) cells were cultured as embryoid bodies in Stem Line II medium supplemented with chemically defined nutrients with bFGF and BMP-4. In initial experiments EB derived (EBD) cells were isolated and analyzed for hematopoietic markers c-kit, KDR, CD31, CD34, CD43, CD44, CD45 by FACS. EBD cells were also subjected to RT-PCR analysis to look for early mesoderm and hematopoietic commitment and to clonogenic assays for progenitors. To look for generation of definitive multi-potential hematopoietic stem cells, EBD cells were plated on MS-5 stromal cells under optimal erythro-myeloid and lymphoid conditions respectively. Cells were monitored over 2-3 weeks for generation of identifiable myelo-lymphoid stem/progenitor cells. EB produced in the presence of BMP4 and bFGF, were able to generate of CD10+ granulocytic precursors and CD56+ NK cells. In addition, EBD were able to generate CD34+ cells for up to 4 weeks after re-plating on MS-5 stroma. RT-PCR analysis showed early expression of mesodermal marker brachyury, (day 2 and 4) which preceded the expression of SCL and GATA-1 indicating a sequential mesoderm to hematopoietic commitment. Similar experiments have been performed by re-plating purified CD34+ cells generated from hEB. Our latest results to define the elusive definitive HSC and its possible precursor cell will be discussed. We thus aim to identify, isolate and characterize the developmental hierarchy of mesodermal commitment to hematopoietic progeny including multipotent transplantable HSCs.

CIRM SCHOLAR: XIAOHUA JIANG

TITLE: **hESC as tools to study neural crest origin of Ewing's sarcoma**

AUTHORS: **Xiaohua Jiang, Ynnez Gwye, and Elizabeth Lawlor**

Background: Ewing's sarcoma family tumors (ESFT) are the second most common bone and soft tissue malignancy of children and young adults. All ESFT express characteristic fusion oncogenes, most commonly EWS-FLI1. The EWS-FLI1 Background: Ewing's sarcoma family tumors (ESFT) are the second most common bone and soft tissue malignancy of children and young adults. All ESFT express characteristic fusion oncogenes, most commonly EWS-FLI1. The EWS-FLI1 fusion protein behaves as an aberrant transcriptional activator and is believed to contribute to ESFT development. However, the mechanism of EWS-FLI1-induced transformation remains obscure, largely because it induces growth arrest and apoptosis in most differentiated cells, hampering basic understanding of ESFT biology. Given the putative neural crest (NC) origin of ESFT we hypothesize that EWS-FLI1 expression in neural crest stem cell (NCSC) and disruption of NC differentiation are initiating events in ESFT.

Aim: Investigate the consequences of EWS-FLI1 expression on NC differentiation from human embryonic stem cells (hESCs)

Materials and Methods: We used a modification of the method of Pomp and colleagues (Pomp O et al., 2005) for human embryonic stem cells to elicit NC differentiation from both H1 and H9 cells. In brief, on Day 0 approximately 40 mechanically dissociated H1 or H9 colonies were plated on gelatin-coated, PA6 seeded 35mm Petri dishes. Induction media were added on Day 0 and changed on days 4 and 6. On day 8, the medium were further modified to a defined neural media and were replaced every 2 days for the duration of experiments. Successful NC induction and differentiation were confirmed by weekly morphologic, genetic and immunocytochemical methods.

Results: After 5 days of culturing hESCs with PA6, a distinct morphological change in the hESC colonies was observed. Undifferentiated hESCs cultured with mouse fibroblasts appeared as dense, round, or oval monolayer colonies. Differentiating hESC colonies, by contrast, were irregular in outline with cells migrating out of the colonies after 5 days of SDIA treatment. Three weeks after seeding H9 on PA6 cells, massive neuronal differentiation was observed. More than 90% of the colonies were Tuj-1+ with extensive networks of stained axons. Among them, 78.1% of the colonies contained Tuj-1+/peripherin- CNS-like neurons, and 17.7% contained Tuj-1+/peripherin+ PNS-like neurons. In addition, after 1 week of co-culture, approximately 50% of the colonies contained cells which stained positive for the low-affinity neurotrophin receptor p75, which is expressed in migrating neural crest cells. Our RT-PCR result also supported the induction of NCSC from H9. We found several NC markers, including p75, Sox9, Bmi-1, Trkc and Msx-1, were all dramatically induced relative to naive cells after 1 week of SDIA treatment and were reduced after 3 weeks of treatment. The upregulation of these genes in the 1 week cultures, and the subsequent decrease in their expression by 3 weeks of culture, are consistent with the presence of NC stem-like cells in the 1-week cultures, and their terminal differentiation into neurons by the third week.

Discussion: Our results suggest that we are able to induce NC differentiation from hESCs and this system can be used for studying the consequences of EWS-FLI 1 expression in the almost inaccessible human NC.

CHILDREN'S HOSPITAL OF LOS ANGELES S 010

CIRM SCHOLAR: LAURA PERIN

TITLE: **In vivo applications of amniotic fluid stem cells**

AUTHORS: **Laura Perin, Stefano Giuliani, Sargis Sedrakyan, Casey Brewer, Anthony Atala, David Warburton, and Roger De Filippo**

Aim of study: The growing shortage of donor organs has raised interest in alternative strategies for tissue regeneration. I have been studying a new population of cells derived from human and mouse amniotic fluid (hAFS, mAFS) that express both embryonic and mesenchymal stem cell markers to apply these cells for regenerative purposes.

These cells express stem cell markers (Oct-4, SSEA-4), are clonogenic, and are able to differentiate into all three germ layer derivates in vitro. Previous histological and molecular analysis revealed that hAFS were capable of contributing to primordial kidney structures including renal vesicle, C and S-shape bodies that ultimately form the glomerular and tubular components of the mature kidney once injected into mouse embryonic kidneys. With this project, I would like to determine the capacity of amniotic fluid stem cells to differentiate into normal kidney structures in vivo.

Methods: hAFS and mAFS were transfected with lentivirus codifying for GFP, Lac-z or luciferse. The labeled AFS are then intravenously (tail vein) or directly injected into previously glycerol-damaged kidneys of nude mice. The animals were monitored using bioluminescent detection at specific time points and then were sacrificed for histological and molecular analysis to determine the ability of AFS to survive, replicate and differentiate in an in vivo model.

Main results: Our in vivo results demonstrate that AFS survive after both tail vein and direct injection into damaged kidneys as shown by luciferase detection. Histological analysis of serial sections show that mAFS and hAFS replicate and contribute to kidney structures. The injected stem cells were able to differentiate into tubular structures expressing renal markers such as Aquaporin2, Dolichos Biflorus, agglutinin and Peanut Agglutinin and in addition they are able to express GDNF, a specific branching inducer, of glomeruli differentiation.

Conclusion: The preliminary in vivo data suggest that AFS can survive and integrate once injected into an animal model. Further investigations need to be performed in order to confirm the viability and the functional differentiation of the stem cells for potential use in renal regeneration. AFS could, therefore, represent a limitless source of ethically neutral, unmodified stem cells that may prove useful as a novel alternative for whole organ regeneration or cell therapeutic techniques in the future.

CHILDREN'S HOSPITAL OF LOS ANGELES S 011

CIRM SCHOLAR: KEN SAKURAI

TITLE: **Lineage-specific lentiviral vectors for hESC differentiation.**

AUTHORS: **Ken Sakurai, Roger Hollis, Arya Khosravi, and Donald B. Kohn**

hESC have potential to be used for regenerative medicine, by providing sources of specific tissue cell types. Genetic modification of hESC may have important roles as experimental tools to manipulate and characterize ES biology and differentiation. Treatment of genetic diseases using hESC and somatic nuclear transfer will also require gene modification or correction. Lentiviral vectors provide the most efficient and non-toxic method for permanently modifying hESC. Non-integrating lentiviral vectors may be used for transient gene expression in hESC. Lentiviral vectors with lineage-specific promoters may be useful to identify cells that have progressed along specific differentiation pathways. Sox17 and Brachyury are transcriptional factors and are expressed in endodermal and mesodermal cells of early embryos, respectively. Sox17 is an HMG box transcription factor and a key component of the molecular pathway during gastrulation and is essential for endoderm formation. Brachyury is also a transcriptional factor that is required in formation of posterior mesoderm and meso-endoderm and in axial development. We have developed lentiviral vectors that use the human Sox17 and brachyury gene promoters and are assessing their ability to be expressed specifically in cells of the endodermal and mesodermal lineages, derived from hESC. These vectors may be useful for identifying and isolating hESC that have undergone initial stages of differentiation and for assessing methods that direct differentiation.

CIRM SCHOLAR: CINDY TAI

TITLE: FGF10 signaling enhances intestinal epithelial progenitor cell proliferation

AUTHORS: C. C. Tai, F. G. Sala, P. M. Del Moral, J. L. Curtis, S. De Langhe, H. R. Ford, T. Grikscheit, K. S. Wang, and S. Bellusci

Purpose: While Fibroblast Growth Factor 10 (FGF10) is a key regulator of colonic epithelial survival and proliferation during gut organogenesis, Fgf10 is expressed at low levels in the newborn or adult mouse ileum during gut homeostasis. We have previously shown that Fgf10 is upregulated in the ileum after massive small bowel resection, suggesting that FGF10 may be a positive regulator of gut adaptation. FGF10 acts through tyrosine kinase transmembrane receptors, of which the main receptor Purpose: While Fibroblast Growth Factor 10 (FGF10) is a key regulator of colonic epithelial survival and proliferation during gut organogenesis, Fgf10 is expressed at low levels in the newborn or adult mouse ileum during gut homeostasis. We have previously shown that Fgf10 is upregulated in the ileum after massive small bowel resection, suggesting that FGF10 may be a positive regulator of gut adaptation. FGF10 acts through tyrosine kinase transmembrane receptors, of which the main receptor is FGFR2b. To further study the role of FGF10/FGFR2b signaling on intestinal epithelial proliferation and differentiation, we have generated transgenic mice with inducible expression of either Fgf10 or dominant negative soluble Fgfr2b. We hypothesize that over-expression of Fgf10 in adult ileum leads to increased intestinal epithelial progenitor cell proliferation and that inactivation of FGFR2b leads to decreased proliferation.

METHODS: CMV-Cre driver mice were crossed with rtTAflox;tet(O)Fgf10 or rtTAflox;tet(O)sFgfr2b responder mice to generate mice capable of inducible pan-somatic expression of Fgf10 or the dominant negative soluble Fgfr2b. Similarly, Villin-Cre mice were crossed with the same responder mice (rtTAflox;tet(O)Fgf10 or rtTAflox;tet(O)sFgfr2b) to generate mice with inducible expression of Fgf10 or the dominant negative soluble Fgfr2b specifically in the gut epithelium. Expression of Fgf10 or soluble Fgfr2b was induced by doxycycline for three days and for three months, respectively. Controls were age-matched wild type (WT) on doxycycline diet for the same period of time. Immunohistochemical analysis was performed with Musashi antibody, proliferating cell nuclear antigen (PCNA), H & E, alcian blue, lysozyme antibody, and chromogranin-A antibody.

RESULTS: Over-expression of Fgf10 leads to an expansion of the progenitor cell population (50% increase in PCNA positive cells) compared to controls. There is no statistically significant decrease in proliferating cells with the expression of the dominant negative soluble Fgfr2b. Over-expression of Fgf10 in the ileum is associated with 100% increase in the number of goblet cells via alcian blue staining, whereas inactivation of FGFR2b signaling is associated with a 30% decrease. There is no significant difference in the number of enterocytes, Paneth cells or enteroendocrine cells by immunohistochemistry.

CONCLUSIONS: We conclude that FGF10 signaling is a key player in intestinal epithelial proliferation and differentiation. Over-expression of Fgf10 is associated with an increase in the progenitor cell population, possibly by maintaining the transit amplifying cells in an undifferentiated state. Other pathways, such as Wnt signaling, are likely involved as well since inactivation of FGFR2b did not lead to a significant decrease in the number of proliferating cells. We have also shown that FGF10/FGFR2b signaling promotes goblet cell differentiation in adult mice. This pathway is likely to be critical in gut adaptation, as goblet cell population is known to increase after massive intestinal resection. We speculate that since goblet cells are an integral part of the intestinal mucosal barrier, disruption of such barrier may play a pivotal role in diseases such as inflammatory bowel syndrome or necrotizing enterocolitis.

CIRM SCHOLAR: ANTHONY BOITANO

TITLE: **Human hematopoietic stem cell screens**

AUTHORS: **Anthony E. Boitano, Michael P. Cooke, and Peter G. Schultz**

Hematopoietic stem cells (HSCs) possess a distinct ability to perpetuate through self-renewal and to generate progeny that differentiate into mature cells of myeloid and lymphoid lineages. They hold great promise in regenerative medicine, tissue repair, and tumor biology. However, our understanding of the mechanisms that determine whether, where and when a stem cell will self-renew or differentiate is still limited, and a better understanding of the molecular mechanisms by which HSCs replicate and differentiate from the perspective of developing new approaches for HSC transplantation is necessary for further advances. With this in mind, we purified CD34+ human HSCs from mobilized peripheral blood and carried out both genetic and high throughput screens of >100,000 synthetic small molecules to identify factors that promote self-renewal or control differentiation. After confirmation and secondary assays, we have identified a class of small molecules that promote self-renewal of CD34+ HSCs and another class that directs HSC differentiation down the megakaryocyte lineage. From the genetic screens we discovered a novel gene that drives hematopoiesis down the myeloid lineage. Identification of the target and mechanism of action these factors will reveal insight into the signaling pathway controlling HSC self-renewal and differentiation, and may ultimately facilitate development of small molecule therapeutics.

CIRM SCHOLAR: RANDOR RADAKOVITS

TITLE: **Integrins control neural stem cell numbers in the developing cerebral cortex**

AUTHORS: **Randor Radakovits and Ulrich Muller**

Stem cells in the developing and adult nervous system resides in stem cell niches that are thought to control the self-renewal and differentiation of stem cells. Stem cell niches contain defined assemblies of extracellular matrix (ECM) glycoproteins that are thought to interact with stem cells and sequester secreted growth and differentiation factors. However, the mechanisms that control the self-renewal and differentiation of neural stem cells and their interactions with the ECM are not known.

Here we show that ECM receptors of the integrin family are crucial for neural stem cell maintenance in the cerebral cortex. Mice that were genetically engineered to lack beta 1 integrins in neural stem cells in the cerebral cortex are born alive, but the cerebral cortex is severely reduced in size.

Immunohistological data and time-lapse video recordings demonstrate that cleavage spindle polarity and survival of stem cells is affected in the absence of beta 1 integrins, leading to defects in stem cell self-renewal and survival.

Collectively, our findings reveal an essential function for beta 1 integrins in neural stem cell maintenance, likely by mediating intereractions with ECM components that define the stem cell niche.

CIRM SCHOLAR: SEUNG-HEE LEE

TITLE: **Control of replication in human pancreatic beta cells and progenitors**

AUTHORS: **Seung-Hee Lee, Ifat Geron, Suzette Farber-Katz, Fred Levine, and Pamela Itkin-Ansari**

Ultimately, any therapy for diabetes based on promoting beta cell regeneration, whether by stimulating the formation of new beta cells from stem cells endogenously or through ex vivo approaches, will require an improved understanding of the regulation of beta cell replication. To facilitate eventual clinical application, and because human and rodent beta cells differ substantially in potentially important characteristics such as the rate of replication and the pattern of expression of cyclin dependent kinase inhibitors (CDKIs), we have focused our efforts on the study of human islets, and in particular on the factors that control the relationship between growth and differentiation in the human beta cell and its progenitor/stem cells. In the course of these studies we have found that p57Kip2 and p21Cip1, are the major cyclin dependent kinase inhibitors expressed in human islets. It is important to note that p57Kip2 is beta cell specific in the pancreas and is sufficient to cause beta cell hyperplasia when deleted in humans (but not in rodents). Utilizing TRM-6, a cell line we derived from human islets, and expressing PDX-1 and NeuroD1 (T6PN) we discovered that E47, a class I basic helix-loop-helix transcription factor and an important transactivator of the insulin promoter, also regulates p57Kip2 and p21Cip1. Furthermore, we have identified the specific binding site for E47 in the Kip2 promoter. Finally, we have confirmed that E47 controls Kip2 expression in human beta cells. We are currently determining the extent to which manipulation of this pathway can be used to control replication of human beta cells and pancreatic endocrine progenitor/stem cells.

CIRM SCHOLAR: JEFF LINDQUIST

TITLE: **Selective patterning of peripheral neurons with the vasculature during stem cell differentiation**

AUTHORS: **J. N. Lindquist, E. Y. Snyder, and D. A. Cheresh**

Throughout the body of the adult vertebrate, the vascular network aligns closely with the neural network suggesting that patterning of the vascular and neural systems may be regulated by a common mechanism. To investigate this, we attempted to model the earliest stages of embryogenesis in culture by employing human embryonic stem cells to measure vascular development in the context of the spontaneous emergence of the fundamental 3 embryonic germ layers.

We observed that 2 different neuronal subpopulations spontaneously arise, one that emerges in synchrony with the vascular network and another that originates spontaneously outside of the early endothelium. Our studies indicate that the neuronal network representing the early autonomic nervous system (neural crest-derived) is closely associated with the developing vasculature. In contrast, a second wave of neuronal investment from the embryoid body representing the central nervous system (CNS) (neural tube-derived) does not co-align with developing vascular networks. This was supported by labeling using lineage and cell-specific immune-markers, some under time-lapse video-microscopy, (Map2alpha, Dopa beta-hydroxylase, ChAT, peripherin, beta-III-tubulin, nestin).

Furthermore, we demonstrated co-dependence between blood vessel and autonomic neural patterning since disruption of neovascularization with a function-blocking antibody to integrin alpha V beta 3 or pharmacological inhibition of VEGFR2 selectively disrupted the vascular associated neural networks, while the CNS neuronal population was unaffected.

These findings suggest that the emergence and patterning of the endothelium is pivotal for insuring the appropriately-patterned innervation of emerging vasculature by the autonomic nervous system and then plays a role in the mutually-coordinated and appropriate co-patterning of both CNS innervation and perfusion of tissues.

CIRM SCHOLAR: YUHONG PANG

TITLE: **A conserved signaling mechanism for regulation of human and mouse ES cell differentiation by Shp2 Tyrosine Phosphatase**

AUTHORS: **Dongmei Wu, Yuhong Pang, Yuehai Ke, Zhao He, Lutz Tautz, Tomas Mustelin, and Gen-Sheng Feng**

Recent studies from a number of laboratories have suggested distinct biological properties and molecular signaling mechanisms between human and mouse embryonic stem cells (hESCs and mESCs). Here we report that Shp2, an intracellular tyrosine phosphatase with two SH2 domains, has a conserved role in promoting signals resulting in differentiation of both hESCs and mESCs. Homozygous ablation of Shp2 in mESCs resulted in impaired differentiation into all three germ layer cell lineages accompanied by improved self-renewal. siRNA-mediated Shp2 knockdown in hESCs resulted in a very similar phenotype of impaired differentiation. Furthermore, small molecule inhibitors for Shp2 enzyme suppressed both hESC and mESC differentiation in vitro. Shp2 modulates LIF and BMP signals flowing through Erk, Stat3 and Smad pathways in mESCs and also regulates FGF and BMP signaling through the Erk and Smad pathways in hESCs. These results reveal a common signaling mechanism shared by hESCs and mESCs via Shp2 modulation of overlapping and distinct pathways. Pharmaceutical intervention of Shp2 activity might be a practical means for amplification of hESCs in vitro.

CIRM SCHOLAR: TODD MACFARLAN

TITLE: **Analysis of REST and its corepressors' function in neurogenesis**

AUTHORS: **Todd MacFarlan, Soo-Kyung Lee, Yan Zhang, Thomas J. Baiga, Gordon Gill, Su-Chun Zhang, Joseph P. Noel, and Samuel L. Pfaff**

Diseases and injuries of the nervous system affect the quality of life and productivity of an estimated 50 million Americans each year. Many of these diseases could potentially be treated with stem cells or stem cells differentiated in vitro into neurons and glia. It is therefore one of our goals to characterize the fundamental gene regulatory processes that are necessary for hES cells to become neurons.

To ensure that neuronal cell fates are properly segregated during development, transcription factor repressor systems exist to restrict neuronal genes from being expressed too early and in non-neuronal cells. The RE-1 silencing factor REST has emerged as a critical transcription factor for controlling global level neuron gene expression. REST is a zinc finger protein that binds to a consensus DNA element (called RE1) found near the promoters of ~1000 neuronal genes. In non-neuronal cells, REST recruits a growing list of corepressors to establish a repressed chromatin state. Less is known about the function of REST and its corepressors in stem cells and neuronal progenitors. Since some of the corepressors have enzymatic activities, they may serve as drugable targets. We have therefore begun to analyze the expression and function of REST corepressors in neurogenesis with particular emphasis on the small CTD phosphatase SCP1. We have obtained the crystal structure of SCP1 bound to a CTD peptide and are using this information for structure based inhibitor design. We have also begun to explore the potential of using hES cell differentiation into motor neurons as a tool to study factors that regulate neurogenesis and as a testing ground for pro-neural drugs.

CIRM SCHOLAR: GERALD PAO

TITLE: **Tumor suppressor BRCA1 ubiquitylates Histone H2A and regulates heterochromatin integrity in neural stem cells**

AUTHORS: **Gerald Pao, Quan Zhu, Nina Tonnu, Alexis Huynh, Shenju Chou, Fred H Gage, Dennis M O'Leary, and Inder Verma**

During the course of studying silencing mechanisms operative in embryonic stem cells we observed that BRCA1 was highly upregulated in embryonic neural progenitors. To study the function of BRCA1 in this compartment we generated a neural stem cell conditional knockout of BRCA1 with a nestin CRE driver. The resulting animal displayed a molecular phenotype in which repetitive DNA known as satellite transcripts are transcribed which is abnormal given that these are some of the most tightly repressed regions of the genome. This phenotype is essentially identical to the phenotype displayed by the Dicer knockout ES cells, suggesting the possibility of a common pathway in the silencing process. BRCA1 is a tumor suppressor whose loss leads to breast and/or ovarian cancer. As mentioned previously, the brain specific knockout of BRCA1, that cells devoid of BRCA1 display a defect in constitutive heterochromatin formation/maintenance. Loss of BRCA1 by CRE mediated deletion leads to a decrease of heterochromatic centers and the concomitant downregulation of cellular HP1 levels and loss of transcriptional silencing at pericentromeric satellite repeats and alterations in the expression of selected imprinted genes. The heterochromatic silencing of BRCA1 is cell autonomous, as this defect can be recapitulated in cultured embryonal and postnatal neural stem cells. BRCA1 binds to satellite repeats in the mouse as well as human pericentric heterochromatin as determined by chromatin immunoprecipitation. Ubiquitin-Histone H2A adducts, an epigenetic modification associated with silencing is enriched at mouse minor and major satellite repeats. We have shown that purified recombinant BRCA1 possesses a RING ubiquityl ligase activity, and preferentially monoubiquitylates Histone H2A in vitro. The silencing of pericentromeric heterochromatin is dependent on the integrity of the BRCA1 RING domain. The ubiquitylation of Histone H2A by BRCA1 is corroborated by the in vivo observation that the deletion of BRCA1 is accompanied by the loss of the Histone Ubiquitin-H2A associated with mouse satellite repeats. The loss of BRCA1 in neural stem cells leads to increased apoptosis as well as a defect in proliferation in the mouse developing brain, which leads to numerous defects in brain structure and organization. These findings open the possibility of a requirement for heterochromatin integrity in neural proliferation and development. The present findings are not restricted to neural tissues as fibroblasts and human epithelial cell lines deficient in BRCA1 also exhibit a defect in heterochromatic silencing. We therefore propose, that cells devoid of BRCA1 are impaired in heterochromatin formation by a failure to ubiquitylate histone H2A in these regions. A function in heterochromatin integrity may reconcile large numbers of disparate functions attributed to BRCA1. We have generated human ES cells in which BRCA1 has been downregulated by siRNA and in the process of analyzing the silencing phenotype in these cells.

SALK INSTITUTE FOR BIOLOGICAL STUDIES S 020

CIRM SCHOLAR: ODED SINGER

TITLE: **The role of miRNA regulation during differentiation of human embryonic stem cells**

AUTHORS: **Oded Singer and Inder M. Verma**

Several miRNAs that are differentially expressed during differentiation of hES cells had been identified in our lab. miRNAs had been suggested before as an important component that govern the transition between stem cells and differentiated cells. The study of the importance of miRNAs is hampered due to the inability to generate gene knockouts using homologous recombination in hES cells. In order to address this question we chose to directly interfere with the maturation and action of miRNAs by expression of dominant negative molecules. Two approaches had been tested: 1) Over expression of a short hairpin RNA targeting a specific protein (DGCR8) required for the maturation step of miRNAs. 2) Over expression of an RNAi suppressor protein. We have identified a viral RNAi suppressor protein able to inhibit the action of miRNAs. Although the precise mechanism of suppression is yet unknown, it is thought that the suppressor bind to miRNA precursors in way that prevent its incorporation into the effector complex, RNA induced silencing complex (RISC). Suppression of RNAi in human ES cells will able us to generate a Dicer-knockout-like phenotype and study the effect of ablation of all functional miRNAs on the ability of ES cells to self renew and differentiate.

CIRM SCHOLAR: MATHEW BLURTON-JONES

TITLE: **Neural stem cells improve cognition in transgenic models of Alzheimer disease and neuronal loss**

AUTHORS: **Mathew Blurton-Jones, Tritia Yamasaki, Masashi Kitazawa, Hilda Martinez-Coria, Kim N. Green, Franz-Joseph Müller, Jeanne F. Loring, and Frank M. LaFerla**

Stem cell transplantation may offer a viable therapeutic approach for the treatment of a number of devastating brain injuries and disorders. It remains unknown, however, whether stem cell-based therapies can ameliorate the cognitive deficits associated with Alzheimer Disease (AD) or other disorders that involve hippocampal neuronal loss. Here we utilized the 3xTg-AD mouse model of AD and an inducible transgenic model of neuronal ablation (Tet-DT/CaM mice) to determine the impact of neural stem cell (NSC) transplantation on learning and memory. 3xTg-AD mice recapitulate many of the salient features of AD including age-related memory impairment and the accumulation of extracellular beta-amyloid plaques and intracellular neurofibrillary tangles (NFTs). In humans, AD, stroke, and traumatic brain injury are also characterized by hippocampal neuronal loss, which we can model using tetracycline-regulatable expression of Diphtheria Toxin within CA1 neurons.

Haplotype-matched murine neural stem cells (NSCs) derived from GFP transgenic mice were injected into the hippocampus of aged 3xTg-AD that exhibit well established AD and NFT pathology. Within one month of transplantation, NSC-injected mice are significantly improved in a hippocampal-dependent novel object recognition task and the Morris water maze versus vehicle-injected controls. To examine the impact of NSCs on cognition in a model with robust hippocampal neuronal loss, we genetically induced a lesion in Tet-DT/CaM mice and transplanted GFP-NSCs. Within 4 months of NSC transplantation, Tet-DT/CaM mice exhibit a significant improvement in hippocampal-dependant novel object recognition.

GFP-NSCs in both the 3xTg-AD and Tet-DT/CaM mice differentiated into all 3 NSC lineages: neurons, astrocytes, and oligodendrocytes. Notably, NSC-transplanted mice exhibited increased hippocampal synaptic density and NSCs expressed neurotrophins suggesting a plausible neurotrophic mechanism of cognitive rescue. Ongoing studies will determine the influence of NSC transplantation on beta-amyloid and tau pathologies, synaptic plasticity, and the role of NSC-neurotrophin expression in the cognitive rescue.

This work was supported by a CIRM Postdoctoral Scholar Award to Mathew Blurton-Jones and by NIA-R01AG027544 to Frank M. LaFerla.

CIRM SCHOLAR: CLAIRE DUBOIS

TITLE: **Defining the function of the pancreatic progenitor cell maintenance factor, Sox9, for endocrine function and regeneration in adult mice**

AUTHORS: **Claire Dubois, Philip Seymour, Kristine Freude, James Behrmann, Man Tran, and Maike Sander**

The transcription factor Sox9 is required for expansion and maintenance of the pancreatic progenitor cell pool, and thus, normal pancreas development. During development Sox9 is found exclusively in the pluripotent undifferentiated precursor cell pool, whereas in adulthood, expression is restricted to pancreatic ductal and centro-acinar cells – a cell population that has been speculated to have stem-cell characteristics. This compartment also expresses the transcription factor HNF1-beta, heterozygous mutations in which cause maturity-onset diabetes of the young (MODY) 5 in humans. In order to determine whether Sox9 haploinsufficiency causes MODY-type diabetes during adulthood, mice with heterozygous-deleted Sox9 were examined from birth until 20 weeks of age using glucose tolerance tests and endocrine mass measurements. Although Sox9-heterozygous mutant mice do not become diabetic even at 8 months of age, we found their ability to clear glucose from the blood after a glucose challenge to be significantly impaired compared to littermate controls as early as 6 weeks of age. In previous research our lab has found that Sox9 heterozygous-deleted mice are born with half the endocrine cell mass of normal mice. Surprisingly, although endocrine cell mass was still reduced in Sox9-heterozygous mutant pancreata by 6 weeks of age, the difference between mutants and their controls was much smaller than at birth. This suggests an adaptive mechanism in the postnatal pancreas to compensate for reduced endocrine cell mass. We have shown that cell hypertrophy does not account for the postnatal increase in endocrine cell mass, and we are currently investigating whether increased endocrine cell proliferation or reduced apoptosis is the underlying mechanism. Because the observed ~20% reduction in insulin cell numbers may not be sufficient to explain the abnormal glucose tolerance tests in Sox9-haploinsufficient mice, we are also examining endocrine cell function by measuring whole-pancreas insulin content and insulin secretion from isolated islets in vitro.

Given the crucial role of Sox9 in pancreatic progenitor-cell maintenance and cell neogenesis during embryonic life, we are determining whether Sox9 also has a function in adaptive cell growth of the adult pancreas. This possibility will be explored by comparing cell expansion and regeneration in response to high fat diet, partial pancreatectomy or ligation of the pancreatic duct.

This work was supported by the CIRM Scholar Award to Claire Dubois and Kristine Freude, research grants from the NIH/NIDDK (1R01-DK68471-01) and the Juvenile Diabetes Research Foundation (2-2001-728) to Maike Sander and a postdoctoral fellowship from the Juvenile Diabetes Research Foundation to Philip Seymour.

CIRM SCHOLAR: WEIWEI FAN

TITLE: **Introduction of a joint missense and frameshift mutation into the mouse germline resulted in the loss of the frameshift mutation and created the first mouse model of cardiomyopathy caused by a mtDNA mutation**

AUTHORS: **Weiwei Fan, Katrina Waymire, Grant MacGregor, Pinar E. Coskun, Navneet Narula, Peng Li, Christophe Rocher, Jagat Narula, Mani Vannan, and Douglas C. Wallace**

A mouse cell line harboring a joint mitochondrial DNA (mtDNA) COI T6589C missense mutation and ND6 13885insC frameshift mutation was isolated in cell culture. The linked mutations were successfully introduced into the mouse germline via female mouse embryonic stem (mES) cells. The founder female mouse with 100% of the COI mutation and 47% of the ND6 mutation had a partial complex I and complex IV defect and showed mild myopathy and cardiomyopathy. The COI mutation remained homoplasmic entirely in her offspring while the ND6 mutation was rapidly lost within three generations due to a mechanism during oogenesis that selectively eliminated cells harboring severe mtDNA mutations. Mice harboring only the homoplasmic COI mutation had a 37%-48% decrease of the complex IV activity in the brain, heart, liver, and skeletal muscle. Electron microscopy showed increased mitochondrial proliferation, disordered mitochondrial distribution, and loss of mitochondrial cristae in their hearts. Echocardiograms in these animals further showed a 26% increase of the left ventricular wall thickness (LVWT), a 30% decrease of the left ventricular diastolic internal dimension (LVIDd), a 39% decrease of the circumferential strain, and a 74% decrease of the radial strain. Therefore, we are confident that we have firstly created a mouse model of cardiomyopathy caused by a mtDNA missense mutation.

CIRM SCHOLAR: HELEN FONG

TITLE: The role of Sox2 in the pluripotency of human embryonic stem cells

AUTHORS: Helen Fong, Kristi A. Hohenstein, and Peter J. Donovan

Human embryonic stem (hES) cells are pluripotent cells that have the ability to become any cell in the body. Because of this unique feature, hES cells hold much promise in the future of understanding human development, as well as in the field of therapeutic medicine. Determining how these cells decide when and how to generate, regenerate, and maintain various cell types and tissues is of considerable importance if these cells are to be used one day in biomedical applications. Studies in mice have identified a number of factors that play a critical role in regulating the pluripotent state of hES cells. These factors include the transcriptional regulators, Oct4 (POU5f1), Nanog and Sox2. The functions of Oct4 and Nanog in regulating pluripotency have been confirmed by functional studies in hES cells but the role of Sox2 remains unclear. In mice, Sox2, a member of the HMG-domain DNA-binding-protein family, is essential for normal pluripotent stem cell development and maintenance of the undifferentiated cell type. To determine its role in hES cells we used RNA interference to knockdown Sox2. We demonstrate here its importance in maintaining pluripotency. The knockdown of Sox2 expression in hES cells results in a loss of the pluripotent phenotype indicated by the presence of differentiated cells in culture. The transfected cells display a distinct change in phenotype by acquiring a flattened morphology and a change in surface antigen expression as assayed by flow cytometry and immunofluorescence. The consequent of loss of Sox2 activity in hES cells will be discussed in relation to the role of the other factors known to regulate potency in hES cells, namely Oct4 and Nanog.

CIRM SCHOLAR: KRISTINE FREUDE

TITLE: **SOX9 regulates pancreatic morphogenesis and cytodifferentiation in a dosage-dependent manner**

AUTHORS: **Kristine K. Freude, Philip A. Seymour, Man N. Tran, Nisha A. Patel, Ralf Kist, Gerd Scherer, and Maike Sander**

One crucial yet poorly understood aspect of pancreas development is that of pancreatic stem/progenitor cell (PPC) maintenance. We recently identified the HMG box transcription factor SOX9 as a novel selective marker of PPCs throughout the development of the pancreas and possibly beyond, in the adult. To examine SOX9 function during pancreas development, we analyzed mice in which Sox9 was selectively inactivated in PPCs using Pdx1-Cre-driven recombination of a floxed Sox9 allele. Pancreas-specific inactivation of both Sox9 alleles resulted in severe pancreatic hypoplasia and perinatally lethal diabetes due to depletion of the PPC pool. In the absence of Sox9, PPCs displayed reduced proliferation, increased apoptosis and precocious cell cycle exit, revealing a crucial role for SOX9 in pancreatic stem/progenitor cell maintenance. Interestingly, deletion of a single Sox9 allele manifested in islet-specific hypoplasia, phenocopying the human Sox9 haploinsufficiency disorder Campomelic dysplasia. The observed two-fold reduction in all endocrine cell types was preceded by a similar two-fold depletion in committed endocrine progenitor cell number. Moreover, despite a consistent 25% reduction in pancreas size prior to the secondary transition, Sox9 haploinsufficient pancreata exhibited normal (wild-type equivalent) mass at birth, indicative of subsequent compensatory growth. These results extend our previous findings, revealing that the crucial requirement for Sox9 in PPC maintenance and pancreatic morphogenesis is dosage-dependent with endocrine differentiation being particularly sensitive. A more comprehensive understanding of the functional role(s) of SOX9 in pancreas development and pancreatic stem cell maintenance will be of future application for the in vitro generation of insulin-producing cells for diabetes therapies.

UNIVERSITY OF CALIFORNIA, IRVINE S 026

CIRM SCHOLAR: DARIUS GLEASON

TITLE: **Neural stem cells are located in the ependymal layer and are activated by injury in the mammalian forebrain**

AUTHORS: **Darius Gleason, James H. Fallon, Magda Guerra, Peter J. Bryant**

Evidence is presented to show that neural stem cells (NSCs) are located in the ependymal layer surrounding the ventricles of the rat forebrain, and that these cells can be activated to proliferate by a combination of injury and growth factor stimulation. Several markers of ACD, a characteristic of true stem cells, are strongly expressed and asymmetrically localized in the ependymal layer. Thus the G protein G-alpha-i and its binding partner LGN, as well as atypical protein kinase C-zeta and the stem-cell marker nestin show apical localization, whereas Numb shows basolateral localization. Homologs of these proteins have been shown to have similar asymmetric localization in well-documented stem cells in model systems. In the Subventricular Zone (SVZ) underlying the ependymal layer, which has previously been thought to house stem cells, these ACD markers are either weakly or not expressed, and when they are expressed they do not show asymmetric localization. When the brain is treated with a combination of local chemical injury by 6-hydroxydopamine and growth stimulation by infusion of transforming growth factor-alpha, the ependymal cells are stimulated to incorporate DNA precursors and divide, and they appear to feed progeny into the SVZ where additional growth stimulation occurs. Initial results using a lentivirus to mark cell clones are consistent with the proposed clonal relationship between ependymal stem cells and SVZ cells. Ependymal stem cells may have been missed in many previous studies because they are normally slowly dividing and are activated to divide only by specific treatment combinations. The results reveal a similarity between the mammalian brain and the spinal cord of both mammals and lower vertebrates, where ependymal cells respond to injury by proliferating and upregulating the stem-cell marker nestin. Ependymal cells that divide with the spindle axis at an oblique or vertical angle relative to the ventricle surface are predicted to distribute ACD determinants differentially to the daughter cells while mitoses with the spindle axis parallel to the surface will distribute determinants symmetrically. These divisions are therefore expected to be differentiative or self-renewing, respectively. If the hypothesis that ependymal cells are NSCs is proven correct it implies that SVZ cells are transit amplifying cells. Either or both cell types may have the capacity to proliferate in response to injury, but correct identification of the adult NSC is important because SVZ cells may be more restricted in potential and therefore less effective for therapeutic applications than the true stem cells of the ependymal layer.

CIRM SCHOLAR: CHIHUI LI

TITLE: **BRCA1 in mammary stem cells regulation**

AUTHORS: **Chihui Li and Eva Lee**

BRCA1 (breast cancer susceptible gene)-associated mammary tumors possess a basal phenotype. The basal subtype of breast cancer is negative for estrogen receptor-alpha, progesterone receptor (PR), and human epidermal growth receptor 2 (Her2). It has been found that the mouse mammary stem cells (MSC) enriched population does not express ER-alpha, PR, or ErbB2/Her2. Thus, the mouse MSCs share the common properties with the poor-prognosis basal breast cancer. This suggests potential functions of BRCA1 in the regulation of stem cells.

In a mouse model carrying somatic mutations of p53 and Brca1 alleles in mammary epithelial cells using the Cre/loxP system, we found a significant increase of the percentage of MSCs (CD29hiCD24med). This suggested that BRCA1 is involved in MSC renewal and that loss of BRCA1 function leads to the expansion of stem cells. To investigate the biological functions of BRCA1 in stem cell regulation, MSCs with the Brca1/p53 knockout were transplanted into fat pad-cleared immunodeficient RAG1-/- mice. The expected outgrowth of glands from the Brca1/p53 knockout MSCs was successfully observed. However, mammosphere formation, which is regarded as an indication of MSC activity in vitro, was not observed.

Accumulating evidence has suggested that somatic stem cells may be the targets of transformation during carcinogenesis and that virtually all cancers are clonal, each representing the progeny of a single cell. It has also been suggested that chemoresistance results from the slow cycling of the stem cells. The increased MSC population in Brca1 mutated mice could therefore contribute to their chemoresistance.

This work was supported by a CIRM Scholar Award to Chihui Li.

CIRM SCHOLAR: SOPHIA LIAO

TITLE: **Novel synthetic extracellular matrix for mesenchymal stem cells**

AUTHORS: **Sophia Liao and Zhibin Guan**

The natural extracellular matrix (ECM) – composed of a hierarchical organization of proteins and glycosaminoglycan (GAG) – is the noncellular component where cells reside. Inspiration from natural ECMs is used in our lab to design functional synthetic ECMs for cell culture and tissue engineering. Specifically, our laboratory has recently developed a novel class of biomaterials derived from peptide and saccharide building blocks using de novo synthesis. Enzymatic crosslink-linking of the hydrophilic copolymers resulted in the formation of hydrogels. The mechanical modulus of the resulting hydrogel can be tuned independently through tyrosine incorporation or enzymatic concentration during crosslink formation. Minimal cytotoxicity for mesenchymal stem cells (MSCs) was found for polymer concentrations up to 10 mg/mL. Initial cell adhesion studies were also conducted. The following hydrogel properties, such as the mechanical stiffness, cell adhesion ligand type and density, degradability, and the surfaces charges will be independently varied with the aim to control cell fate.

This work is supported by a CIRM Scholar Award to Sophia Liao.

CIRM SCHOLAR: YUTING LIN

TITLE: **Tracking stem cells with a combined fluorescence tomographic imaging and MRI system**

AUTHORS: **Yuting Lin, Hao Gao, Orhan Nalcioglu, and Gultekin Gulsen**

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. Molecular imaging has the potential to fill this need by visualizing the migration, proliferation, and survival of stem cells longitudinally after they are transplanted into the animal or patient. Among the various in vivo molecular imaging modalities, planar fluorescence imaging (PFI) has several advantages over others, including low energy radiation (compared to nuclear imaging) and high sensitivity (compared to magnetic resonance imaging(MRI)). We are building a novel fluorescence tomographic imaging (FTI) system which is capable of acquiring cross-sectional or three-dimensional images of fluorophores using a mathematical approach. A novel data acquisition method is used to take measurements to calculate the optical properties of the small animal in addition to the fluorescence signal. We demonstrate in this study that the optical properties of the whole animal have a significant effect on locating and quantifying the fluorophores embedded inside the animal. In addition, our FTI system employs additional information from different imaging modalities to obtain higher resolution images with better accuracy than is obtainable with standard FTI systems. In our design, we obtain MRI data and FTI measurements simultaneously. MRI provides the high resolution structural images of the small animal's internal organs, while FTI is especially sensitive to low amount of fluorophores. By combining these two modalities, we are able to localize and quantify the amount of fluorophores that are carried by stem cells in vivo. We have shown the feasibility of this approach in a simulation study. In a heterogeneous mouse-shaped model, if the optical properties of the animal are estimated with a 20% error, the fluorophores can not be located at all. When we apply our novel data acquisition method without MRI, the fluorophore position can be located but the calculated signal strength from the fluorophore is not accurate compared to its original strength. However, when we incorporate the MRI structural image into the FTI calculation algorithm, both the location of the fluorophore and the signal strength from the fluorophore are obtained accurately. The results demonstrate that our combined MRI-FTI system is capable of assessing the position and amount of fluorophores inside the animal. We are now carrying out experiments using tissue-mimicking materials with the combined MRI-FT system, to show experimentally the feasibility of this approach.

This work was supported by a CIRM Scholar Award to Yuting Lin.

CIRM SCHOLAR: SHARYN ROSSI

TITLE: **Human embryonic stem cell-derived motor neuron progenitors enhance neurite outgrowth and have neuroprotective effects in vitro: implications for spinal cord injury**

AUTHORS: **Sharyn L. Rossi, Adriana Guiterrez, Gabriel I. Nistor, Alexsandra Poole, and Hans S. Keirstead**

Human embryonic stem cells (hESCs) are a valuable biological tool due to their ability to self-renew and develop into essentially any human cell type. Our lab and collaborators have devised protocols for the controlled differentiation of hESCs into high purity populations of oligodendrocyte and motor neuron progenitors (OPCs and MNPs, respectively). HESC-derived OPCs remyelinate axons and restore functional recovery following spinal cord injury (SCI) and have been shown to secrete trophic factors that increase neuronal survival and process outgrowth both in vitro and vivo. Because OPCs and MNPs are derived from a common neural lineage, we sought to investigate if growth factors secreted from hESC-derived MNPs also have neuroprotective effects which could help restore function following spinal cord injury. To address this, we cultured rat primary cortical neurons in the presence of control media or MNP conditioned media and assessed neuronal health and survival. Conditioned media (CM) from MNPs enhanced neurite outgrowth of cortical neurons after 7 days in culture, an effect that was attenuated by antibody inhibition of growth factors. As SCI is accompanied by an acute inflammatory response and axonal severing, we sought to determine whether MNP CM has protective effects following these insults in vitro. MNP CM enhanced cortical neuron survival and viability following insult with LPS-activated microglial CM. Following axotomy of cortical neurons in an isolated microfludic culture platform, MNP CM enhanced axonal regeneration. These findings indicate that hESCs have the potential to not only replace cells lost to injury but to act as a vehicle for sustained neurotrophic release, thus protecting neural tissue following SCI.

CIRM SCHOLAR: DESIREE SALAZAR

TITLE: **Delayed transplantation of human central nervous system stem cells into spinal cord injured NOD/SCID mice at 30 days post-injury results in functional recovery and increased engraftment compared to 9 day post-injury transplants.**

AUTHORS: **Desiree L. Salazar, Brian J. Cummings, Mitra J. Hooshmand, Nobuko Uchida, Stan Tamaki, and Aileen J. Anderson**

We previously reported that human central nervous system stem cells grown as neurospheres (hCNS-SCns) mediate functional locomotor recovery in immunodeficient NOD/SCID mice when transplanted 9 days after a contusion-induced spinal cord injury (SCI). hCNS-SCns were isolated from fetal brain tissue using fluorescence-activated cell sorting (FACS). FACS isolated CD133+ cells can be expanded in defined serum-free conditions as neurospheres, and can differentiate into neurons or glia in vitro and in vivo. hCNS-SCns terminally differentiate into myelinating oligodendrocytes and synapse-forming neurons, in this paradigm suggesting integration of human cells with the mouse host as a possible mechanism for locomotor improvements. Selective ablation of human cells using Diphtheria toxin (DT) abolishes locomotor recovery in this 9 day sub-acute post-SCI paradigm, demonstrating that continued survival of engrafted cells is necessary to sustain locomotor recovery.

In the present study, we assessed engraftment, migration, differentiation, and locomotor recovery in NOD-scid mice when transplantation was delayed 30 days after SCI. 75,000-hCNS-SCns were transplanted into NOD-scid mouse spinal cord 30 days after a moderate spinal cord contusion (50 kdyne) injury at T-9 using the IH Impactor. Locomotor recovery was assessed using open-field BMS testing and CatWalk gait analysis. Improved locomotor recovery was observed on the BMS scale in this paradigm, 6.4 vs. 4.8 for vehicle controls at 16 weeks post-transplantation. Additionally, improvements in swing speed and stride length parameters were observed using CatWalk gait analysis, 1.21 m/s and 54.85 mm for hCNS-SCns and 1.08 m/s and 51.38 mm for vehicle controls respectively. Human fibroblast controls were not significantly different from either vehicle controls or hCNS-SCns transplanted animals for BMS or CatWalk analysis. hCNS-SCns engraftment was quantified 16 weeks post-transplantation using unbiased stereology and an antibody to human cytoplasmic protein (SC121) in both the 9 day sub-acute and 30 day delayed paradigms. Comparison of these groups revealed an average of 145,553 ± 12,941 engrafted cells in the 9 day sub-acute paradigm vs. an average of 215,711 ± 48,977 engrafted cells in the 30 day delayed paradigm. Differentiation of hCNS-SCns was assessed 16 weeks post-grafting using double labeling for human cytoplasm or human nuclei and terminal differentiation markers for neurons (beta-tubulin III), oligodendrocytes (APC-CC1), and astrocytes (GFAP), as well as makers for undifferentiated (Nestin) and dividing cells (Ki67) using confocal microscopy. Preliminary analysis suggests astrocytic differentiation is also rare when hCNS-SCns are transplanted after a 30 day delay. These data demonstrate that 30 day delayed transplantation of hCNS-SCns produces locomotor recovery as well as improved engraftment in comparison with a sub-acute 9 day post-SCI transplantation paradigm. These data suggest that hCNS-SCns not only promote recovery 9 days post-injury, but may also prove beneficial at longer time-points, perhaps months post-injury.

CIRM SCHOLAR: CHRIS SCHAUMBURG

TITLE: **Human embryonic stem cells and remyelination in a viral model of multiple sclerosis**

AUTHORS: **Chris S. Schaumburg, Maya N. Hatch, Hans S. Keirstead, and Thomas E. Lane**

Multiple sclerosis (MS) is a chronic inflammatory disease that most often results in progressive neurodegeneration of the central nervous system (CNS), e.g. demyelination and axonal loss, culminating in extensive disability through defects in neurologic function. Using current human embryonic stem cell (hESC) technology, we have initiated studies to determine if intraspinal transplantation of hESC-derived oligodendrocyte progenitor cells (OPCs) can promote myelin regeneration in a viral model of MS. Intracranial infection of susceptible mice with mouse hepatitis virus (MHV) results in an acute encephalomyelitis followed by an immune-mediated demyelinating disease similar to the pathology observed in MS patients. Our preliminary studies indicate that implanted human OPCs survive within spinal cords of recipient mice up to 2 weeks post-transplant, but are undetectable thereafter despite daily treatment with the immunosuppressant cyclosporine A (CsA). Nonetheless, our results suggest that the implanted cells in the presence of CsA promoted remyelination by 3 weeks post-transplant when compared to control mice receiving media alone. To determine if hESC-derived OPCs can directly remyelinate damaged axons in MHV-infected mice we are currently testing an alternative immunosuppression regimen of FK506 in combination with rapamycin, which has been shown to extend human neural stem cell survival in mice compared to CsA. Further, to differentiate between a direct and/or indirect effect of the hESC-derived OPCs on remyelination we are using transplant recipient mice with a deletion in the Olig1 gene that renders the endogenous murine oligodendrocytes unable to regenerate myelin on damaged axons. Therefore, any observed remyelination following viral-induced demyelination can only be attributed to repair directed by the human OPC transplant population.

CIRM SCHOLAR: BRUNO BLANCHI

TITLE: **Genetically modified MeCP2 deficient hESC derived neurons to unravel mechanisms of Rett Syndrome**

AUTHORS: **Bruno Blanchi, Jun Xu, Zhiping Pang, Hao Wu, Kevin J. Kim, Thomas C. Südhof, and Yi E. Sun**

Using human embryonic stem cells (hESC) derived neurons, we have generated and analyzed the first human cellular model for Rett Syndrome (RTT), a severe neuro-developmental disorder that is caused by mutation of MeCP2. We have used anti-MeCP2 shRNA carrying lentivirus to infect hESC and generate MeCP2 deficient hESC sub-clones, or to ectopically infect hESC-derived human neural progenitor cells (hNPC). Using a protocol established in the laboratory to differentiate hESC into hNPC and human neurons, we have generated MeCP2 deficient human neuronal cultures. Knockdown of MeCP2 expression in HSF-6 hESC derived neurons causes a dramatic shift from the primarily glutamatergic excitatory spontaneous synaptic activity observed in control neurons to a predominantly GABA-ergic inhibitory synaptic activity in MeCP2 deficient human neurons. Gene expression analysis of wild type and MeCP2 deficient human neurons has identified 254 genes whose expression is altered due to MeCP2-deficiency, among them mRNAs coding for various synaptotagmin isoforms, for proteins involved in Glutamate and GABA neurotransmissions such as receptors and transporters, and for nuclear proteins such as several histones. Interestingly, more than 80% of the mis-expressed genes are downregulated in MeCP2 deficient human neurons which was unexpected since MeCP2 is described as a transcription repressor. Using quantitative RT-PCR for about 200 human microRNAs, we have found abnormal expression of several microRNAs in MeCP2 deficient human neurons, which might in turn be responsible for abnormal mRNA expression. Alternatively, abnormal histone expression might alter nuclear organization and contribute to abnormal RNA expression observed in MeCP2 deficient neurons. We have identified a striking phenotype of MeCP2 deficient human neuronal culture, i.e. a dramatic imbalance of spontaneous synaptic activity in favor of inhibition, which might be involved in some of the manifestations of Rett Syndrome. Furthermore, microRNA and mRNA expression analysis using this first human cellular model for RTT will allow us to better characterize the molecular mechanisms of this devastating disorder and to identify potential molecular targets for a future therapeutic intervention.

CIRM SCHOLAR: MATTHEW FRANK

TITLE: **TCL1 family control of embryonic, germ cell, and immune system development**

AUTHORS: **Matthew Frank, Josh Troke, Anthony Dodson, Jane Suh, Amander Clark, and Michael Teitell**

TCL1 family genes (3 in human, 7 in mice) encode 13 15-kDa, non-enzymatic intracellular binding proteins that regulate RAS-MAPK-ERK and PI3K-AKT signal transduction pathways. When dysregulated, TCL1 and MTCP1 family members cause T and B cell leukemias and lymphomas in humans and mice(2) Recently, Tcl1 was shown to control mouse ESC self-renewal(3)(4) and proliferation(5), and the PI3K-AKT signaling pathway was shown to control hESC survival(6). Combined with breeding defects in Tcl1 knockout mice(7), a role for TCL1 family members in regulating early embryonic and lineage-specific development in humans and mice is strongly suggested. However, the expression of TCL1 family genes and proteins in hESC and early development is unknown, prompting the initial phase of this study. We determined that TCL1 was highly expressed in oocytes, with expression markedly decreased during cleavage divisions up to day 3 following zygote formation. RT-PCR analysis of HSF1 hESCs also showed expression of TCL1 and MTCP1 but not TCL1b; protein level determinations in HSF1 and other hESCs are ongoing. Undifferentiated H9 hESCs were differentiated to embryoid bodies on ultra-low adhesion plates in KSR medium without FGF-2. RT-PCR showed stable TCL1 expression through day 10 of EB differentiation, with protein level determinations ongoing. Robust TCL1 protein expression was also identified in primordial germ cells seeding the developing gonad in a 5-week human fetus by immunohistochemistry, consistent with a key but unknown role for TCL1 genes in controlling developing germ cells and lymphocytes. Thus far, Tcl1 has been implicated in mouse ESC self-renewal and proliferation by unknown mechanisms and is known to regulate AKT survival signaling. In early human development, relevant TCL1 family members have not yet been established. Lineage-specific TCL1 expression has been shown in adult germ cell and lymphoid lineages; however, TCL1 expression is markedly repressed during human zygote cleavage divisions and is silenced with cleavage divisions in mice(7). Our ongoing work now focuses on mechanisms controlling silencing and re-expression of human family members with lineage development and determining which family member in early human embryogenesis may parallel the role for mouse Tcl1 in controlling self-renewal and proliferation of hESCs.

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CIRM SCHOLAR: WEI GUO

TITLE: **c-Myc overexpression/beta-catenin activation leads to self-renewable leukemic stem cell formation and MPD to T-ALL transition in Pten null leukemia mice**

AUTHORS: **Wei Guo, Joseph L. Lasky III, Chun-Ju Chang, Xiaoman Lewis, Yun Xiao, M. Luisa Iruela-Arispe, Marileila Varella-Garcia, and Hong Wu**

The PTEN-PI3K pathway has been implicated in human leukemogenesis. While acute deletion of the murine Pten gene in adult hematopoietic stem cells (HSC) leads to defects in HSC self-renewal and homing, this also causes a brief myeloproliferative disorder (MPD) followed by the development of acute leukemia. However, leukemia initiating cells, or leukemic stem cells (LSC), responsible for this transplantable disease, have not been identified and, more importantly, the molecular mechanisms responsible for LSC self-renewal and MPD to leukemia transition remain to be elucidated. Here we report the establishment of a new leukemia model in which Pten is conditionally deleted in fetal liver HSCs. The resulting animals developed a similar MPD phenotype with a much prolonged latency towards acute T lymphoblastic leukemia (T-ALL). In this model, LSCs are enriched in the c-Kit(mid)CD3(+)Lin(-) compartment where activated beta-catenin can be detected. Conditional ablation of one allele of beta-catenin significantly decreases the incidence and delays the occurrence of T-ALL caused by Pten loss. Moreover, a recurring chromosomal translocation T(14;15), involving c-Myc and the T cell receptor alpha/delta (TCR alpha/delta) cluster, results in aberrant over-expression of the c-Myc oncogene in c-Kit(mid)CD3(+)Lin(-) LSCs and CD3+ leukemic blasts, recapitulating a subset of human T-ALL. Our study suggests that multi-step genetic or molecular alterations cooperatively contribute to LSC transformation, and underscores the importance of combinatorial therapy for tumors that are initiated by cancer stem cells.

CIRM SCHOLAR: ICHIRO NAKANO

TITLE: **Characterization of maternal embryonic leucine zipper kinase in brain tumor stem cells**

AUTHORS: **Ichiro Nakano and Harley I. Kornblum**

Introduction: Recent advances in stem cell research have allowed for the demonstration of the existence of cancer stem cells in several cancers including some brain tumors. Cancer stem cells in each organ exhibit some genetic and/or cellular similarities with their somatic stem cells. Previously, we found that a gene encoding the serine/threonine kinase, maternal embryonic leucine zipper kinase (MELK), is highly expressed in tumor spheres derived from brain tumor stem cells (BTSC), and regulates their survival and proliferation. Therefore, we hypothesized that inhibitors for MELK eradicate BTSC, resulting in inhibition of malignant brain tumor growth.

Methods: (1) We screened MELK inhibitors using cultured BTSC from pediatric GBM and MB. (2) We characterized the identified MELK inhibitors to determine whether the inhibitors interfere with survival of BTSC, their derivatives, and/or normal neural stem cells.

Results: (1) Several compounds were identified by our screening and they were found to inhibit MELK expression. (2) The identified compounds contained those which cause apoptosis of BTSC, but not their derivatives or normal neural stem cells.

Conclusions: MELK is highly expressed in some BTSC and is required for their survival. Inhibition of this gene can target stem cell component in pediatric malignant tumors, leading to growth inhibition of the entire tumor mass.

CIRM SCHOLAR: JOANNE LEUNG

TITLE: **Adipose stem cells for bladder tissue engineering**

AUTHORS: **Joanne Leung, Ben Wu, and Larissa Rodriguez**

Tissue engineering of the urinary bladder had shown some promises, but with limited success. First, there had been no report to-date on acquiring the appropriate cell source through a procedure with minimal discomfort, where researchers typically obtained cells from bladder biopsies, or acquired bone marrow stem cells for smooth muscle cell (SMC) differentiation. Second, the most popular choices of scaffold materials such as Small Intestinal Submuscosa and PLGA have major disadvantages, namely xenogenic rejection and limited mechanical stretchability respectively. The bladder is a dynamic organ that maintains an isotonic internal pressure through the changes in volume, and it had been suggested that SMC show increased late differentiation marker expression when seeded onto an elastomeric substrate under cyclical mechanical stimulation. We had created a fast-degrading elastomeric scaffold by electrospinning polyglecaprone25 to mimic the native organ's pliability, onto which we had seeded both differentiated and undiffetrentiated autologous adipose derived stem cells (ASC). We intend to improve the neo-organ's cellularity and state of differentiation by coating the scaffold with various extracellular matrices (ECM) and growth factors (GF) that had been implicated to promote cell attachment or SMC differentiation.

We have found that the ASC's attachment and survival on the ECM/GF-coated porous polyglecaprone25 scaffolds compared to the uncoated scaffolds were improved up to: (1) 20 fold with a coating of 420 μg collagen I + 5 μg laminin/scaffold; (2) 6 fold with 420 μg collagen I + 10 ng TGF-beta-1/scaffold; (3) 8 fold with fibrin glue + 40 ng HGF/scaffold; and (4) 4 fold with 420 μg collagen/scaffold with 25% caprolactone blended, detected by fluorescence microscopy of DiI labeled cells followed by semi-quantitative image analysis. These results suggest that a dramatic increase in cellularity can be achieved by surface coating the scaffolds with ECM and/or GF, which is desirable in a tissue engineering perspective. Second, the potential for undifferentiated ASC to differentiate into smooth muscle cells after being seeded onto various ECM/GF-coated scaffold groups had been evaluated. The majority of the coated scaffold groups that had sufficient cell numbers for molecular analyses demonstrated a smooth muscle actin upregulation similar to what had been observed in the 2D culture supplemented by smooth muscle induction media detected by RT-PCR. ASCs seeded on the fibrin glue + 40 ng HGF-coated scaffold showed particularly high upregulation of smooth muscle specific markers. Third, progress had been made towards applying the tissue engineered construct to the in vivo rat bladder injury model with improved architecture, mechanical properties of the biodegradable scaffold and cell seeding method that will allow us to move forward with the investigation of ECM/GF coatings' effect in vivo after confirmation of the preliminary in vitro data.

CIRM SCHOLAR: ROGER LO

TITLE: **Context-dependent TGF-beta activation promotes human melanocytic dermal invasion**

AUTHORS: **Roger S. Lo and Owen N. Witte**

Accumulation of distinct sets of genetic/epigenetic alterations is thought to contribute to stepwise progression of human cutaneous melanomas. We investigated the causative roles of melanoma-associated genetic/epigenetic alterations in human organotypic skin cultures. Although PTEN deficiency or Braf activation in immortalized human melanocytes individually supported anchorage-independent growth, each alteration only conferred benign, hyperplastic growth in a skin environment. While PTEN deficiency combined with Braf activation failed to collaborate functionally without a proper skin architecture, these alterations, found to coexist in human melanomas, together induced a melanoma in situ-like phenotype without dermal invasion in organotypic skin cultures. In contrast, Ras activation in immortal melanocytes conferred dermal invasion in skin cultures. Further addition of cell-autonomous TGF-beta activation in the context of PTEN deficiency and Braf activation promoted dermal invasion in skin cultures, despite failing to confer a growth advantage as monolayer cultures or as subcutaneous tumors. Thus, distinct stages of human melanoma are genetically tractable in the proper tissue architecture, and evidence of genetic interactions among PTEN deficiency, Braf activation, and cell-autonomous TGF-beta activation suggests a strategy of combined targeted therapy in the appropriately selected patients.

CIRM SCHOLAR: MARCUS OHLSSON

TITLE: **Human adult and embryonal stem cells in a translational rat model of traumatic spinal cord injury**

AUTHORS: **Marcus Ohlsson and Leif A. Havton**

Trauma to the lumbar portion of spine may result in a cauda equina/conus medullaris injury of the spinal cord. Clinically, these patients present symptoms like paraparesis/paraplegia, decreased sensation of the lower body, neuropathic pain, as well as bladder, bowel, and reproductive dysfunction. Persons with spinal cord injuries rank recovery of these functions as "most important" or "very important" therapeutic targets. In experimental rat studies, a unilateral lumbosacral ventral root avulsion (VRA) of L5-S2 results in a loss of autonomic and motoneurons in the spinal cord, thereby emulating the typical clinical scenario.

Adult human neural stem cells are harvested with the patient's informed consent during intracranial neurosurgical procedures. These cells have been shown to develop into functional neurons in vitro. Here, in collaboration with Dr. Westerlund and Dr. Svensson, Karolinska Institutet, Sweden, a total of 45,000 adult human neural stem cells were implanted into the L6-S1 portion of the conus medullaris in rats subjected to a lumbosacral ventral root avulsion and implantation. At 1 week after transplantation, the grafted cells were spherical with only few extensions. At 4 and 8 weeks after transplantation, cytoplasmatic extensions were common and some stellar-like cells were found. Of the >90 % Nestin+/p75+ cells transplanted, 5.1 % survived for 8 weeks after transplantation. Of these cells, 35 % stained positive for GFAP and a few beta-III-tubulin positive cells were found. Interestingly, we found occasional axons positive for human Neurofilament extending into the implanted root.

Next, using the above rat injury model, we will study the effects of implanting human spinal motoneurons from human embryonic stem cells (hESC), provided by Dr. S. A. Goldman (Singh Roy et al, Exp Neurol 2005, 196:224-34). In this collaborative study, the morphological fate of the implanted cells is studied with immunohistochemistry, and functionally with urodynamics including cystometrogram and EMG recordings from the external urethral sphincter to study connections with the lower urinary tract.

CIRM SCHOLAR: YIN SHEN

TITLE: **X-inactivation in female hESCs is non-random and subject to epigenetic alternations**

AUTHORS: **Yin Shen, Youko Matsuno, Shaun D. Fouse, Nagesh Rao, Sierra Root, Renhe Xu, Matteo Pellegrini, Art D. Riggs, and Guoping Fan**

X-chromosome inactivation is an essential dosage compensation mechanism for female somatic cells. However, controversial data exist regarding the XCI status in several lines of female human embryonic stem cells (hESCs). Previously, we have used XIST RNA FISH and immunostaining of histone H3 lysine(K)27 tri-methylation, which is another XCI hallmark, and observed varied XCI statuses in the same hESC cell line (HSF6 and H9). Loss of XCI marks in HSF6 and H9 cells are tightly associated with DNA hypermethylation at the XIST gene promoter, confirming the involvement of epigenetic factors in XCI regulation. These data argue that XCI status in female hESCs is unstable and loss of XCI marks can happen under stressed culture conditions. Nevertheless, loss of XCI marks did not show any obvious phenotypes and only leads to partial reactivation of silenced X-linked genes (two out of eight) when we analyzed the expression of X-linked polymorphic genes. These results suggest that additional mechanism(s) exist in female hESCs to balance the dosage of X-linked genes, even in the absence of XIST expression. We speculate that other epigenetic mechanisms such as histone modifications may help locking in the expression levels of X-linked genes and maintain dosage compensation.

To further dissect the molecular machinery in the regulation of XCI in hESCs, we examined global DNA methylation patterns on X chromosome of hESCs either with or without XCI using methylated DNA immunoprecipitation coupled with DNA microarray technology (mDIP-Chip) technique. The DNA samples (e.g. hESCs with XCI, hESC without XCI) which are pulled down by an antibody against 5-methylcytidine, and input DNA are labeled and co-hybridized onto the Agilent human whole genome CpG island array. Using mDIP-Chip, we asked whether DNA methylation patterns on the X-chromosome are different between hESCs with or without XCI. Among the 419 X-linked promoter/1st exon CpG islands, 52 CpG islands (49 genes) showed higher methylation level in hESCs with XCI marks, while 7 CpG islands showed more methylation in hESCs without XCI. When we compared the differentially methylated list with the list of X-linked genes showing differential expression, we identified 10 genes that had hypomethylation in their promoter CpG islands coupled with up-regulation of expression levels in hESCs without XCI. Taken together, our data suggest that loss of XCI marks may be causally linked to the demethylation on the previously silenced allele and the partial reactivation of a subset of X-linked genes in female hESCs.

CIRM SCHOLAR: ROBERT SIGNER

TITLE: **Age-related hematopoietic defects underlie the myeloid dominance of adult leukemia**

AUTHORS: **Robert A.J. Signer, Encarnacion Montecino-Rodriguez, Owen N. Witte, Jami McLaughlin, and Kenneth Dorshkind**

Chronic Myeloid Leukemia (CML) is an adult onset myeloproliferative disorder (MPD) arising from transformation of hematopoietic stem cells (HSC) by the BCR-ABL fusion oncogene. Despite originating in HSC, BCR-ABL induced leukemia presents predominantly as CML and rarely causes lymphoid disease. We determined that age-associated declines in lymphopoiesis underlie this myeloid biased disease presentation. Using a murine model, we demonstrate that young bone marrow (BM) cells transformed with BCR-ABL initiate both MPDs and lymphoid leukemia, while BCR-ABL transformed old BM cells recapitulate the human disease by inducing MPDs with rare lymphoid involvement. We further document that BCR-ABL induced MPDs derived from old BM cells are characterized by a reduced tumor burden when compared to those derived from young BM cells and show this is related to heretofore unappreciated developmental defects present in aged myeloid progenitors. Furthermore, transplantation experiments indicate that leukemia stem cells generated from BCR-ABL transformed old BM cells exhibit these age-related lympho- and myelopoietic defects. Taken together, our results provide a biological explanation for both the myeloid dominance of CML and other adult leukemias, and demonstrate the impact of senescence and stem cell aging on the development of hematopoietic malignancies.

CIRM SCHOLAR: APARNA SUBRAMANIAN

TITLE: **Differentiation of functional macrophages from human embryonic stem cells**

AUTHORS: **Aparna Subramanian, Beichu Gao, Matthew Marsden, Zoran Galic, and Jerome Zack**

Human embryonic stem cells (hES) have been induced to differentiate into (1) B cells, (2) dendritic cells, (3) NK cells, (4) macrophages, and (5) by our group into T cells. The focus of the current work is to optimize and characterize differentiation of hES cells into macrophages, a primary target for HIV. We have successfully obtained macrophage differentiation from hES by co-culture with the bone marrow stromal cell line OP9. In addition we have developed a highly efficient method to obtain macrophages by promoting the formation of embryoid bodies from hES cells. In order to optimize the conditions we have compared 3 different ES cell lines for their hematopoietic potential and found the H1 line to be most efficient in the context of embryoid body formation and macrophage differentiation. We show here that our hES derived macrophages are functionally similar to peripheral blood mononuclear cell derived macrophages. To demonstrate that human ES derived macrophages can be genetically manipulated we successfully introduced a lentiviral vector expressing GFP into hES and observed its expression in the differentiated macrophages. Our results thereby demonstrate the viability of genetically manipulating hES cells and their potential utility in the treatment of various immunological disorders such as AIDS.

CIRM SCHOLAR: HAO WU

TITLE: **Integrative genomic and functional analyses reveal neuronal subtype differentiation bias in human embryonic stem cell lines**

AUTHORS: **Hao Wu, Jun Xu, Zhiping P. Pang, Weihong Ge, Kevin J. Kim, Bruno Blanchi, Caifu Chen, Thomas C. Südhof, and Yi E. Sun**

The self-renewal and differentiation potential of human embryonic stem cells (hESCs) suggests that hESCs could be used for regenerative medicine, especially for restoring neuronal functions in brain diseases. However, the functional properties of neurons derived from hESC are largely unknown. Moreover, because hESCs were derived under diverse conditions, the possibility arises that neurons derived from different hESC lines exhibit distinct properties, but this possibility remains unexplored. To address these issues, we developed a protocol that allows step-wise generation from hESCs of cultures composed of 70-80% human neurons that exhibit spontaneous synaptic network activity. Comparison of neurons derived from the well characterized HSF1 and HSF6 hESC lines revealed that HSF1- but not HSF6-derived neurons exhibit forebrain properties. Accordingly, HSF1-derived neurons initially form primarily GABAergic synaptic networks, whereas HSF6-derived neurons initially form glutamatergic networks. microRNA profiling revealed significant expression differences between the two hESC lines, suggesting that microRNAs may influence their distinct differentiation properties. These observations indicate that although both HSF1 and HSF6 hESCs differentiate into functional neurons, the two hESC lines exhibit distinct differentiation potentials, suggesting that they are pre-programmed. Information on hESC line-specific differentiation biases is crucial for neural stem cell therapy and establishment of novel disease models using hESCs.

CIRM SCHOLAR: ZHICAO YUE

TITLE: **Identify stem cell property through a functional signaling network analysis**

AUTHORS: **Zhicao Yue, Fengfeng Zhuang, Yi-Hsin Liu, and Chih-Ming Ho**

Cells are composed of many proteins, RNA / DNA, carbohydrate and lipid molecules, however the cell property is not a simple additive effect of all these components. To identify a cell's property based on the expression profiles are often not satisfactory. Here we present evidence that a cell's characteristic response pattern to external stimuli through its intrinsic signaling network is a fundamental property of the cell.

In mouse embryonic stem cells, the maintenance of stem cell property depends on a growth factor LIF. LIF withdraw initiated mES cell differentiation. Functional assays suggested mES cells start to lose the stem cell property as early as 24 hours after LIF removal, and by 48 hours very little stem cell function was left. No significant change at the expression level of many important genes such as Oct4, Nanog and Sox2 was observed even after LIF removal for 96 hours. However during this time period, the cell's signaling network responsive pattern to LIF changed dramatically. At 48 hours, the cells initiated a much weaker response at the Erk level (but not p38a), and although at the Akt level it was activated to the same extent as in mES cells, the downstream effector Gsk3 a/b was not activated.

These results suggested that a signaling network analysis revealed stem cell property loss at a much early time point. Also it provided a functional framework to test the role of many targets from microarray expression analysis. We propose that the pattern of signaling network responsiveness provides an intrinsic functional test of the cell identity.

CIRM SCHOLAR: ANN ZOVEIN

TITLE: **Tracking VE-cadherin progeny from origins in the midgestation aorta to final residence in adult bone marrow.**

AUTHORS: **Ann C. Zovein, Jennifer J. Hofmann, Kirsten A. Turlo, and M. Luisa Iruela-Arispe**

While the midgestation aorta is thought to harbor the origination of hematopoietic stem cells, this has not yet been demonstrated by careful lineage tracing. Here we address, through genetic mapping, the contribution of VE-cadherin progeny from the AGM region (aorto-gonado-mesonephros). Conditional fate tracing, which employs tamoxifen inducible Cre/lox technology, maps a VE-cadherin population restricted to the dorsal aorta at midgestation, thus allowing the detection of these cells and their progeny within their contextual milieu, and without the complication of newly introduced cohorts from the circulating pool. The data reveals that this restricted VE-cadherin population autonomously contributes to definitive hematopoiesis. In addition, the limited population emerges contiguously with the endothelium and later migrates to the fetal liver. While fetal liver migration had previously been presumed, recent reports of VE-cadherin+ populations within the fetal liver required further clarification within the system. Cultures of fetal liver and AGM, induced separately for VE-cadherin tracing, revealed that the early VE-cadherin fetal liver population is not independent of the dorsal aorta. Furthermore, the progeny of the VE-cadherin cells in the midgestation aorta could later be detected in adult bone marrow up to one year of postnatal age, and constituted all adult bone marrow lineages. Progeny was also noted in additional adult hematopoietic sites, namely the thymus and spleen. Lastly, as the fate tracing employed a known endothelial protein, it revealed endothelial labeling predating that of neighboring hematopoietic stem cells, which may suggest a possible endothelial origin of hematopoiesis. Together the data solidifies in vivo, through fate mapping, a small cohort of VE-cadherin cells in the dorsal aorta capable of supporting long term definitive hematopoiesis.

CIRM SCHOLAR: CARLA DEMETERCO-BERGGREN

TITLE: **Beta-cell regeneration in type 2 diabetes**

AUTHORS: **C. Demeterco-Berggren, S-H. Lee, I. Geron, F. Levine, and P. Itkin-Ansari**

The endocrine pancreas has a remarkable capacity to adapt to conditions of increased insulin demand, such as in obesity, pregnancy, cortisol and growth hormone excess, by increasing its functional beta-cell mass. However, 10–20% of individuals fail to adapt and become diabetic with time. It is accepted that beta-cell regeneration pays a role in the adaptive increase in beta-cell mass observed during increased insulin demand. However, it is unclear whether regeneration occurs by replication of preexisting beta-cells or by neogenesis from stem or progenitor cells. Previously, we have shown that non-endocrine epithelial cells of the adult human pancreas can be induced to differentiate into beta-cells when cotransplanted with human fetal pancreatic cells. We are interested in understanding the nature of the signals that are inducing beta-cell differentiation and whether those signals are present in the adult pancreas in pathological processes such as Type 2 diabetes. Wnt signaling is an important regulatory pathway controlling cell growth and differentiation in many tissues, both during embryogenesis and in the adult. However, its role in the adult endocrine pancreas has not been well studied. We have been studying Wnt signaling in Type 2 diabetic patients. Our current model is that this pathway plays a role in the pathogenesis of Type 2 diabetes and suggests manipulation of Wnt signaling as a new approach to beta-cell directed diabetes as well as pre-diabetes therapy.

CIRM SCHOLAR: YOUNGJUN KIM

TITLE: **A conserved phosphatase cascade that regulates nuclear membrane biogenesis**

AUTHORS: **Youngjun Kim, Matthew S. Gentry, Thurl E. Harris, Sandra E. Wiley, John C. Lawrence, Jr., and Jack E. Dixon**

A newly emerging family of phosphatases that are members of the haloacid dehalogenase superfamily contain the catalytic motif DXDX(T/V). A member of this DXDX(T/V) phosphatase family known as Dullard was recently shown to be a potential regulator of neural tube development in Xenopus. (Satow R., et al (2002) Biochem. Biophys. Res. Commun. 295, 85-91). Herein, we demonstrate that human Dullard and the yeast protein Nem1p perform similar functions in mammalian cells and yeast cells, respectively. In addition to similarity in primary sequence, Dullard and Nem1p possess similar domains, they show similar substrate preferences, and both localize to the nuclear envelope. Additionally, we show that human Dullard can rescue the aberrant nuclear envelope morphology of nem1 yeast cells, functionally replacing Nem1p. Finally, Nem1p, has been shown to deposphorylate the yeast phosphatidic acid phosphatase Smp2p (Santos-Rosa et al (2005) EMBO 24, 1931-41) and we show that Dullard dephosphorylates the mammalian phospatidic acid phosphatase, lipin. Therefore, we propose that Dullard participates in a unique phosphatase cascade regulating nuclear membrane biogenesis and that this cascade is conserved from yeast to mammals.

CIRM SCHOLAR: FREDERICK LO

TITLE: **Reconstructing the signaling pathway of amino-terminal Dickkopf-1 in 293T and embryonic stem cells**

AUTHORS: **Frederick Lo, Ruchika Gupta, Rene Haverslag, Hiroko Matsuo, Mark Mercola, and Shankar Subramaniam**

Dickkopf-1 (Dkk1) has a well-known function sequestering the Lrp class of Wnt co-receptors, thereby behaving as an inhibitor of Wnt/beta-catenin signaling. However, earlier studies in Mercola Lab have demonstrated that this Dkk1 activity is due to the C-terminal region of the protein, whereas cells treated with conditioned media containing the N-terminal region (N1) produced a separate, novel pro-migratory response. Further studies in our lab indicated that N1 upregulated certain heart markers and cooperated with other Wnt antagonists during embryogenesis. Given these studies and the need for fine control over Wnt/beta-catenin signaling during the induced cardiomyogenesis of embryonic stem cells (ESCs), N1's precise activity should be well-characterized prior to Dkk1's inclusion in a cardiomyogenesis protocol. Our goal is to rebuild the signaling pathway triggered by N1, starting with a simpler 293T model followed by its refinement to encompass ESCs. As a first step, our lab has utilized and continues to refine a scratch-wound assay and a related cell-tracking assay to validate N1 activity, using purified N1 protein. For both assays, a pipette tip is used to create a channel in confluent wells of 293T cells. In the scratch-wound assay, the cell-free area is imaged over time and analyzed using a custom Matlab script, permitting the determination of the rate of wound healing, and thus the rate of cell migration. For the cell tracking assay, the velocities of individual cells are recorded over 20 hours with the assistance of a high-throughput microscope and the MTrackJ plugin for ImageJ. Next, we intend to use mass spectrometry to identify proteins significantly up- or downregulated in 293T cells by N1. This set of significantly regulated proteins will be sent to a third-party service to determine their expression levels and phosphorylation states over multiple timepoints after N1 treatment. We had also planned to search for N1 inhibitors using small molecules and NMR spectroscopy. Unfortunately, peaks on the N1 spectrum overlap peaks occupied by the small molecules, prohibiting the identification of N1 inhibitors by this method. By combining the data from the expression and phosphoprotein assays, exhaustive literature and database searches, and systems biology and bioinformatics approaches, we expect to be able to trace the N1-initiated signaling cascade. These processes will be adjusted and repeated with ESCs once we are confident in their efficacy in 293T cells.

CIRM SCHOLAR: RHIANNON NOLAN

TITLE: **Axonal transport defects lead to altered APP metabolism in human embryonic stem cell-derived differentiating neurons.**

AUTHORS: **Rhiannon L. Nolan, Jessica D. Flippin, and Lawrence S. B. Goldstein**

Alzheimer's Disease (AD) is a neurodegenerative disorder characterized pathologically by 1) extracellular senile plaques (SP) principally composed of amyloid beta (Abeta) peptide, 2) intracellular neurofibrillary tangles (NFT) and 3) cell death. It is commonly believed 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 deficits 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. To test further the effects of axonal transport disruptions on APP metabolism in human neurons with endogenous levels of APP, we have made human embryonic stem cell (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 can also lead to altered proteolytic processing and/or secretion of amyloid beta. These preliminary results support the hypothesis that axonal transport defects such as those induced by KLC1 reduction lead to altered APP metabolism in human neurons.

CIRM SCHOLAR: ROBERT O'BRIEN

TITLE: Proteome profiling identifies Utf1 as a protein highly enriched in ES cells

AUTHORS: Robert O'Brien, Zhouxin Shen, Kiyoshi Tachikawa, and Steve Briggs

Embryonic Stem cells have two characteristics that make them exciting to the field of regenerative medicine. First, they are pluripotent and able to differentiate into cells derived from all three embryonic germ layer lineages. Second, they are self-renewing and highly proliferative in vitro, giving them potential regenerative capacity that adult stem cells lack.

The Oct4 and the Oct4/Sox2 heterodimer act as master regulators of the twin processes of pluripotency and self-renewal in ES cells. We have produced whole and phosphoproteome profiles of ES cells and their cancerous counterparts in order to identify those proteins and phosphoproteins enriched in the undifferentiated state. From these profiles, we have identified several pathways and transcription factors that are upregulated in undifferentiated human and mouse ES and EC cells. One of the most striking of these factors was the ES-specific transcriptional co-activator, Utf1 which is in the top 5 hits in all profiles of undifferentiated cells and is completely absent in our profiles of differentiated cells.

Utf1 is a direct target of the Oct4/Sox2 heterodimer in both mouse and human ES cells. I has been reported to be sufficient to rescue the slow growing phenotype that results from rescuing Oct4-/- ES cells with a chimeric Oct4/Oct6 protein containing the Oct4 DNA binding domain and the Oct6 transactivation domain. There are no reports that describe whether Utf1 is necessary for ES cell proliferation or self-renewal. If it is, then Utf1 becomes the first gene downstream of Oct4/Sox2 in an ES cell specific self-renewal pathway.

For this reason, we have set out to determine whether Utf1 is indeed necessary for self-renewal or rapid proliferation in ES cells.

CIRM SCHOLAR: SEUNGHAN OH

TITLE: **Control of embryonic stem cell culture and differentiation using nanotechnology**

AUTHORS: **Seunghan (Brian) Oh, Karla Brammer, and Sungho Jin**

Stem cells, which are capable of both self-renewal and multi-lineage differentiation, offer exciting possibilities of treating a wide range of diseases. The promising applications of stem cell therapies rely on specific stem cell differentiation into well-defined lineage and relatively easy and reproducible growth of stem cells. 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 stem cell differentiation so that only those relevant cells are preferentially produced from the embryonic or adult stem cells while a spontaneous differentiation into undesired lineage is prohibited. It would also be desirable if one can control and accelerate the differentiated cell enrichment process. To date, many of in vitro researches relating to stem cell differentiation have been based on chemical (or gene) treatment by adding or removing chemicals (or genes) showing capabilities of stem cell differentiation into desired lineage. The usefulness of such in-vitro research may be somewhat limited in terms of possible applications to human clinical therapies, since most of stem cell research relies on two-dimensional culture systems on the surface of cell-culture dishes, rather than 3-Dimensional cell cultures occurring in real in-vivo systems. So, it is highly desirable to develop novel culture systems for stem cell differentiation and growth toward desirable types and configurations of cells.

In this research, we have begun to investigate the exciting yet unexplored territories of possible direct control of stem cell differentiation by applying nano-science approaches. Recent advances in nanotechnology are incorporated in the proposed work to physically induce changes in local temperature and mechanical strain. The effects of such physical stimuli in novel stem cell culture system are examined to enhance the understanding of the nature of stem cell differentiation, as well as to find controllable processes for stem cell differentiation and growth. We also investigate the effects of nanoscale, engineered substrate materials on controlled growth of stem cells.

CIRM SCHOLAR: KATHERINE RUBY

TITLE: **Genetically modified human embryonic stem cells in spinal axon development and regeneration**

AUTHORS: **Katherine Ruby and Binhai Zheng**

Background, Significance, and Rationale: Spinal cord injury, a neurological condition that results in paralysis and disability primarily due to the severing of axonal pathways, has currently no cure or effective therapies. The inability to regenerate axons in the mature mammalian central nervous system (CNS) underlies the poor prognosis for patients suffering from spinal cord injuries. Even though medical advancement has significantly increased the life span of those who suffered a spinal cord injury in the last century, very little has been offered to enhance functional recovery of the injured patients.

Human embryonic stem cells (hESCs) offer great promise as an alternative approach to treat neurological conditions such as spinal cord injury. Oligodendrocyte progenitors derived from hESCs have been used in transplantation studies to alleviate demyelination of the axonal tracts to enhance functional recovery after experimental spinal cord injury with modest success (Keirstead, et al. 2005). However, this approach will not be useful to repair axons that have been severed in spinal cord injury. HESC-derived neurons can, in theory, replace damaged neurons and re-form functional circuitry.

Specific Aims: We will test the idea that hESCs can be used to study the development, differentiation and functional integration of hESC-derived cells into the immature and mature CNS and particularly the corticospinal system. Such an approach will potentially provide novel strategies for studying the role of human genes in spinal tract development and spinal cord repair.

CIRM SCHOLAR: SHAUNA YUAN

TITLE: **Establishing human embryonic stem cells as human neurodegenerative disease model**

AUTHORS: **Shauna H. Yuan and Lawrence S.B. Goldstein**

Alzheimer's disease (AD) is a devastating neurodegenerative disease that currently has few treatment options and no cure. Although animal models have contributed to our understanding of the disease mechanisms, they are inadequate because of their inability to fully demonstrate the human disease pathologies. A human disease model is the most ideal system; however tissue procurement is mostly post-mortem, and the supply is a limited and unreliable resource. We propose an alternative source, by engineering human embryonic stem cells (hESC) to mimick heritable AD. The goal is to establish a human disease model of AD, for the purpose of studying disease mechanisms and as a tool for drug development.

CIRM SCHOLAR: JUSTIN VOOG

TITLE: **A role for escargot in stem cell niche maintenance**

AUTHORS: **J. Voog, G. Hime, M. Rocha, M. Boyle, M. Fuller, and L. Jones**

The somatic hub at the tip of the Drosophila testis is a primary component of the male germ-line stem cell niche. Hub cells secrete the ligand Unpaired (Upd), which activates the JAK-STAT pathway in adjacent germline stem cells (GSCs) to specify stem cell self-renewal. This study's objective is to characterize shutoff (shof), an allele of escargot (esg), a member of the Snail family of transcription factors. In adult shof males we observe a loss of GSCs and somatic stem cells, also known as cyst progenitor cells (CPCs). Immunofluorescence analysis of testes from shof adults revealed a loss of hub markers, including FasIII, DE-cadherin, DN-cadherin, and Center-divider (Cdi). Furthermore, loss of expression of upd and shotgun (shg), the gene that encodes DE-cadherin, was observed in shof testes from larval (L3) males. These findings suggest that hub cells may be lost and niche function compromised during development in shof males. Adherens junctions, composed of homotypic Cadherin dimers, anchor GSCs to hub cells and are concentrated between hub cells. We hypothesize that stem cell loss in shof mutants is due in part to loss of adherens junctions between hub cells and between hub cells and stem cells. Germline stem cell clones homozygous mutant for shg were not capable of long-term self-renewal, supporting the notion that DE-cadherin expression is necessary for stem cell maintenance. Mammalian homologs of esg have been shown to directly regulate E-cadherin, an essential component of adherens junctions. Furthermore, in Drosophila esg has been shown to act genetically upstream of shg in the trachea. Therefore, we propose that escargot acts to regulate shg expression within the niche. Characterization of genetic programs that regulate stem cell-niche cell interactions and stem cell niche maintenance will be important for the realization of regenerative therapies.

CIRM SCHOLAR: SAMANTHA ZEITLIN

TITLE: **Role of ATM-directed chromatin remodeling during neural lineage differentiation**

AUTHORS: **S.G. Zeitlin, J. Y. Wang, L. S. B. Goldstein and and D.W. Cleveland**

Background, Significance and Rationale: Ataxia Telangiectasia (A-T) patients walk normally until onset at age 2-3 years. The majority of cases result from autosomal dominant truncations of the A-T mutated kinase, ATM. ATM knockout mice exhibit similar elevated cancer rates and immune dysfunctions to those seen in patients. Surprisingly, the ATM knockout mouse does not phenocopy the neurodegeneration and ataxia seen in patients.

The wide range of affected tissues in A-T patients suggests that it is a stem cell disease. All of the phenotypes, including premature aging, high cancer rates, immune dysfunction and neurodegeneration in patients with A-T may be to global chromatin disruption in stem cells. A histone H3 variant called centromere protein A (CENP-A), normally restricted to centromeric chromatin, is chronically mislocalized in primary cells from A-T patients.

Hypothesis: Mislocalization of CENP-A due to decreased activity of ATM could restrict the differentiation potential of neural lineage precursors by interfering with normal transcriptional regulation.

Approach: HUES-9 cells were differentiated in vitro into embryoid bodies (EBs) and then into neuroprogenitor cells (NPCs) using a wide range of growth conditions and differentiation procedures. Cell morphology and differentiation markers were examined by confocal microscopy. The differentiation ratios of nestin+, TUJ1+ (e.g. neuronal) and GFAP+ (e.g. astrocytic) cells were determined for each growth condition.

Future plans: CENP-A-GFP will be delivered to HUES-9 cells using lentivirus. Quantitative assays on a high-content automated microscope will be used for live cell imaging of differentiation, with and without DNA damage and inhibition of ATM by small molecule inhibitors.

CIRM SCHOLAR: DAVID BUCHHOLZ

TITLE: **Phenotype stability in retinal pigment epithelial cells**

AUTHORS: **David Buchholz, Sherry Hikita, Kathryn Blaschke, Christy Curletti, Monte Radeke, Natasha Gallo, Linc Johnson, and Dennis Clegg**

Retinal pigment epithelial (RPE) cells provide crucial support functions for photoreceptors in the retina, and have been implicated in blinding diseases such as Age-related Macular Degeneration. Human embryonic stem cells will spontaneously differentiate to an RPE-like cell (hES-RPE) after removal of FGF, although how closely these cells resemble RPE is not yet clear. We have compared structure, function and expression of RPE markers in hES-RPE and RPE cells in situ, and analyzed the stability of the RPE phenotype in hES-RPE after repeated passage in culture. We found that hES-RPE ultrastructure closely resembled that of native RPE, with development of polarized cell structure characterized by apical microvilli, tight junctions, and melanin granules. hES RPE expressed RPE-specific proteins EMPRIN, Apo-E, CRALBP and others in a pattern that is close but not identical to native RPE. Lipofuscin accumulation is absent in cultured hES-RPE. Analysis of cell morphology after 5 passages showed that hES-RPE pigmentation is lost then regained after passage, and cells appear more fibroblastic after time. cDNA array analysis indicated that hES-RPE at early passage are similar to human fetal RPE, but marked changes in expression occur after 5 passages.

CIRM SCHOLAR: NAREG DJABRAYAN

TITLE: **Analysis of genes required for the switch from pluripotency to committed differentiation in the C. elegans embryo**

AUTHORS: **Nareg J.-V. Djabrayan and Joel H. Rothman**

The development of methods for efficient directed differentiation of Embryonic Stem Cell populations requires a mechanistic understanding of the developmental programs which guide cell lineages to their respective fates. Model organisms such as C. elegans provide an array of high throughput assays for the functional analysis of genes involved in the cell fate decisions of the early embryo. We have devised a screen to identify key regulators of the pluripotency to commitment switch. In earlier studies, we demonstrated that the END-3 GATA factor, which is essential to specify the endoderm, is sufficient to convert all normally non-endodermal somatic progenitors into endoderm when activated ectopically under heat-shock control. These studies further revealed that the competency of cells to respond to END-3 is temporally restricted to a particular period during embryogenesis. This "competency window" indicates that a temporally controlled program restricts the developmental potential of embryonic cells. We have screened 800 embryonic lethal genes from the Ahringer RNAi library for genes that, when inactivated, allow cells to respond to END-3 and activate endoderm-specific markers much later in development. From this screen, we have preliminarily identified 134 genes that allow for distinct sets of cells to be reprogrammed at various stages of development when debilitated. One gene from our screen, lin-26, encodes a transcription factor required for specification of non-neural ectoderm. In lin-26 mutants, cells that normally become hypodermis instead become neuron-like. We found that a chromosomal mutation of lin-26 extends the "competency window" to a stage at which neurons have begun to differentiate (6 hours past the two-cell stage). This suggests that LIN-26 acts not only in the neuron vs. hypodermis decision, but also in regulating the committed state of differentiation. We have found that cells ectopically expressing an elt-2::gfp fusion, a direct target of end-3, also stain for the gut-specific antigen recognized by antibody MH33, suggesting activation of much of the endodermal program. Other genes from the screen indicate that additional transcriptional regulatory processes, chromatin remodeling, and signal transduction may collaborate to restrict the developmental plasticity of cells during the progressive events in embryonic development. Current studies are being directed towards characterizing candidates who's homologues in H. sapiens are up-regulated during the differentiation of Human Embryonic Stem Cells. Candidates which provide robust competency for directed differentiation will be chosen for a translational study in Embryonic Stem Cell culture.

CIRM SCHOLAR: MIN-JEONG KYE

TITLE: **MicroRNA profiling of H9 human embryonic stem cell in different culture conditions**

AUTHORS: **Min-Jeong Kye, Na Xu, Kaiqin Lao, and Kenneth Kosik**

MicroRNAs are relatively recently discovered small RNA molecules. Their primary function seems to be to regulate gene expression. MicroRNAs accomplish this by binding to partially complementary sequences in the 3' untranslated region (UTR) of mRNAs, when bound the microRNAs prevent expression by either signaling for the degradation of the mRNA or by inhibiting their translation. It has been shown that miRNAs are involved in multiple biological processes such as development, neuronal plasticity, immune function, proliferation and apoptosis. But function of miRNAs in stem cell differentiation is not well studied. Although there are several reports showing miRNAs are involved in pluripotency or self-renewal of stem cells, understanding about function of individual miRNA is very poor. We profiled 466 human microRNA gene expressions in H9 human embryonic stem cell using multiplexed real time PCR. Some of the miRNAs showed different expression pattern in different culture condition such as feeder cell and concentration of FGF. Interestingly, a majority of miRNAs showing difference are human specific.

CIRM SCHOLAR: NA XU

TITLE: **Human embryonic stem cells: from epigenetics to microRNAs**

AUTHORS: **Na Xu, Min-Jeong Kye,Kai Q Lao, and Kenneth Kosik**

Human embryonic stem cells have the intriguing property of self-renew and pluripotency in vitro, thereby being an ideal system for studying the most fundamental questions in human development. One of the questions is what are the roles of microRNA genes, a group of novel non-coding RNA genes, in the regulation of stem cell fate. To address that question, our group have investigated the expression profile of 466 human microRNAs (miRNAs) using multiplex realtime RT-PCR approach. The differentially expressed miRNAs during the transition from undifferentiated hESC to the differentiated state might be candidates for repressing either the stem-cell-specific or lineage-specific genes, thereby facilitating the transition.

CIRM SCHOLAR: H.-R. JESSIE HSU

TITLE: **Molecular cooperation of EWS-FLI1 and Bmi-1 in Ewing tumors**

AUTHORS: **H.-R. Jessie Hsu, Dorothea Douglas, Srinivas Somanchi, Long Hung, John van Doorninck, Xiaohua Jiang, Aaron Cooper, Hiroyuki Shimada, and Elizabeth R. Lawlor**

Ewing tumors (ET) characteristically express a chimeric EWS-FLI1 fusion gene. Although EWS-FLI1 functions as an oncogene in ET, in primary cells it commonly causes growth arrest and/or cell death implying that the cell of origin of ET must be permissive for oncogene expression. We have previously shown that the stem cell self-renewal gene Bmi-1 is highly expressed by ET and that it promotes ET cell proliferation and anchorage-independent growth. In these studies, we are investigating whether oncogenic cooperation between Bmi-1 and EWS-FLI1 is central to the pathogenesis of ET.

We have shown that Bmi-1 abrogates EWS-FLI1-mediated growth arrest in primary human fibroblasts. Further, in studies of ET cell lines as well as primary human fibroblasts and mesenchymal stem cells we find that ectopic over-expression of EWS-FLI1 leads to induction of Bmi-1 and preliminary results suggest that this is associated with altered regulation of p16. While these data implicate EWS-FLI1 in Bmi-1 deregulation, studies of ET cell lines grown in different culture conditions indicate that other mechanisms are also likely to be involved. First, withdrawal of serum dramatically reduces Bmi-1 promoter activity. In addition, anchorage-independent spheroids express less Bmi-1 than monolayer cultures. Immunocytochemical staining shows that this is due to the fact that, unlike their adherent counterparts, many cells in ET spheroids do not express Bmi-1. Interestingly, some primary ET also display this non-uniform expression of Bmi-1 at the cellular level. These data suggest that a cellular hierarchy, as defined by Bmi-1 expression, may exist in some ET.

We propose that deregulation of Bmi-1 by EWS-FLI1 is critical for malignant transformation and that continued expression of Bmi-1 may be required for ET cell self-renewal and tumorigenicity. Future studies will test this hypothesis.

CIRM SCHOLAR: CHUN-PENG LIAO

TITLE: **Attempts to identify cancer epithelial stem / progenitor cells in a mouse model of prostate cancer**

AUTHORS: **Chun-Peng Liao, Gohar Saribekyan, and Pradip Roy-Burman**

Existing therapies for prostate cancer eliminate the bulk of the androgen-sensitive cells within a tumor. However, most patients go on to develop androgen-depletion independent (ADI) cancer that is incurable by current treatment strategies. Evidence is accumulating to imply that intra-tumor cancer cell heterogeneity is organized as a hierarchy of cells originating from "cancer stem cells" that are responsible for maintaining the tumor. Recent studies have shown that Lin-Sca-1+CD49f+ cell subpopulation isolated form the normal murine prostate can self-renew to form spheroids in vitro for multiple generations, and can differentiate to produce prostatic tubule-like structures in vivo. Considering that this type of selection is likely to yield enrichment to putative stem cell-like cell population of the prostate, we applied this approach to examine the occurrence of such cells in the adenocarcinoma tissue of the Pten deletion mouse model of prostate cancer. We find that sorting of cells with a Lin-Sca-1+CD49+ antigen profile results in an eight-fold enrichment in primary tumors (32.7%, n=5) than in normal littermate or age-matched controls (4.1%, n=5). The percentage of this subpopulation is noted to be slightly increased in the ADI cancer (42.3%, n=2), although the results are only very preliminary and all analyses remain to be expanded in numbers to derive a statistical significance. There is an approximate eight-fold increase in the Lin-Sca-1+ cell population in the primary (63.5%, n=4) or ADI cancer (69.6%, n=2) relative to the normal controls (8.3%, n=3). The enrichment is, however, only three-fold when the Lin-CD49f+ cell population in the primary (55.1%, n=4) or ADI cancer (55.8%, n=2) is compared to the normal controls (18.8%, n=3). In biological assays with the Lin-Sca-1+CD49f+ cells isolated from primary tumors, we find that they are able to form colonies on the NIH3T3 feeder layer and, when co-cultured with prostate myofibroblasts derived from either the normal or the tumor tissues, to form spheres in matrigel. Taken together, the results provide a basis for the following contentions. (1) Lin-Sca-1+CD49f+ enriched population isolated from the mouse model of prostate adenocarcinoma does have the capacity to self-renew and differentiate. (2) The increased percentage of Lin-Sca-1+Cd49f+ cells in the cancer relative to the normal counterpart tissues argues for an important role for them in tumor growth, and describes Sca-1 as a prominent cell surface marker of stem /progenitor cells both in normal and neoplastic context of the mouse prostate. (3) Continued presence or even further enrichment of Lin-Sca-1+Cd49f+ cells in ADI cancer might serve as a clue in the strategies to better understand the development of recurrent prostate cancer.

CIRM SCHOLAR: HAIHUI LU

TITLE: **Reconstitution of V(D)J recombination in a purified system and suppressing NHEJ to improve gene targeting efficiency for human embryonic stem cells**

AUTHORS: **Haihui Lu and Michael R. Lieber**

Antigen receptor genes are assembled by V(D)J recombination during B and T lymphocyte development. The recombination events are initiated by specific cleavage at the paired V, D and J gene segments by RAG proteins, which result in formation of two coding end hairpins and two blunt signal ends. The DNA ends are processed by NHEJ to form coding joints and signal joints. Here V(D)J recombination was reconstituted in an in vitro system for the first time using purified proteins: mouse core RAG1/RAG2, as well as human proteins HMG1, Ku70/Ku80, Artemis, DNA-PKcs, DNA ligase IV:XRCC4, XLF, polymerases mu, lambda, and TdT. VDJ substrates consisting of DNA oligonucleotides were incubated with the protein factors to achieve coding joint formation, which were then detected by PCR using radioactive labeled primers. The PCR products were cloned and sequenced to reveal the junction sequences. The reconstituted reactions exhibited the expected sequence profile of the junctions and protein requirements. The coding joint formation requires the RAGs complex, Artemis, DNA:PKcs and DNA ligase IV:XRCC4, whereas the RAGs complex alone is sufficient for hybrid joint formation. Furthermore, we demonstrated that DNA-PKcs activates Artemis and enhances the activity of RAGs and DNA ligase IV:XRCC4 under the same conditions as in the reconstitution. Our data strongly support the role of DNA-PKcs as a coordinator in coding joint formation. This in vitro system provides a novel tool for detailed mechanistic analysis of V(D)J recombination. Besides its indispensable role in V(D)J recombination, NHEJ is also the primary repair pathway in all double-strand DNA break repair in mammalian cells. Gene targeting of human embryonic stem cells (hESCs) has been extremely difficult because the desirable repair pathway for successful gene targeting is homologous recombination (HR). Here we try to suppress NHEJ in order to optimize the HR mediated gene targeting in hESCs.

CIRM SCHOLAR: SUPARNA MISHRA

TITLE: **In utero stem cell gene therapy for cystic fibrosis**

AUTHORS: **S. Mishra, X. C. Wang, D. Senadheera, K. C. Bui, D. F. Chang, N. Smiley, and C. Lutzko**

Cystic Fibrosis (CF) is a common genetic disorder, resulting from mutations in the CFTR gene, which codes for a cAMP activated chloride channel expressed in epithelial cells. We propose that gene delivery to epithelial stem cells in vivo may provide long-term therapy for CF patients. Prenatal screening for CF may provide an opportunity for in utero gene therapy to correct the defect before birth. The goal of the current study is to optimize the in utero gene delivery of recombinant lentiviral vectors, carrying an eGFP reporter gene, to epithelial stem and progenitor cells in the tissues affected by CF.

Lentiviral supernatant (~4.5x10^7.IU/fetus) was injected directly into the amniotic fluid of each fetus and the effect of gestational age on transduction efficiency was evaluated. From the E12.5, E14.5 and E16.5 injections, 1/12, 6/12 and 7/7 pups were born naturally, respectively. At P0, P7 and P28, 2-3 pups from each litter were sacrificed and tissues harvested for analysis of gene transfer and eGFP transgene expression. Analysis of gene transfer efficiency was done using quantitative PCR, while transgene expression was evaluated using immunofluorescence. In pups injected at E14.5 and E16.5, proviral sequences were detected in the skin, trachea, esophagus, intestine and lung. eGFP expression was observed in skin, trachea, lung and liver. Identification of the cell lineage in the eGFP positive tissues is in progress, but clearly demonstrate transgene expression in epithelial cells in the lung.

Our preliminary results show promise for in utero stem cell gene therapy with lentiviral gene delivery method, as evidenced by the integration and expression of the eGFP transgene in multiple tissues affected by CF. Future studies aimed at delivering the normal CFTR cDNA to epithelial stem cells in tissues affected by CF will determine the therapeutic potential of this method.

CIRM SCHOLAR: ESZTER PAIS

TITLE: **Characterization of Lentiviral vector constructs designed to induce the controlled proliferation of pancreatic beta cells and their progenitors**

AUTHORS: **Eszter Pais, Shundi Ge, Kit Shaw, Gay M. Crooks, and Donald B. Kohn**

Background: Although pancreatic beta cell transplantation might serve as a potential cure for diabetes mellitus, limited donor tissue availability poses a major challenge. Aiming to overcome this shortage, our study utilizes a novel approach with the potential to induce the controlled proliferation of pancreatic beta cells and their progenitors (e.g., human embryonic stem cells). The methodology is based on the Lentiviral delivery of a novel fusion protein (F36V-cmet) into target cells. Based on previous studies utilizing similar vector constructs, the fusion protein is only active in the presence of a synthetic ligand (AP20187), thus allowing the controlled stimulation of selected signal transduction pathways. Our construct was designed to specifically activate the HGF (cmet) pathway that is known to be involved in beta cell development and proliferation. Thus, the aim of our study was to characterize this novel vector construct and to test the hypothesis that controlled beta cell proliferation can be achieved using this approach.

Methods: We designed third generation, dual-promoter Lentiviral vector constructs encoding the novel fusion protein, F36V-cmet and the eGFP reporter. Vector particles were generated via the transient transfection of 293T cells; vector preparations were concentrated by ultracentrifugation yielding titers as high as 3 5X109 TU/ml. For the initial evaluations we utilized murine insulinoma cell lines (Nit-1, beta-TC6) as a model system. Our studies included: 1) FACS analysis for eGFP expression; 2) Southern blot experiments to test vector intactness and integration; 3) Northern blot to confirm transcription from the vector. In addition, cell proliferation studies were performed to assess vector performance.

Results: High transduction efficiency of the novel vector construct was revealed. Our experiments confirmed that the intact vector is carried into the cells achieving long-term, stable transgene expression. Notably, both transgenes encoded by the dual promoter vector were effectively transcribed by the target cells. Cell proliferation studies confirmed the significant, selective growth advantage of the transduced cells cultured in the presence of the AP20187 stimulatory synthetic ligand.

Conclusions: Our experiments revealed that transduction with the newly developed vector construct results in long-term, stable transgene expression. The encoded fusion protein showed the expected biological activity in the presence of the synthetic ligand. Our study thus establishes a novel approach to induce the controlled proliferation of primary pancreatic beta cells and their progenitors, such as hESC.

CIRM SCHOLAR: JINGJING SUN

TITLE: **Msx genes function downstream of BMP signaling pathway in regulating the production and migration of primordial germ cells**

AUTHORS: **Jingjing Sun, Mamoru Ishii, and Robert Maxson**

Genetic information is transmitted by the germ cell lineage from one generation to the next. In mammals, the induction of the primordial germ cells (PGCs) from the proximal epiblast marks the initiation of the germline development. It is well known that BMP signaling pathway plays critical roles during this process. As immediate downstream targets of BMP signaling pathway, we found Msx genes are also involved. At E6.5, when PGC precursors receive the BMP signal from the extraembryonic ectoderm, both Msx1 and Msx2 transcripts can be detected in the extraembryonic ectoderm. Along with the emergence of the mesoderm during gastrulation, their expression first becomes more restricted to the posterior end of the primitive streak, then by E7.25 exclusively in the mesoderm, including the allantois, which houses the newly-formed PGCs. Here we demonstrate that the expressions of Msx1 and Msx2 at these stages are controlled by BMP signaling pathway. Loss of function in both genes causes a 50% reduction in the number of PGCs by E8.5. Our result also showed that the homing of PGCs from the hindgut to the genital ridges is greatly delayed in Msx1, 2 double mutant embryos, and significantly more PGCs remain in the caudal region of these embryos at E12.5, which suggests that Msx genes regulate the migration of PGCs as well.

CIRM SCHOLAR: STEVEN TSAI

TITLE: **The role of OCT-4 isoforms in hESC self-renewal**

AUTHORS: **Steven Tsai, David Chang, Xiao-Jin Yu, Xingchao Wang, and Carolyn Lutzko**

Self-renewal and pluripotency are two unique properties that characterize embryonic stem cells derived from the inner cell mass of human pre-implantation blastocysts. The ability to grow indefinitely and differentiate into tissues from all three germ layers is regulated by a complex network of transcription factors, a key one being OCT-4. OCT-4 is a POU domain transcription factor that is highly expressed in self-renewing, pluripotent cultures of human embryonic stem cells (hESC) and is routinely used as a quality control marker for hESC culture. POU5F1, the human OCT-4 gene, encodes two isoforms known as OCT-4A and OCT-4B. Each isoform has a unique N-terminal transactivation domain while sharing a common 225 amino acid C-terminal domain. Current research on OCT-4 generally does not consider the alternative OCT-4B transcript. In our present study, we have focused on evaluating the expression of both OCT-4 isoforms. We hypothesize that the existence of OCT-4 isoforms with different N-terminal transactivation domains is functionally important and will have differential effects on hESC self-renewal and pluripotency. RT-PCR using isoform specific primers showed that both isoforms are expressed in H9 hESC, embryoid bodies derived from H9 cells, and teratocarcinoma cell lines Tera-2, PA-1, and NCCIT. To study subcellular localization of OCT-4A and OCT-4B, cDNA was amplified from H9 RNA and cloned into pcDNA3.1, a mammalian expression construct. Immunofluorescence microscopy on 293A cells transiently transfected with OCT-4A and OCT-4B expression constructs demonstrated strong nuclear staining for OCT-4A. In contrast, OCT-4B showed cytoplasmic localization despite having a nuclear localization signal in its common C-terminal domain. Preliminary results from western blot analysis of H9 lysates suggest that OCT-4A protein is strongly expressed in the nucleus but not cytoplasm, whereas OCT-4B may not be endogenously expressed in hESC. Future studies include developing lentiviral vectors to evaluate the biological effects of long-term overexpression and gene knockdown of OCT-4A and OCT-4B in hESC.