Cell Stem Cell
ISSCR: Meeting Report

Stem Cells in the Land of the Rising Sun: ISSCR 2012

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The 2012 annual meeting of the International Society for Stem Cell Research (ISSCR) marked the Tenth Anniversary of the ISSCR. Held in Japan, the meeting showcased recent discoveries and surveyed the remarkable progress that has been made in a decade of stem cell research.

Over 3,500 attendees from 58 countries converged on Yokohama, Japan, for the ISSCR Tenth Annual Meeting, June 13–16, 2012, to learn about the state of the art of stem cell research spanning basic discoveries to clinical trials. No longer a fledgling, the society, as well as the field in general, is maturing rapidly and showing early signs of its long-touted therapeutic promise. This was the first time the annual meeting was held in Japan, a country with a firm commitment to the field of stem cell research. A highlight of the meeting was a personal appearance by the Emperor and Empress of Japan at a ceremony commemorating the Tenth Anniversary of the ISSCR. The scientific core of the meeting consisted of 7 plenary sessions and 20 concurrent sessions spread over 5 days. Additionally, and in response to a record number of abstract submissions, over 1,400 posters were also presented. Here we review highlights of the plenary presentations.

Pluripotency

The official program began with the Presidential Symposium chaired by ISSCR President Fred Gage. The mayor of Yokohama, Fumiko Hayashi, welcomed the attendees, and Rob and Cheryl McEwen were presented with the ISSCR Public Service Award. Rudolf Jaenisch (Whitehead Institute, USA), recipient of the 2012 McEwen Award for Innovation, reviewed recent progress in the understanding of cellular reprogramming, providing more evidence of how quickly this field is moving. While the application of induced pluripotent stem cells (iPSCs) to model complex human disease is gaining traction, the technology is still evolving. Jaenisch underscored that while all somatic cells can potentially be reprogrammed to an iPSC state, reprogramming is a continuous, stochastic process, and variability in the level and stoichiometry of the reprogramming factors make the process highly inefficient. Not all iPSC clones are fully reprogrammed, and not all of them reach the same state. Analysis of single-cell gene expression at different stages in the reprogramming process is beginning to shed light on the mechanism of reprogramming and was used to identify genes, for example, *Esrrb* or *Utf1*, that are highly predictive of future iPSC colony formation. Jaenisch presented a novel gene expression analysis of reprogramming where a stochastic phase is followed by a more deterministic phase of gene expression. He established a Bayesian network that revealed a genetic hierarchy to reprogramming.

Austin Smith (University of Cambridge, UK) described mouse embryonic stem cells (ESCs) cultured in defined medium that includes inhibitors of two kinases (MEK and GSK3), a condition known as "2i" (Leitch et al., 2010). These growth conditions, along with the cytokine leukemia inhibitory factor (LIF), establish a self-renewing ground state with less morphological heterogeneity and more consistent expression of key pluripotency genes than ESCs cultured in serum. Smith showed that the transcriptome and epigenome profiles of serum- and 2i-grown ESCs are quite distinct. A relative absence of lineage-related gene expression in ESCs grown in 2i medium suggests that multilineage priming may be an induced condition and not an intrinsic feature of self-renewing ESCs. The 2i conditions more effectively block differentiation signals and may more accurately represent the naive state of pluripotent cells in the inner cell mass of the blastocyst. Furthermore, Smith was recently able to derive embryonic germ cells from primordial germ cells reproducibly and at a high efficiency in 2i conditions supplemented with LIF; thus LIF and 2i were sufficient to rebuild pluripotency in this unipotent population.

Kazutoshi Takahashi (Kyoto University, Japan) addressed the subject of reprogramming errors and epigenetic memory in iPSCs by comparing the global gene expression, DNA methylation, and exon sequences of over 50 human pluripotent stem cell lines. For the most part, gene expression and epigenetic signatures were comparable between pluripotent iPSC and ESC lines, as was the ability to undergo neural differentiation. However, a small fraction of iPSC clones were resistant to differentiation and formed teratomas when transplanted into immune-deficient

Incoming ISSCR President Shinya Yamanaka escorted the Emperor and Empress of Japan to a ceremony commemorating the Tenth Anniversary of the society. The mayor of Yokohama, the governor of the Kanagawa Prefecture, and the senior vice president of Education, Culture, Sports, and Medicine were also in attendance. ISSCR Founding President Leonard Zon reviewed accomplishments of the ISSCR's first decade.

mice. Interestingly, subcloning these "bad" clones produced subclones that produced differentiated cells and no teratomas, as well as "bad" subclones. Microarray analysis of these clones and subclones identified three genes that were consistently upregulated in cells that did not differentiate. These genes contained LTR promoters for human retroviruses. Takahashi concluded that there appears to be epigenetic diversity among iPSC clones, and further, that specific genes may serve as markers for clones that are resistant to differentiation.

John Gurdon (The Gurdon Institute, UK) has been taking advantage of the amphibian oocyte in order to analyze nuclear reprogramming and identify mechanisms underlying an inherent resistance to this process. His strategy is to transplant mammalian cell nuclei into amphibian oocytes to study the mechanisms of reactivation and silencing of embryonic genes and to identify key oocyte reprogramming factors. There are significant differences between somatic nuclei obtained from cells of different developmental stages in their resistance to oocyte reprogramming. To understand the mechanisms underlying these differences, one approach is to progressively remove proteins and RNA from somatic nuclei in order to determine those responsible for resistance to reprogramming. These are thought to be components that help stabilize the state of gene expression in the differentiated states (Pasque et al., 2011). One conclusion is that resistance to reprogramming in nuclear transfer experiments is caused, at least in part, by restrictive histone marks that block reactivation.

Tissue Formation and Cancer

Jane Visvader (Walter and Eliza Hall Institute, Australia) spoke about the mammary stem cell (MaSC) hierarchy in order to understand the relationship between breast tissue stem and progenitor cells and the cells of origin of breast cancer. Similar to the hematopoietic system, a hierarchy of stem cells appears to exist within the mammary gland, including unipotent and multipotent cells that likely play different roles in the morphogenesis and maintenance of the mammary epithelium (Van Keymeulen et al., 2011). The mammalian epithelial hierarchy consists of luminal and myoepithelial lineages, and the luminal lineage can be further subdivided into ductal and alveolar sublineages.

Interestingly, MaSCs lack expression of estrogen and progesterone receptors, but are highly responsive to steroid hormones. Using genome-wide transcriptome analyses of mammary epithelial populations, a number of potential mediators of hormone action were identified. Apparently the morphogenic effects of steroid hormones on the mammary gland occur through paracrine signaling, mediated, at least in part, by luminal cell expression of RANK Ligand that signals to MaSCs to increase their pool size (Asselin-Labat et al., 2010). Hormone-stimulated expansion of the MaSC pool helps explain the tumor-suppressing effects of ovariectomy and antiestrogen compounds. Visvader recently extended these studies by determining the epigenomes of primary mammary epithelial subtypes. Epigenetic profiling of MaSCs, luminal progenitors, and mature cells suggests that H3-K27 methylation increases with lineage commitment. Moreover, H3-K27 methylation appears to be modulated by hormones. With H3-K27 methylation knocked out, progenitor proliferation decreases and pregnancy-induced proliferation is lost. Furthermore, no mammary growth was observed when H3-K27 methylation-deficient MaSCs were transplanted into the mammary fat pad, an indication of reduced "stemness" potential. The demonstration that hormones can induce changes in the epigenome may be important not only for understanding normal breast function, but also for understanding breast cancer.

Yoshiki Sasai (RIKEN Center for Developmental Biology, Japan) presented a striking 3D, self-organized reconstruction of the mouse and human retina arising from ESCs in culture. Culture of mouse ESCs to form first retinal epithelium and then a complex six-layered retinal structure, including a layer of retinal pigment epithelia (RPE), demonstrated a self-driven morphogenesis of the retinal epithelium, and confirmed that the processes of optic vesicle evagination and formation of the optic cup are internally programmed (Eiraku et al., 2011).

Sasai showed evidence that WNT signaling is responsible for neural retina versus RPE patterning. When an ESC-derived optic vesicle was cocultured with WNT3-expressing cells, both neural retina and RPE were formed. Using this model, he went on to show that autoregulatory secretion of a WNT inhibitor,

DKK1, in the distal epithelium suppresses RPE differentiation. Conversely, WNT signaling in the proximal epithelium suppresses expression of DKK1. This bistable mutual inhibitory circuit between WNT and WNT inhibitor regulates neural retina to RPE specification. Later, the distal epithelium expresses FGFs that promote differentiation of neural retina at the cost of RPE, stabilizing cell identity. In vivo, the self-organizing ability of the developing retina is also coordinated with extrinsic signals, such as FGFs from the cornea and lens, as well as WNTS from the periocular mesenchyme. Sasai also proposed a relaxation-expansion model to explain self-driven optic cup morphogenesis. The governing features include a relatively soft neural retina, strong apical constriction at the hinge region, and enormous expansion of the neural retina.

Sasai's final topic concerned human retinal generation and species-specific tissue mechanics. Sasai and colleagues were able to generate a human ESC-derived optic cup in culture, confirming an intrinsic retinal morphogenesis program across species (Nakano et al., 2012). However, the human retina was much larger and thicker than the mouse retina, and took much longer to form. Furthermore, the isolated human ESC-derived NR demonstrated an intrinsic tendency to develop an apically convex curvature, not observed in the mouse ESC-derived NR. Apparently, eversion is dependent on beta-integrin signaling from the basement membrane but does not require Rho/ROCK signals. Neutralizing beta1-integrin antibody blocked eversion and also altered apical basal cell patterning, a feature that contributes to apical curvature. The overall message of this talk was not only that a novel model exists for studies of eye development, but also that 3D human ESC-derived tissues can be produced in culture, with potential utility for disease modeling, drug screening, toxicology, and cell-based therapy.

Elaine Fuchs (Rockefeller University, USA) presented a panoramic view of the role of skin stem cells in homeostasis, wound repair, and cancer. A key concern is how stem cells in the epidermis, hair follicle, and sweat gland balance self-renewal and differentiation. Similar to mammary glands, sweat glands derive from epidermal progenitors, but they appear to have limited tissue regenerative capacity. During development, multipotent progenitors in the sweat ducts transition to distinct unipotent progenitors, and following injury they remain unipotent, so that healing occurs through activation of epidermal, myoepithelial, and luminal-specific progenitors (Lu et al., 2012).

Regeneration of hair follicles relies on the activation of quiescent stem cells regulated through multiple signals, including BMPs, WNTS, and FGFs. Fuchs addressed the role of TGF-β signaling in this transition by describing a paracrine transmitter, TGF-β2, produced by the dermal papilla that activates transient Smad2/3 signaling in hair follicle stem cells. Intracellular signaling pathways activated by growth factors and niche signals appear to mediate the crosstalk between dermal papilla and stem cells that brings the stem cells out of quiescence. Fuchs described two approaches taken to identify additional regulators of long-term skin stem cell self-renewal. The first used RNAi to screen genes differentially regulated in these cells, uncovering TBX1 as a modulator of stem cell self-renewal in the hair follicle (Chen et al., 2012). Fuchs is also doing a genome-wide screen using in utero intra-amniotic cavity injections of fluorescently labeled lentiviruses carrying shRNA to achieve selective genetic alteration of epidermal progenitors, thus enabling rapid assessment of gene function.

This year's Ernest McCulloch Memorial Lecture was very fittingly given by Irving Weissman (Stanford University, USA) who discussed normal and neoplastic stem cells, building on the classic work by Till and McCulloch half a century ago. Weissman described the poor outcome in metastatic breast cancer patients given myeloablative therapy followed by autologous mobilized peripheral blood transplants. He highlighted contamination by circulating tumor cells as a cause, and presented data supporting the strategy of "purifying" hematopoietic stem cells (HSCs) to eliminate the cancer cells. In a small clinical trial started in 1996, women with metastatic breast cancer treated with high-dose chemotherapy followed by transplantation of purified HSCs showed better than expected overall survival as well as freedom from progression.

Weissman also discussed the clinical need for targeted depletion of endogenous HSCs in order to facilitate their replacement with allogeneic or gene-corrected stem cells. For example, anti-CD117 (c-Kit) monoclonal antibody eliminates native HSCs and facilitates engraftment of purified donor HSCs in a mouse model of severe combined immunodeficiency (SCID) (Czechowicz et al., 2007). More recently, a humanized anti-human CD117 monoclonal antibody has been identified as a potential candidate for the treatment of SCID. Weissman ended his presentation with a glimpse at a very exciting potential anticancer strategy emerging from the observation that CD47, a "don't eat me" signal that allows cells to evade phagocytosis by macrophages, is overexpressed on a wide array of human cancer cells. This defense can be breached by use of an anti-CD47 antibody, a strategy that has been used to treat acute myeloid leukemia, primary human lymphoma, and other tumors in immune-deficient mice. Similarly, anti-CD47 antibodies have inhibited the growth and prevented metastasis in a range of orthotopically xenotransplanted human solid tumors including ovarian, breast, colon, and brain (Willingham et al., 2012). Targeting CD47 in human cancers is an attractive anticancer strategy and plans are in place to move it rapidly to the clinic.

Pathways to the Clinic

The last decade of research into the fundamental nature of stem cells, progenitor cells, and their differentiated cell types has laid the foundation for informed attempts to translate these findings into new regenerative therapies. In a session on clinical translation, the opportunities and challenges for bringing new therapies to patients were explored. A central theme that emerged in this session was that preclinical studies are beginning to show efficacy, and that initial introduction of stem cell derivatives into human subjects can be safe. This session also explored ethical issues surrounding stem cell treatments, including the selection of subjects and use of unproven treatments.

Three presentations focusing on varying uses of neural stem cells suggested promise in this important area. Ann Tsukamoto (Stem Cells, Inc., USA) described a purified population of human neural stem cells derived from the central nervous system that can be expanded in vitro and banked. She presented data suggesting that when introduced in vivo, these cells can proliferate

and reside in neurogenic areas, giving rise to neurons, astrocytes, and oligodendrocytes, and can also provide paracrine support to neighboring cells. Initial trials in human patients with Batten disease, a devastating lysosomal storage disease, have shown safety of the cells. New trials in demyelinating disorders, spinal cord injury, and age-related macular degeneration are underway or soon to be started.

Unlike the organ-derived cells described by Tsukamoto, Masayo Takahashi (RIKEN Center for Developmental Biology, Japan) and Hideyuki Okano (Keio University, Japan) described the potential use of iPSC-derived neural cells. Takahashi has shown the efficient generation of RPE cells from human ESCs and iPSCs that closely resemble endogenous RPE cells. These are the cells that are defective in the wet form of age-related macular degeneration (AMD), and Takahashi showed that iPSC-derived RPEs can be transplanted in animal models of AMD. So far, these cells have not formed tumors. The next hurdle will be translating these findings into a human clinical trial, and the enrollment for this trial should begin soon.

Similarly, Okano tested neural stem/progenitor cells derived from multiple human iPSC lines, and injected them into a mouse model of spinal cord injury. Interestingly, some of these iPSC lines were tumorigenic, but others were not and could be selected and tested in mice for safety. Use of neural progenitor cells from the "safe" iPSC lines in a nonhuman primate model of spinal cord injury revealed improved myelination, synapse formation of iPSC-derived neurons with existing neurons, and most importantly, improved locomotion in animals with spinal cord injury. These studies hold promise but the issue of tumorigenicity will need to be considered. Given the limited time window for intervention after spinal cord injury, new approaches for direct reprogramming of somatic cells to neuronal stem cells may offer an attractive source of cells for transplantation.

Another cell type that has been used extensively in early human clinical trials is the mesenchymal stromal cell (MSC). Katarina Le Blanc (Karolinska Institute, Sweden) presented a summary highlighting the overall safety of these cells when they are introduced intravenously. MSCs may show benefit in the treatment of some conditions, but while one hypothesis is that they exert a paracrine effect that limits inflammatory responses, the exact mechanism of their action remains unknown. Determining the best methods of expanding and growing MSCs and testing their efficacy in animal models of human disease are both important steps that remain before their true therapeutic potential can be fully assessed.

As stem cell therapies advance into people, the ethical standards and implications deserve consideration. Jan Helge Solbakk (University of Oslo, Norway) spoke to concerns of "rogue" stem cell clinics preying on patients. Many clinics worldwide are offering unproven therapies without proper trials but claiming "proven" efficacy. Rigorous ethical standards are increasingly important as we go forward, and clear ways for patients to determine if treatments are proven or being sold without evidence will be critical. In addition, we need to consider the populations that are tested with new therapies, how they are selected, and how treatments are financed in an ethical fashion. These and other ethical issues need to remain in the forefront as clinical translation of stem cells accelerates in the coming years.

Systems Biology, Epigenetics, and an Award

The availability of methods for mapping chromatin modifications and transcription factor binding on a genome-wide level has had a dramatic impact on ESC research. Bing Ren (University of California, USA) pointed out how *cis*-regulatory sequences, and a better understanding of their function and regulation, are important to understand cell-type-specific expression patterns. Ren reported conclusions from their epigenetic data mapping survey of pluripotent cells (including iPSCs and ESCs) and various differentiated cell types. Typically, chromatin modifications at promoters remain largely invariant during differentiation, but distal regulatory regions, i.e., enhancers, display much greater dynamics in chromatin modification, associated with active chromatin marks in a cell-type-specific manner (Hawkins et al., 2011). This agrees with the notion that enhancers are key to the regulation of cell-type-specific expression patterns. However, finding the gene that is regulated by a specific enhancer is not straightforward because a significant number of enhancers may not target the closest promoter. Ren added a new wrinkle with the report that the genome is spatially organized into domains of coordinately regulated enhancers and promoters (Shen et al., 2012). The invention of chromosome conformation capture-based technologies has allowed the mapping of DNA-DNA contacts in mammalian cells. Using Hi-C, Ren demonstrated the existence of thousands of megabase-sized chromosome interaction domains, termed topological domains, which function as locally coregulated regions where short range DNA-DNA contacts, i.e. those between enhancers and promoters, occur (Dixon et al., 2012; Shen et al., 2012). A recent report by Edith Heard's laboratory showed that disruption of a boundary between two of these topological domains caused a dramatic change in long-range DNA contacts and widespread transcriptional misregulation (Nora et al., 2012).

Anjana Rao (La Jolla Institute for Allergy & Immunology, USA) reported new insights into the role of Tet proteins, their regulation, and 5-methylcytosine (5mC) oxidation in ESCs and HSCs. Tet enzymes diminish DNA methylation by converting 5mC to 5-hydroxymethylcytosine (5hmC) and additional oxidized products. Rao reviewed that both TET1 and TET2 are present in ESCs, are regulated by OCT4, and are important for 5hmC production in these cells and for their differentiation potential, although double knockout mice are viable (Koh et al., 2011). Using an approach that distinguishes 5hmC from 5mC, Rao discussed the genome-wide distribution of 5hmC in ESCs depleted for TET1 or TET2 and addressed how TET protein stability is regulated (Pastor et al., 2011).

Huck Hui Ng (Genome Institute of Singapore, Singapore) presented an intriguing follow-up on his genome-wide RNAi screen (Chia et al., 2010). For this screen, a GFP reporter driven by *Oct4* regulatory sequences was introduced into human ESCs, and the knockdown of several hundred genes resulted in reduced GFP reporter expression. While much attention has focused on deciphering the role of transcription factors, chromatin regulators, and signaling pathways in ESCs cells, Ng's talk focused on one of the factors identified in the screen that does not fall into any of these classical categories. He revealed that a novel spliceosome-associated RNA-binding factor is important for the control of splicing of a subset of genes in ESCs. Together with a report by Blencowe and colleagues (Gabut et al., 2011),

this finding highlights a pluripotency-specific control of mRNA splicing.

Cedric Blanpain (Université Libre de Bruxelles, Belgium), winner of the 2012 ISSCR-University of Pittsburgh Outstanding Young Investigator Award, summarized his research focusing primarily on the role of stem cells during epithelial homeostasis and cancer initiation. He first described clonal analysis of interfollicular epidermis. It had been previously proposed that skin homeostasis can be explained by the population dynamics of a single stem cell type with no intermediate transit amplifying population. Blanpain reinvestigated this issue using CreER constructs driven by either Keratin 14 (K14) or Involucrin to follow the fate of basal progenitor epidermal cells over time. He found that clones marked by each of the two drivers had different survival characteristics and size distributions. By mathematically modeling the population dynamics, Blanpain demonstrated the existence of two distinct populations, one of slower cycling stem cells (K14+) that gives rise to a second, faster-dividing intermediate progenitor. Interestingly, the K14+ stem cell population, but rarely the committed progenitors, participate in long-term repair of the epidermis after wounding (Mascré et al., 2012).

In the second part of the talk, Blanpain focused on lineage tracing in the mammary gland. During pregnancy and puberty, K14 marks the myoepithelial mammary lineage and is unipotent. Meanwhile, K8 marks the luminal lineage, which is likewise unipotent. In contrast, when cells are dissociated and used to reconstitute mammary tissue in the fat pad, the myoepithelial cells can reconstitute both myoepithelial and luminal cells (Van Keymeulen et al., 2011).

Finally, the topic turned to cancer. Here, Blanpain used genetic labeling of tumor cells and clonal analysis to dissect the mode of tumor growth in squamous skin tumors (Driessens et al., 2012). In benign papilloma, the majority of tumor cells disappear, indicating that they behave as transient amplifying progeny that are lost through differentiation, whereas a fraction expand massively. This leads to a model where a cancer stem cell is established that divides very quickly, but still undergoes stochastic cell fate choices allowing self-renewal and differentiation, reminiscent of the hierarchical organization of the normal epidermis. When invasive squamous cell tumors were examined, some clones showed evidence of cell differentiation, while others gave rise to all undifferentiated daughters, consistent with the emergence of one population of tumor-initiating cells with limited differentiation potential.

RNA Control of Stem Cell Behavior

Narry Kim (Seoul National University, South Korea) spoke about the biochemical function of LIN28, a pluripotency factor expressed in ESCs. She showed that LIN28 suppresses Let7 microRNA accumulation by oligouridylation of Let7 RNA. This oligouridylation blocks Let7 processing by Dicer, shunting it to a degradation pathway. To find the enzymatic activity associated with LIN28/Let7 responsible for oligouridylation, Kim and colleagues analyzed proteins associated with pre-Let7 in LIN28 transfected cells, and found that the noncanonical polyA polymerase TUT4 is the uridylyl transferase that acts in concert with LIN28 (Heo et al., 2009). In new work, she examined the function of monouridylation and found that, in contrast to oligouridylation, it enhances Dicer processing of the pre-microRNA. Finally, Kim described work examining targets of LIN28. Through UV crosslinking and immunoprecipitation, she defined the LIN28 target motif. Further functional studies showed that LIN28 does not affect target mRNA abundance, but rather reduces translational efficiency as assayed by ribosome footprint analysis.

Plenary Session VI continued with Yumiko Saga (National Institute of Genetics, Japan) describing her work on the function of NANOS2, an RNA-associated factor in germ cells. NANOS is a zinc finger motif protein expressed in male germ cells starting at E13.5 where it prevents meiosis by inhibiting Stra8 expression until after birth (Suzuki and Saga, 2008). Examination of NANOS2 expression in postnatal testes, as well as lineage tracing using a *Nanos2* enhancer, showed that NANOS2 is also expressed in the spermatogonial stem cells. Overexpression of NANOS2 in spermatogonial stem cells prevents their further differentiation. Interestingly, overexpression of NANOS2 in female germ cells promotes expression of male germ cell features.

Cell and molecular analysis has shown that NANOS2 is localized to P-bodies that are structures involved in mRNA degradation. Of note, NANOS2 interacts with components of the deadenylation complex involved in the initial step of RNA degradation (Suzuki et al., 2010). New work looking for other Nanos-interacting partners identified the RNA-binding protein DND1. Examination of transcripts that coimmunoprecipitate with NANOS2 showed 48 sequences from transcripts that are also upregulated in the NANOS2 knockout mouse. These results indicate that NANOS2 is essential for regulating abundance of key transcripts in the male germline.

Attention then turned to epigenetic switches in breast cell transformation and bistable switches in the maintenance of cancer stem cells in a lecture given by Kevin Struhl (Harvard University, USA). He made the point that an important class of epigenetic switches is positive feedback loops that generate a "heritable" state of transcription factor activity. It is the transcription factors that then determine the pattern of histone modification, laid down by recruiting chromatin-modifying activities. In the first part of his talk, Struhl described an inflammatory feedback loop involved in SRC-mediated transformation of the breast epithelial cell line MCF-10A. The initial SRC activation triggers a series of changes resulting in activation of an inflammatory positive feedback loop involving activation of NFkB, IL6, and LIN28, which normally inhibits LET7. Importantly, high levels of IL6 also activate NFkB, establishing a positive inflammatory feedback loop (Iliopoulos et al., 2009).

In the second part, Struhl presented work analyzing how intracellular communication between cancer stem cells (CSCs) and non-cancer stem cells (NCSCs) via IL6 is critical for maintaining a stable proportion of the two cell types in a population over time. Struhl then further described the intracellular switch between the CSC and NCSC states. CSCs downregulate microRNAs 128, 125b, 103, and 145 that normally regulate polycomb genes SUZ12, BMI1, ZEB1/2, and KLF4. In turn, ZEB1/2 inhibits the microRNAs, which results in a reinforcing circuit that acts as a bistable switch (Polytarchou et al., 2012). Evidence indicates that this circuitry is relevant in triple negative breast cancer, where cells are low in these microRNAs, resulting in upregulation of these important targets. The talk ended describing work to identify agents that would target CSCs that are resistant to

normal chemotherapy treatments. Metformin was identified as selectively killing CSCs. Administration of metformin plus the standard chemotherapy agents increased tumor regression and prolonged remission in a mouse xenograft model (Iliopoulos et al., 2011).

Stem Cell and Fate Control

In Plenary Session VII, the final session of the Yokohama annual meeting, we heard exciting published and unpublished studies on the regulation of intestinal, neural, and skin stem cells.

Using impressive animations, Hans Clevers (Hubrecht Institute, Netherlands) illustrated the behavior and lineage emergence of intestinal epithelia from LGR5-expressing stem cells (Leblond cells). LGR5 is part of the LRP-WNT-Frizzled receptor complex and mediates Rspondin (Rspo)-enhancement of Wnt signaling. Clevers' laboratory has shown that Leblond cells are long-lived and actively dividing and give rise to all cell types that are constantly replaced in the villi of the intestinal epithelium. Sorted individual LGR5-expresing cells cultured in the presence of RSPO1 and other factors give rise to self-organizing organoids called "miniguts." Interestingly, and in collaboration with the Watanabe laboratory, Clevers and colleagues showed that miniguts derived from single LGR5+ cells give rise to normal gut epithelium after transplantation. The cells integrate and can repair damaged epithelial lining. In vivo, Leblond cells sit next to Paneth cells. LGR5+ cells cocultured with Paneth cells give rise to miniguts with much higher efficiency compared to LGR5+ cells alone. Clevers then described the use of these miniguts for disease modeling and drug screening using cystic fibrosis as a model. Finally, Clevers presented beautiful multicolor lineage analysis that established that all 15 stem cells present in the crypt of each villus divide and contribute to intestinal lineages. However, with time, one stem cell per villus populates the entire crypt (Snippert et al., 2010). Interestingly, APC loss in LGR5 progenitors results in progeny with normal appearance, but in which Wnt signaling cannot turn off, giving rise to lateral growth that forms polyps or adenomas. These adenomas contain interspersed LGR5+ cells that serve as a population of tumor stem cells. Collectively, this work illustrates the power of somatic stem cells in normal tissue homeostasis, regeneration, and the understanding of cancer.

Hongjun Song (Johns Hopkins University, USA) presented an innovative new way to lineage trace the progeny of neural stem cells in the adult hippocampus. A population of radial astrocytes, also known as type I progenitors or radial glial-like cells, function as the primary progenitors for the generation of new neurons in the dentate gyrus of the hippocampus in the adult mammalian brain. Using very low concentrations of tamoxifen in mice carrying Nestin enhancer-CreER and a floxed reporter, Song's laboratory was able to label individual radial astrocytes and show that these cells can give rise to neurons, astrocytes, or other radial astrocyte cells (Bonaguidi et al., 2011). Interestingly, this observation suggests that a small subpopulation of progenitors in the adult brain is capable of both self-renewal and multilineage differentiation. Clonal analysis suggests that about 70% of these cells generate progeny: neurons and astrocytes. They also observed that about 16% of the clones at 1 month and about 30% of the clones at 2 months no longer contained a radial astrocyte, suggesting some level of stem cell depletion with age. These observations show that the behavior of stem cells in the adult hippocampus is heterogeneous, dividing either symmetrically to amplify the population of primary progenitors, or asymmetrically to produce progeny that include astrocytes and neurons, but not oligodendrocytes. Song went on to present exciting new observations showing that radial astrocytes respond tonically to GABA through $GABA_A$ receptors. Interestingly, conditional depletion of this receptor on the radial astrocytes modified their clonal behavior and induced increased symmetrical division. This is an exciting finding, suggesting that the neurotransmitter concentration next to adult neural stem cells may play an important role in regulating their behavior. Specifically, GABA may regulate symmetrical versus asymmetrical division of primary progenitors and therefore control neurogenesis (Song et al., 2012).

Oliver Hobert (Columbia University, USA) presented a comprehensive approach to understanding the genetic control for generation of neuronal diversity. Using *C. elegans*, his laboratory has determined the set of genes specifically expressed by ASE gustatory neurons. Among these more than 1,000 genes, none were unique to these cells, suggesting that a combinatorial strategy is used to make the different neuronal types (Etchberger et al., 2007). Using transgenesis they identified minimal regulatory elements present in more than a dozen of the ASE-specific transcripts. The identified ASE motif was essential for expression of transcription factors in these neurons. In addition, using mutagenesis, they found a zinc finger transcription factor, *che-1*, that is essential for the expression of an ASE-specific neuropeptide. Loss of *che-1* does not cause the loss of ASE neurons, but results in the loss of the expression of ASE-specific genes. Interestingly, *che-1*, which specifically binds the ASE motif, is exclusively expressed in ASE neurons through an autoregulatory loop. These results suggest that distinct features of a neuronal type are due to regulatory elements commonly regulated by unique transcription factors. Importantly, transcription factors similar to che-1, essential for the regulation of a neuron-specific set of genes, are also found in other *C. elegans* neuronal types: terminal selector transcription factors. Misexpression of *che-1* during neurogenesis induces the expression of ASE-specific genes in other neurons. Interestingly, with the exception of a few other chemosensory neurons, the ectopic expression of *che-1* induces transdifferentiation into ASE neurons only during a restricted period of neurogenesis. Knockdown of *lin-53* permits the ectopic expression of ASE markers past this developmental permissive period (Tursun et al., 2011). Interestingly, this induction was only observed in germ cells, and within a very specific subpopulation of these cells, the mitotic germ cells. This talk beautifully illustrated what could be a common genetic mechanism of specification of different types of neurons. The surprising observation that a subpopulation of germ cells is susceptible to reprogramming into specific subtypes of nerve cells by elimination of single epigenetic factor is extremely interesting.

Finally, Fiona Watt (King's College London, UK) delivered the Anne McLaren Memorial Lecture. Watt discussed how engineered extracellular matrix (ECM) influences human epidermal stem cell fate. Using small engineered ECM islands, her laboratory previously showed that differentiation of epidermal progenitor cells is inversely related to the adhesion surface area they are cultured on. This response to restriction in spreading is

dependent on F-actin and growth factor signaling through JUNB and c-FOS. This work was done with cells grown on glass that has much higher stiffness compared to the natural environment where epidermal stem cells reside. By creating polydimethylsiloxane substrates of varying stiffness linked to ECM components, Watt showed evidence that substrate stiffness had no effect on spreading or differentiation. Yet, when polyacrylamide hydrogels were used to vary stiffness, they found that keratinocytes spread and remained undifferentiated on hard substrates, but not on soft substrates (Trappmann et al., 2012). Interestingly, this was due to the porosity of the hydrogel and consequent differences in the anchoring of collagen and not related to stiffness. Indeed, this work suggests that the low anchoring density of the ECM in a porous substrate prevents integrin clustering, resulting in decreased ERK activation, dampening JNK phosphatase, increasing AP1-dependent transcription, and driving differentiation. These exciting findings highlight the importance of physical cues in the environment in the activation of specific signaling responses within stem cells.

Watt also presented recent data on chromatin regulators of this same population of cells. siRNA-based genetic screens of several hundred chromatin modifiers in human epidermal stem cells revealed a network that includes EZH2, UHRF1, ING5, BPTF, and SMARCA5. They find that these chromatin factors regulate differentiation through two distinct gene sets including integrin ECM receptors that mediate anchorage of epidermal stem cells to their niche (Mulder et al., 2012). The work begins to integrate epigenetic factor and specific matrix anchorage properties of human epidermal stem cells in the regulation of their differentiation.

Much has been accomplished in the last 10 years, most of it not predictable at the time of the founding of the ISSCR. One can only guess at the achievements of the next 10 years, but the first inklings will be on view in Boston when the ISSCR meets there in 2013.

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