Cell Stem Cell
ISSCR: Meeting Report

On the Streets of San Francisco: Highlights from the ISSCR Annual Meeting 2010

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The 2010 Annual Meeting of the International Society for Stem Cell Research (ISSCR) was held in San Francisco in June with an exciting program covering a wealth of stem cell research from basic science to clinical research.

Nearly 4000 scientists from 49 countries filled the vast auditorium of Moscone West in San Francisco for the opening session of the 8th Annual Meeting of the ISSCR. The stellar science presented in 8 plenaries and 15 concurrent sessions by 108 speakers over the ensuing four days led many to conclude that this was the best ISSCR annual meeting to date.

As promises of new stem cell therapies move closer to reality, ISSCR President Irving Weissman opened the meeting reiterating the society's commitment to champion the responsible clinical translation of stem cell research and highlighting the ISSCR's recent initiative that resulted in the June 2010 launch of a new public education resource, A Closer Look at Stem Cell Treatments (www.closerlookatstemcells.org).

In his Keynote Address on neuronal plasticity and diversity, Fred Gage (Salk Institute, USA) led off with a beautiful review of neurogenesis in the adult brain and then focused on the role of long interspersed nuclear element (LINE-1) retrotransposons in modulating gene expression in developing neurons (Singer et al., 2010). Although LINE-1 elements constitute nearly 20% of the mammalian genome, most are truncated copies that are fixed in position. In fact, in the human genome there are approximately 150 full-length potentially mobile LINE-1 elements, and probably only a handful of these are responsible for most retrotransposition activity. New LINE-1 insertions occur predominantly in developing neural cells, both during embryonic neurogenesis and in the adult brain. It is believed that LINE-1 activation, regulated by the *Wnt* signaling pathway, can affect gene expression and thus cellular phenotype. Gage maintains that LINE-1 induced somatic mosaicism may ultimately influence cognition and behavior—adding a whole new interpretation to the notion of ''changing your mind''!

The winner of the 2010 ISSCR Outstanding Young Investigator Award, Joanna Wysocka (Stanford University, USA), also discussed developmental plasticity, examining how epigenetic processes influence gene expression in the neural crest. Mutations in the gene encoding chromodomain helicase DNA-binding domain (CHD)7 cause a complex disorder with prominent craniofacial malformations, termed CHARGE syndrome. In order to confirm the long-standing hypothesis that CHARGE syndrome represented a neural crest disorder, Wysocka's laboratory established an in vitro model of neural crest-like cell (hNCLC) formation from hESCs and demonstrated that hNCLCs expressed high levels of CHD7. She showed that siRNA-mediated downregulation of this gene perturbed cell migration and reduced the transcription of genes specifying neural crest migration and specification. Recently, her laboratory discovered the association of CHD7 with another chromatin remodelling protein, PBAF (polybromo- and BRG1-associated factor containing complex) (Bajpai et al., 2010). This research has broad implications, providing an example for the synergistic control of distal enhancers by complexes of chromatin remodeling proteins.

This year, the Anne McLaren Memorial Lecture was delivered by Brigid Hogan (Duke University, USA), who discussed epithelial stem and progenitor cells in lung development, homeostasis, and repair—a timely topic given that 2010 is the Year of the Lung. Highlighting important differences in size and structure between mouse and human lung, Hogan pointed out that undifferentiated basal cells, which express the transcription factor *Trp-63* (*p63*) and cytokeratins 5 (*Krt5*) and 14 (*Krt14*), are restricted to the trachea in mice but are found throughout the small airways in the human lung. Lineage-tracing studies in the mouse demonstrated that tracheal basal cells gave rise to ciliated and secretory cells and that viable basal cells could be isolated from tracheal epithelium by virtue of their expression of the nerve growth factor receptor (*Ngfr*, *p75*). Growth in a clonogenic assay in vitro revealed that basal cells could renew and differentiate in the absence of stroma. Translation of this work to human lung epithelium revealed similar clonogenic properties in human lung basal cells purified on the basis of their combined expression of *NGFR* and *ITGA6* (Rock et al., 2009). Currently, her laboratory is dissecting the pathways that bias basal cell differentiation toward either secretory or ciliated epithelium.

Toward Cell Therapies—Work in Progress

A session devoted to cell therapy opened with an update of the much-publicized clinical trial to evaluate the role of human embryonic stem cell (hESC)-derived oligodendrocytes in the

President Irving Weissman opened the ISSCR 8th Annual Meeting in the Ballroom, Moscone West, San Francisco.

The poster boards and exhibit areas were a constant hive of activity.

treatment of acute thoracic spinal cord damage. Joseph Gold (Geron Corporation, USA) explained that stored oligodendrocytes will be injected into the spinal cord lesion in patients within 2 weeks of injury. On the basis of preclinical studies in rats, it is hypothesized that the oligodendrocytes might have a beneficial effect by restoring the damaged myelin sheath around nerve bundles. The trial was temporarily put on hold after the identification of microscopic epithelial cysts in the spinal cord of some recipient rats. It appears that these were benign, composed of endothelial cells, allowing the clinical hold to be lifted on July 30, 2010 and the trial to proceed.

Despite significant advances in the generation of cardiomyocytes from human pluripotent stem cells, several speakers indicated that using these cells for therapy still seemed remote. Joseph Gold, Gordon Keller (McEwen Centre for Regenerative Medicine, Canada), Kenneth Chien (Massachusetts General Hospital, USA) and Christine Mummery (Leiden University Medical Center, Netherlands) described high yields of cardiomyocytes from hESC and human induced pluripotent stem cells (hiPSCs), with cultures under defined growth factor conditions reproducibly generating over 50% cardiomyocytes. Although the spectacular beating sheets of immature cells should be well-suited to transplantation, there remained significant additional hurdles to overcome. In addition to hazards posed by xenoreagents, residual undifferentiated pluripotent cells and immune rejection, cardiac cell transplantation presents risks of inducing arrhythmias and difficulties in achieving proper cell alignment. However, both Keller and Chien presented potential tissue engineering solutions to the latter problems. Geron's cardiomyocyte studies are now being extended to the guinea pig and pig—larger animal models in which it would be easier to detect adverse effects and to meet challenges related to cell preparation, administration, and safety associated with the larger cell doses required for human cell therapy.

In addition to improving the efficiency of stem cell differentiation, for many applications it would also be useful to identify cell-surface markers that could be used to enrich for viable progenitors or differentiated cells. Ali Nsair (University of California, Los Angeles, USA) proposed that tripotent cardiac progenitors expressing the transcription factor *Isl1*, usually identified by expression of the VEGF2-receptor *flk1*, could be more readily identified with a combination of antibodies directed against the tyrosine kinase receptors *flt1* and *flt4*. Cells selected from differentiating hESCs, hiPSCs, and human fetal heart with this strategy were enriched for cardiomyocyte, endothelial, and smooth muscle differentiation potential.

Diseases affecting the skin and the cornea have also been targeted for stem cell therapies. Daniel Miller (University of Washington, USA) presented a combined cell (keratinocyte) and gene therapy approach to a severe blistering skin disease, epidermolysis bullosa simplex, caused by a dominantly inherited mutation in *KRT5* or *KRT14.* Miller used an adeno-associated virus vector to target and repair the mutation in human keratinocytes and showed that these genetically repaired cells, grown on a matrix scaffold, organized into a normal skin epithelium, which could be successfully grafted to athymic mice (Petek et al., 2010).

Impressive progress in stem cell therapy has also been made for some eye diseases. Graziella Pellegrini (University of Modena and Reggio Emilia, Italy) described the use of stem cells from the corneal limbus in the treatment of corneal disease. Limbal stem cells cultured from the contralateral healthy eye were expanded in vitro and transplanted to repair the damaged cornea. Long-term follow-up revealed that successful ongoing corneal regeneration was dependent upon the frequency of high proliferative potential, *p63*-expressing "holoclones" within the transplanted population (Rama et al., 2010). This avenue of research is particularly promising because there are options to use either autologous or allogeneic limbal stem cells, given that the cornea is not sensitive to immune rejection.

Alexandra Capela, from StemCells, Inc., USA, discussed the use of human fetal central nervous system stem (hCNS-SCns) cells for the treatment of age related macular degeneration. hCNS-SCns cells have been shown to be neuroprotective in a mouse model of infantile neuronal ceroid lipofuscinosis (Tamaki et al., 2009) and are now under evaluation for the treatment of fatal neurodegenerative disorders in children. Capela argued that retinal degenerative diseases might similarly benefit from neuroprotective strategies to reduce photoreceptor loss. In rats predisposed to postnatal retinal degeneration, injection of

hCNS-SCns cells successfully preserved photoreceptors and maintained near-normal visual acuity. The opportunity to test this therapy in humans is eagerly awaited since hCNS-SCns cells have already been banked for use in clinical trials for neurodegenerative diseases.

The generation of human pancreatic beta cells as cell therapy for type 1 diabetes is an area of intense research interest for groups around the world. Kevin D'Amour (ViaCyte, Inc., USA; formerly Novocell, Inc.) described their recent progress in the differentiation of hESCs to pancreatic progenitors. Previously, they published that hESCs differentiated to pancreatic endoderm reversed type 1 diabetes in a mouse model following a 3 month in vivo maturation stage (Kroon et al., 2008). D'Amour described hESC differentiation in cellular aggregates, rather than as a monolayer, in order to enable upscaling to the cell numbers required for clinical use. The differentiated product was high in purity for pancreatic precursors, could be cryopreserved, and differentiated efficiently in vivo to yield structures that were very similar to pancreatic islets. D'Amour also described an encapsulation device that would address issues of patient safety, by simultaneously protecting the graft from immunological attack and blocking egress of any unwanted proliferating cells, while still enabling graft endocrine function.

Metabolism and Stress Response

Two plenary sessions were devoted to metabolic regulation and stress response in stem cells, which attested to the strong interest in basic stem cell biology. Celeste Simon (University of Pennsylvania, USA) described her ongoing work on oxygen deprivation and hypoxia-inducible factors (HIFs) in stem cells and cancer. The HIFs and their interacting partner, ARNT, modulate several essential stem cell effector pathways, including *Notch*, *Wnt/β-catenin*, and *Oct4* that influence stem cell proliferation, differentiation and pluripotency in low oxygen concentrations. She highlighted the importance of these regulatory mechanisms for neuronal and hematopoietic stem cell (HSC) maintenance in their respective hypoxic niches. Ricardo Pardal (Instituto de Biomedicina de Sevilla, Spain) described hypoxia-induced postnatal neurogenesis in the carotid body, the organ detecting oxygen tension in the arterial blood, through changes in the ratio of quiescent and proliferative glial-like stem cells. Marc Van Gilst (Fred Hutchinson Cancer Research Center, USA) and Yukiko Yamashita (University of Michigan, USA) showed that nutrient availability regulates stem cell numbers and overall tissue architecture. Using the nematode, *C. elegans*, Van Gilst illustrated that regulation of fat expenditure and lipid synthesis controlled the biological activity of germline stem cells and the reproductive status of the worm through the NHR49 pathway (Angelo and Van Gilst, 2009). Yamashita explained that nutrients provided to the fruit fly *D. melanogaster* impacted on centrosome orientation and dictated the rate of cell division in the male germline stem cells through the insulin pathway.

Pier Giuseppe Pelicci (University of Milan, Italy) delivered the first in a series of talks describing the DNA damage response of several stem cell populations that highlighted the prominent roles and contrasting functions of the *p53* and *p21* tumor suppressor genes (Cicalese et al., 2009; Viale et al., 2009). Using X-rays to illustrate genotoxic stress, he discussed the importance of the cell cycle inhibitor *p21* in maintaining the stem cell pool in normal and malignant HSCs and mammary stem cells. Lenhard Rudolph (Ulm University, Germany) discussed the ameliorating effects of *p21* deletion on the shortened lifespan of telomere dysfunctional mice and expanded on the role of *p53*-induced senescence and apoptosis as a response to telomere dysfunction. *p53* played a unique role in protecting adult stem cells against the accumulation of mutations with consequences for aging and cancer development (Begus-Nahrmann et al., 2009). Emmanuelle Passegué (University of California, San Francisco, USA) showed that enhanced prosurvival gene expression and activation of *p53*-mediated DNA damage response ensured the survival of HSCs in response to ionizing radiation. She also presented provocative data indicating that the prevalent DNA repair mechanism active in quiescent HSCs (nonhomologous end-joining mediated repair) is prone to generating mutations in this long-maintained self-renewing population (Mohrin et al., 2010). Cedric Blanpain (Université Libre de Bruxelles, Belgium) reported similar findings in hair follicle bulge stem cells (Sotiropoulou et al., 2010), which suggests that vulnerability to mutagenesis might be a general property of quiescent stem cell populations either normal or cancerous.

Craig Jordan (University of Rochester, USA) postulated that killing cancer stem cells requires combinatorial drug therapies that will more effectively kill all tumor cells by inhibiting developmental pathways and antagonizing protective mechanisms that are active in stem and progenitor cells. As examples of the cancer stem cell targets that new therapies might address, he listed induction of oxidative stress linked with concomitant inhibition of the NFκB-mediated survival pathway, the redox balancing system, heat-shock proteins, and anti-oxidant protective mechanisms.

Refining Reprogramming

The intense interest in cellular reprogramming since the initial presentation of iPSCs at the 4th ISSCR Annual Meeting in 2006 continued during this meeting. Shinya Yamanaka (Kyoto University, Japan and Gladstone Institutes, USA) discussed the requirement for *Myc* genes in reprogramming somatic cells to pluripotency. He concluded that c-*Myc* increased the frequency of iPSC generation but that this was associated with reactivation of the c-*Myc* virus and tumor formation in mice generated with these cells. However, Yamanaka reported reprogramming fibroblasts by using a cocktail of factors in which L-*Myc*, a weakly transforming family member of the *Myc* family, was substituted for c-*Myc*. The inclusion of L-*Myc* led to more efficient generation of iPSC clones that infrequently led to tumors in chimeric mice (Nakagawa et al., 2010).

Work by several laboratories employed different approaches to compare ESCs and iPSCs. George Daley (Children's Hospital Boston, USA) described global DNA methylation analysis that revealed a significant number of differentially methylated regions between ESCs and iPSCs, consistent with the concept that iPSCs retain an "epigenetic memory" that reflected their cell type of origin. Perhaps as a consequence of these epigenetic differences, Daley noted that iPSCs tended to differentiate more efficiently toward cell types related to their cell of origin (Kim et al., 2010). In an elegant series of studies, Konrad Hochedlinger (Massachusetts General Hospital, USA) profiled genetically matched mouse iPSCs that differed in their capacity

to produce high contribution chimeras and "all-iPSC" mice by tetraploid complementation. Surprisingly, he uncovered a single locus on chromosome 12, containing a few mRNAs and microRNAs (miRNAs), whose expression correlated with the developmental potential (Stadtfeld et al., 2010). In most iPSC clones the locus was silenced, and the iPSCs produced low contribution chimeras. In rare iPSC clones in which the locus was expressed, the cells made high contribution chimeras and even all-iPSC mice. Treatment of the silenced clones with a histone deacetylase inhibitor reactivated expression from the locus and induced full developmental potential to the iPSCs.

Christa Buecker (Massachusetts General Hospital, USA) contrasted the distinct leukemia inhibitory factor (LIF)-dependent ESC and fibroblast growth factor (FGF)-dependent epiblast stem cell (EpiSCs) "states" that can be reversibly adopted by mouse pluripotent cells. Human ESCs more closely resemble mouse EpiSCs in their growth rates, factor requirements, and reluctance to passage as single cells. To derive human cell lines that were more similar to mESCs and therefore more amenable to genetic modification, Buecker and colleagues introduced inducible reprogramming factors into human fibroblasts that they cultured in the presence of LIF to derive colonies of cells (denoted hLR5 cells) that morphologically and immunophenotypically resembled mESCs (Buecker et al., 2010). Interestingly, levels of endogenous *OCT4*, *NANOG*, *SOX2*, and *MYC* remained low in hLR5 cells and they remained dependent upon the expression of exogenous reprogramming factors. However, Buecker observed that the hLR5 cells displayed a similar facility for genetic modification to mESCs, generating over 100-fold more stable transfectants than an equivalent number of hESCs.

Direct Fate Conversion

The ability of pigmented retinal epithelium in the chick to develop into lens cells was observed over 30 years ago, and this switch in cellular differentiation was termed "transdifferentiation" (Eguchi and Kodama, 1993). In this case, it was clear that the cells "dedifferentiated" to a stem or progenitor cell state first. Infrequently, the mis-expression of a single gene can convert one cell type into another, without apparent dedifferentiation, as was the case for muscle switching of C3H10T1/2 fibroblasts by *MyoD* (Davis et al., 1987). The advent of somatic cell reprogramming fueled renewed interest in direct cellular fate conversion using multiple genes, thus circumventing a pluripotent intermediate. Following on the heels of the successful conversion of adult pancreatic exocrine cells to beta cells in vivo reported by Douglas Melton (Harvard University, USA) at the ISSCR 6th Annual Meeting in Philadelphia, several speakers presented the results of direct reprogramming studies.

Marius Wernig (Stanford University, USA) described experiments that aimed to generate neural lineages in a single step. He transduced murine fibroblasts with a combination of 19 candidate genes and eventually identified three factors that efficiently reprogrammed fibroblasts to induced neural cells, with a predominantly excitatory cortical neuron phenotype (Vierbuchen et al., 2010). It remains to be seen whether the reprogramming factors can be dispensed with and how readily neurons of different subtypes can be produced. Kevin Eggan (Harvard University, USA) explored the reprogramming of fibroblasts directly to motor neurons, with a view to developing new treatments for amyotrophic lateral sclerosis. He reprogrammed fibroblasts harboring a motor neuron-specific GFP reporter with various combinations of factors. Success eluded him, however, until he also added the three factors used by Wernig. In the final talk on this theme, Deepak Srivastava (Gladstone Institutes, USA) discussed the reprogramming of cardiac-derived "fibroblasts" to cardiomyocytes (Ieda et al., 2010). He used a combination of 14 transcription factors to reprogram the fibroblasts to a cardiac fate. Srivastava refined the list of reprogramming genes, leading to the conclusion that a three factor combination would successfully reprogram fibroblasts to beating, electrically active cells with a phenotype similar to ventricular cardiomyocytes. Importantly, using a lineage tracing strategy, his group showed that the fibroblasts were directly induced into cardiomyocytes, bypassing a progenitor intermediate.

Germ Cells, Imprinting and Reprogramming

Primordial germ cells (PGCs) hold a unique place in the stem cell hierarchy given that it is these cells that give rise to gametes and represent a literal genetic link to the next generation. Several talks were related to the biology of PGCs derived from mammals and nonvertebrate species. Crucial to PGC formation in females is the reactivation of the somatically silenced X chromosome as the PGCs migrate to the urogenital ridge. Kathrin Plath (University of California, Los Angeles, USA) pointed out that, unlike female mESCs, most female hESC lines carry one inactive X chromosome. Recent work from the group of Jaenisch and Mitalipova showed that this may be due to oxidative stress and that female hESCs derived under 5% oxygen conditions retained two active X chromosomes that were randomly inactivated upon further differentiation (Lengner et al., 2010). Interestingly, Plath showed that, like the hESCs, hiPSCs had only one active X chromosome. However, unlike the derivation of hESCs, reprogramming under hypoxic conditions did not support X reactivation (Tchieu et al., 2010). Plath made the important observation that because the inactivated X is retained during reprogramming and differentiation, iPSC clones were well positioned for the study of X-linked diseases (such as Duchenne Muscular Dystrophy) because it would be possible to get clonal lines either expressing the wild-type or mutant allele from the same female patient.

Studies in nonvertebrate species reported examples of gene mutations affecting germ cell development that also affect humans. As an example, Shuyi Chen (Stowers Institute for Medical Research, USA) discussed the role of *Lis1*, which is required for maintaining BMP signaling and the balance between self-renewal and differentiation in *Drosophila* germ cells. A further highlight was the presentation by Mitinori Saitou (RIKEN Center for Developmental Biology, Japan), who studied germ cell specification in mice and the role of the transcription factors *Blimp1* (*Prdm1*) and *Prdm14*, which are coexpressed in the PGCs (Ohinata et al., 2009). Saitou showed that extraembryonic signals were key regulators of germ cell induction. Under defined culture conditions, in the presence of BMP4, most of the isolated epiblast cells in wild-type mouse embryos became *Blimp1*- and alkaline phosphatase-positive PGCs. These culture-induced PGCs developed into sperm upon injection into the testes of aspermic mice and gave rise to viable offspring. Similar signaling pathways appear to be active in mESCs and data

presented also suggested that the dynamics of gene expression were similar whether the PGCs were derived from the epiblast or ESCs.

MicroRNA Regulation of Stem Cells

Robert Blelloch (University of California, San Francisco, USA) discussed the roles for miRNAs in regulating the switch between self-renewal and differentiation in embryonic stem cells (Melton et al., 2010). Blelloch's laboratory discovered antagonistic roles for two families of miRNAs, the *ESCC* and *let-7* miRNAs, which are highly expressed in ESCs and differentiated tissues, respectively. The *ESCC* miRNAs enhance self-renewal of ESCs and promote the dedifferentiation of somatic cells to iPSCs. In contrast, the *let-7* miRNAs promote the differentiation of ESCs and inhibit the dedifferentiation of somatic cells to iPSCs. He also discussed surprising findings that all miRNA function is suppressed in oocytes and preimplantation embryos, postulating that this may be essential for the massive reprogramming that occurs in the early embryo (Suh et al., 2010).

Narry Kim (Seoul National University, Republic of Korea) presented elegant work from her laboratory on the posttranscriptional regulation of *let-7* (Heo et al., 2009). Like all canonical miRNAs, *let-7* is transcribed as a long pri-miRNA. The pri-miRNA is processed first by an RNase, Drosha, to a pre-miRNA and then by another RNase, Dicer, to a mature miRNA. Kim and others have shown that the RNA binding protein Lin28 and the terminal uridylase Tut4 regulate the biogenesis of *let-7*. *Lin28* is highly expressed in ESCs and together with *Tut4* adds uridines at the end of the *let-7* pre-miRNA, destabilizing and inhibiting its further processing by Dicer in ESCs. In contrast, *Lin28* expression is reduced upon differentiation resulting in increased levels of mature *let-7*.

Using an inducible overexpression system, Hao Zhu (Children's Hospital Boston, USA) examined the role of *Lin28* in vivo in the mouse (Zhu et al., 2010). Induction of *Lin28* resulted in a rapid expansion of the intestinal progenitor cell pool, consistent with a block in differentiation and increased proliferation. Interestingly, leaky expression of the *Lin28* transgene led to an increase in size of most organs in the mouse, consistent with the postulated role for *let-7* in suppressing stem cell self-renewal and proliferation.

Leanne Jones (Salk Institute, USA) also discussed the importance of *let-7* in stem cell regulation in the *Drosophila* germline. Her laboratory has studied the molecular mechanisms responsible for the decreasing number of male germline stem cells as the fruit fly ages. The JAK-STAT signaling ligand *Upd*, which is expressed by the niche (hub) cells, is essential for maintaining the germline stem cells and its expression decreases with age. In recent studies she has observed that *let-7* expression increases in the hub cells with age, and such an increase indirectly results in the loss of *Upd*.

Of course, there are other important miRNAs in addition to *let-7*. Xinyu Zhao (University of New Mexico, USA) showed that the epigenetic regulator *MBD1* controls a miRNA, *miR-184*, to regulate neural stem cell (NSC) proliferation and differentiation (Liu et al., 2010). Zhao's laboratory found that MBD1 binds the *miR-184* promoter and inhibits its expression. *miR-184* itself promotes proliferation and inhibits differentiation of NSCs, at least in part by regulating the important NSC regulator *Numb-like*.

Small-Molecule Screening for Stem Cell Regulation

The variety of talks concerned with screens for molecules affecting stem cell behavior or influencing differentiation reflected the importance of small molecules as replacements for recombinant growth factors and as treatments for disease in their own right. Leonard Zon (Children's Hospital Boston, USA) described the results of two recent small-molecule screens in zebrafish. In the first screen, his laboratory identified two small molecules that suppressed growth of rhabdomyosarcomas induced by a mutant *Ras* (G12V) oncogene. Both molecules targeted signaling proteins downstream of the *Ras* pathway and displayed synergy when used in combination. In the second screen, they examined a B-*raf* (V600E):$p53^{-/-}$ melanoma model, in which neural crest stem cell genes, including *crestin*, were highly expressed. Zon's laboratory screened for molecules that would block *crestin* expression, identifying a small-molecule inhibitor of the enzyme *dihydrooratate dehydrogenase*. Inhibition of this enzyme depleted the ribonucleotide pool and, therefore, would be hypothesized to inhibit transcriptional elongation. Indeed, administration of the *dihydrooratate dehydrogenase* inhibitor phenocopied deletion of the RNA polymerase associated factor, *spt5*, a known regulator of transcriptional elongation.

Rodolfo Gonzalez (The Scripps Research Institute, USA) described the development of a library of extracellular and single-pass transmembrane proteins and their use in a pluripotency screen (Gonzalez et al., 2010). Open reading frames encoding these proteins were subcloned, purified, and screened for their ability to maintain *OCT4* expression in hESCs cultured in the absence of bFGF. In addition to bFGF itself, they identified another protein ligand, pigment epithelium derived growth factor (*PEDF*) that maintained hESC marker expression, pluripotency, and chromosomal stability. They are extending their screens to identify molecules that influence hESC differentiation.

Shuibing Chen (Harvard University, USA) discussed the results of small-molecule screens aiming to identify promoters of endoderm lineage differentiation, with the ultimate goal of producing insulin-producing beta cells. She reviewed the results of the screen that led to the identification of indolactam V (an activator of protein kinase C) as an inducer of differentiation of gut endoderm to $PDX1^{+}$ pancreatic endoderm (Chen et al., 2009). She has recently been focusing on the next step in differentiation, from $PDX1^{+}$ pancreatic endoderm to $NGN3^{+}$ endocrine cells. At this stage Chen suggests that there are a couple of promising candidate molecules that not only increase the number of $NGN3^{+}$ cells but also the number of cell clusters containing C-peptide^{+} Glucagon^{-} endocrine cells, consistent with beta cell differentiation.

Justin Ichida (Harvard University, USA) discussed efforts to discover small-molecule replacements for reprogramming genes in mouse iPSC generation. He described a small-molecule inhibitor of TGF-β, which can replace *Sox2* by activating *Nanog* (Ichida et al., 2009). He indicated that a small-molecule replacement for KLF4 had been found, which like the Sox2 replacer, acted late in reprogramming.

Sheng Ding (The Scripps Research Institute, USA) focused on small molecules that promote hESC survival in culture (Xu et al., 2010). His laboratory identified two small molecules, thiazovivin and pyrintegrin, that enhance hESC colony formation following enzymatic dissociation and replating on matrigel.

Both compounds enhance cell-extracellular matrix (ECM) adhesion-mediated integrin signaling, synergizing with growth factors to enhance survival. In addition, thiazovivin enhanced hESC survival in suspension culture, acting to stabilize E-cadherin and directly inhibiting Rho-associated kinase (ROCK).

Stem Cell Niche and Hematopoiesis

In the skin, there are at least three independent stem cell populations—in the bulge of the hair follicle, the sebaceous gland, and the basal layer of the interfollicular skin. They self-renew, differentiate, and have long-term proliferative potential, but differ in their cell cycle status, explained Elaine Fuchs (Rockefeller University, USA). The bulge cells remain largely quiescent, whereas the other stem cell populations proliferate. The quiescent bulge cells are recruited back into cycle after damage and during regular hair growth cycles. The Fuchs laboratory is studying the signaling pathways that regulate the recruitment of bulge stem cells into the transit amplifying cell compartment and the source of that signal within the niche. *Wnt* and *BMP* are among the candidate signaling pathways under investigation. Furthermore, they are studying the misregulation of these pathways in skin cancers. To this end, they have recently developed a novel in vivo screening approach in mouse skin by injecting viruses in utero, which efficiently transduce the embryonic skin and thus will enable large-scale gene knockdown screens (Beronja et al., 2010).

Valerie Horsley (Yale University, USA) focused on the regulatory role of the hair follicle niche, specifically investigating the part that adipocytes play in regulating the hair follicle growth cycle building on the exciting finding that BMPs released by dermal adipocytes regulate the hair cycle (Plikus et al., 2008). The Horsley laboratory has been delving deeper into the nature of the adipocytes, including their cell cycle dynamics during the hair cycle and the relative importance of progenitor versus mature adipocytes in the process.

The role of integrin-ECM interactions in regulating the asymmetric divisions of intestinal stem cells in *Drosophila* was discussed by Ryan Conder (Institute of Molecular Biotechnology, Austria). Knockdown of the *Drosophila* integrin *mew* increased stem cell number and was associated with mislocalization of the Par protein, aPKC, loss of spindle polarity, and loss of asymmetry of expression of the Numb protein. These latter findings are reminiscent of findings in the neuroblast lineage suggesting common mechanisms of asymmetric cell divisions across somatic lineages.

Mark LaBarge (Lawrence Berkeley National Laboratory, USA) discussed the effect of E-cadherin expression levels on cellular organization within mammary tissue. He showed that the mixing of luminal and myoepithelial cells leads to reorganization into ductal structures that is dependent on E-cadherin signaling pathways. Myoepithelial cells express low, luminal cells express intermediate, and progenitors cells express high levels of E-cadherin.

Shahin Rafii (Weill Cornell Medical College, USA) described recent work studying the interplay between endothelial cells (ECs) and HSCs (Butler et al., 2010). With a human umbilical vein endothelial cell coculture system, mouse HSCs could be expanded in vitro, maintaining stem cell marker expression and functional capacity in transplant assays. Similarly, human HSCs could be expanded while still retaining the ability to repopulate NOD-SCID mice. A search for potential factors provided by the ECs uncovered expression of *Notch* ligands, whose significance was confirmed by the concomitant activation of a transgenic *Notch* reporter in the cocultured HSCs. In vivo, *Notch* ligands are provided by the bone marrow sinusoidal endothelial cells. Inhibition of sinusoidal formation with anti-VEGFR2 and anti-VE-cadherin antibodies inhibited *Notch* activation and long-term HSC expansion in coculture. Rafii's laboratory is now searching for additional pathways important in this critical interaction between ECs and HSCs. These findings hold great promise for clinical expansion of HSCs in vitro.

David Scadden (Massachusetts General Hospital, USA) described how microenvironmental deregulations in the bone marrow niche impacted on hematopoiesis and contributed to hematological disorders. He presented the recent finding from his laboratory that inactivation of the miRNA processing gene *Dicer* in osteoblastic progenitors led to a myelodysplastic syndrome that could be relevant to the pathogenesis of Schwachman-Bodian-Diamond syndrome, a human bone marrow failure and leukemia predisposition condition (Raaijmakers et al., 2010).

The role of the *ets* family gene *Erg* in hematopoiesis and myeloproliferative disease was discussed by Benjamin Kile (The Walter and Eliza Hall Institute of Medical Research, Australia). Kile's group showed that *Erg* was required for definitive hematopoiesis, adult hematopoietic stem cell function, and the maintenance of normal peripheral blood platelet numbers (Loughran et al., 2008). They are now examining the role that *ERG* may play in the myeloproliferative disease and acute leukemia associated with Down's syndrome—a tantalizing prospect, given that *ERG* lies in the minimal trisomic region of chromosome 21.

Stem Cells of the Digestive Tract

The characterization of stem cells of the intestinal epithelium, marked by their expression of the orphan G-coupled receptor Lgr5, was discussed by Hans Clevers (Hubrecht Institute, Netherlands). Clevers demonstrated that, in the mouse, cells with stem cell characteristics expressed high levels of an Lgr5-GFP transgenic reporter gene and could be expanded in vitro to form gut-like organoids (Barker et al., 2010). Although these structures grew in the absence of a nonepithelial stromal component, their close association with Paneth cells and evidence of reciprocal signaling between these two cell types suggested that Paneth cells performed a niche-like function in the gut.

In contrast, work on *Drosophila* intestinal stem cells presented by Rongwen Xi (National Institute of Biological Sciences, China) provided evidence that muscle cells maintained the overlying intestinal stem cells. Xi showed that ligands such as *wingless* and *unpaired* are secreted by the muscle cells and regulate stem cell proliferation in a paracrine fashion (Lin et al., 2008, 2010).

Also working in *Drosophila*, David Bilder (University of California, Berkeley, USA), showed that food intake could influence intestinal stem cell self-renewal. He discovered that there is insulin release from the muscle underlying the mid-gut epithelium following feeding, immediately preceding an increase in proliferation of the overlying stem cells. Deletion of the insulin receptor in the stem cells blocks proliferation in response to

feeding while expression of a constitutively active receptor results in increased intestinal proliferation in a feeding-independent fashion.

Markus Grompe (Oregon Health and Science University, USA) discussed ongoing efforts to identify elusive liver stem cells. His laboratory has produced monoclonal antibodies that might separate the different stem cell, progenitor, and differentiated populations in the liver. On the basis of preliminary analysis, Grompe argued that oval cells may not represent the true stem cells because they are not clonogenic. However, Grompe has identified a combination of antibodies that highly enriches for bipotential clonogenic cells. Interestingly, unlike oval cells, these cells do not increase in number after acute injury.

Neural Stem Cells and Regeneration

Amputation of the axolotl tail triggers an amazing and coordinated regeneration of the vertebrae, muscles, spinal cord, and associated peripheral nervous system in which correct embryonic spatial coordinates are remembered. The cellular and molecular control of spinal cord regeneration in the axolotl was the theme of a talk by Elly Tanaka (Center for Regenerative Therapies, Germany). Their recent studies of axolotl limb regeneration revealed that the blastema—the zone of undifferentiated progenitors from which all the tissues will reform—was composed of a heterogeneous collection of restricted progenitor cells rather than just one pluripotent cell type (Kragl et al., 2009). Tanaka is now searching for the growth factors and the genes regulating tail regeneration.

Grigori Enikolopov (Cold Spring Harbor Laboratory, USA) discussed the division and differentiation of adult neural stem cells, highlighting the observation that neurogenesis decreases with age. On the basis of cell-labeling studies, Enikolopov presented the provocative hypothesis that hippocampal quiescent neural progenitor cells, after activation, underwent several cycles of asymmetric division to generate transit amplifying neural precursors before finally differentiating into astrocytes. He argued that the disappearance of stem cells was a direct consequence of their differentiation to produce new neurons. This then begged the question of whether the astrocytes that formed from neural stem cells could be reactivated to generate neurons again.

Magdalena Goetz (Munich University, Germany) also examined features of neural stem cells, looking for key distinguishing features. Multipotent cells that could form renewing neurospheres were isolated by their coexpression of glial fibrillary acidic protein (GFAP) and prominin (CD133), a protein expressed on the stem cell cilium. Live-cell analysis revealed that in vitro these astroglial-like cells divided asymmetrically. Goetz also described reprogramming studies in which astrocytes transduced with single transcription factors were efficiently converted to neurons within a few days without undergoing further cell division (Heinrich et al., 2010). This very exciting work implies that endogenous glial cells may represent a reservoir that could be tapped to generate new neurons to replace those lost from trauma or disease.

Arturo Alvarez-Buylla (University of California, San Francisco, USA) discussed the specification of adult neural stem cells, concluding that neural stem cells in the mouse brain are a regionally diverse collection of progenitors with a restricted set of cell fates (Merkle et al., 2007). His group is now investigating the mechanisms that lead to this stem cell heterogeneity, postulating a role for *Sonic hedgehog* in the specification of cells in the ventral part of the subventricular zone.

Moving Forward

The level of interest in stem cell biology is unprecedented and there is no evidence that the tsunami of new scientific knowledge is abating. Search for the words ''stem cell'' in the publication title in PubMed and you will retrieve references to nearly 3000 articles published in just the past 12 months. In this meeting report we have highlighted the increasing suite of clinical applications of stem cell research that provide tangible evidence for the translation of science to therapy. We have also noted presentations that describe direct cellular reprogramming to convert cells from one cell type to another, demonstrating that it is possible to bypass a ''dedifferentiation'' stage, an idea that was keenly debated in the past. But where will these endeavors lead? Join us in Toronto in 2011 for the next chapter in this fascinating tale!

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