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Covering the Stem Cell Explosion at the 2017 ISSCR Conference in Boston

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The meeting covered a plethora of rapidly evolving approaches and areas, such as organoid cultures modeling tissues and organs; stem cell-specific metabolites revealing new signaling pathways; single-cell technologies discovering new cell types and exploring stem cell niche interactions; novel methods studying stem cells in aging and cancer; lineage-tracing experiments exploring cell plasticity of tissues before and after injury; epigenetic studies illuminating cell reprogramming; new protocols improving cells for regenerative purposes; and several other timely and exciting topics.

This year's ISSCR meeting in Boston amassed several hundred speakers and an audience of almost 4,000 participants (Figure 1). It is safe to say that the current explosion in stem cell research has now penetrated all areas of modern biology, offering new perspectives on developmental disorders including cancer. This report covers a personal selection of exciting presentations with perceived future promise.

Modeling Complex Tissues

In the past few years, it has been recognized that differentiating pluripotent stem cells (PSCs) into structures resembling physiological tissues requires stage-specific manipulation of developmental signaling pathways, achievable through sequential exposure of the cells to different extracellular cues, such as growth factors and niche substrates. Such complex protocols, developed over years of painstaking work, have yielded a bounty of cells with regenerative potential including human PSC-derived insulin-producing and glucose-responsive pancreatic beta cells as well as cardiac atrial and ventricular-like cells with promising *in vivo* functionalities, as reported by Doug Melton and Gordon Keller. However, most organs consist of a mixture of cell types that exhibit complex interactions required for function. In some instances, it has been possible to find PSC culture conditions that permit modeling tissues with more than one specialized cell type, such as the generation of structures that harbor beating cardiac cells surrounded by cardiac endothelial cells (Christine Mummery; Giacomelli et al., 2017).

In other instances, however, the key to faithful reconstruction of complex tissues from PSCs is to first program individual components and then combine them in three-dimensional structures. One striking example was reported by Juergen Knoblich, who a few years ago described the first brain organoids with cerebral cortex structures. A limitation of patterning the forebrain cortex was the variable production and distribution of excitatory pyramidal neurons and inhibitory interneurons in the organoids. During development, interneurons are born in a ventral region and then subsequently migrate into the forebrain, a dynamic process that thus far could not be reproduced in cultures. It turned out that the solution to the problem was to separately generate ventralized organoids containing interneurons and dorsalized organoids containing pyramidal neurons and then to combine them, resulting in fusion. Life imaging showed the migration of interneurons into the dorsalized region, recapitulating normal organogenesis. It was entertaining to watch how cell bodies moved by extending and retracting axons, like octopuses exploring new territories (Bagley et al., 2017). Being able to correctly pattern the cortex and other specific brain areas using patient-derived PSCs has enormous potential for studying neurological diseases and the eventual development of novel drugs.

Another beautiful example of the same strategy, to juxtapose two independently patterned cellular components to recreate a physiological structure, was presented by Magdalena Zernicka-Goetz, working on mouse embryo development, who delivered the 2017 Anne McLaren Memorial Lecture (Figure 2). The blastocyst consists of the epiblast, primitive endoderm, and the trophectoderm that go on to form the embryo, yolk sac, and placenta, respectively. As the embryo develops after implantation into the mother's uterus, a lumen forms that involves both embryonic and extraembryonic structures and that will become the pro-amniotic cavity. How this complex structure is patterned has been a mystery so far. To explore this, her group seeded embryonic stem cells (ESCs) together with separately patterned trophoblast stem cells (TSCs) into an extracellular matrix scaffold (made of Matrigel) as a substitute for primitive endoderm cells. Astonishingly, this led to the formation of so-called "ETS-embryos" (ESC and TSC embryos), strikingly similar in structure and size to normal postimplantation embryos. They contained a polarized epiblast, trophectoderm, and lumen in the correct orientation except for the lack of primitive endoderm. Using these artificial embryos, Zernicka-Goetz was even able to observe

Figure 1. ISSCR 2017
Nearly 4,000 scientists from more than 55 countries attended ISSCR 2017, the largest global stem cell meeting, in Boston, MA, USA, June 14–17, 2017.

the asymmetric formation of mesoderm and germline progenitors (PGC-like cells) at the interface between the epiblast and trophectoderm in response to Wnt and BMP signaling, thus phenocopying embryonic polarity and the onset of gastrulation (Harrison et al., 2017). Lewis Wolpert could hardly have predicted 30 years ago that stem cell research would make such fast strides when he famously wrote that "It is not birth, marriage or death, but gastrulation that is truly the most important time in your life." These studies might shed light on a spectrum of medical problems, from infertility and recurrent miscarriages to congenital conditions such as spina bifida, cancer, and even age-related diseases.

Other examples of the interdependence between stem cells and their niche were demonstrated in the gut and in the lung, using a combination of cell ablation, lineage tracing, and signaling pathway modulation. Studies in the gut were reviewed by Hans Clevers in his plenary talk. One of the most striking observations was that enterocytes in the crypts of the gut have the ability to replenish the pool of self-renewing stem cells by de-differentiating into LGR5 positive cells (Tetteh et al., 2016). Jayaraj Rajagopal, winner of the 2017 ISSCR Susan Lim Award for Outstanding Young Investigators, likewise showed that stem cells can serve as niche cells for their own daughters, turning the niche paradigm on its head. Studying the differentiation of stem-progenitor cells in the airway epithelium of the lung, he showed that stem cells maintain the survival of their progenitors by supplying a Notch ligand that prevents their differentiation into ciliated cells. His group also discovered that airway epithelium has a special form of innate regenerative potential, by harnessing the plasticity of differentiated cells. Ablation of resident stem cells in the airways induces the de-differentiation of secretory cells into stem/progenitor cells (Tata and Rajagopal, 2017). More recent work reported by Rajagopal at the meeting was about the "parental control" of mucus cells by stem cells, mediated by secretion of DLL1, resulting in the activation of the Notch signaling pathway in daughter secretory cells that become activated to become

Figure 2. Anne McLaren Memorial Lecture
Magdalena Zernicka-Goetz, PhD, University of Cambridge, UK, delivers the Anne McLaren Memorial Lecture at ISSCR 2017.

mucus-secreting goblet cells. And, using single-cell analyses, he reported the identification of a novel specialized cell type, the FOXL1 positive "ionocyte," which fundamentally alters our understanding of cystic fibrosis. Precisely charting the composition of the different cell types in the lung and elucidating their complex interplay is crucial to understanding the organ's maladies, including cancer and asthma, and to developing novel therapies.

Stem Cells and Metabolites

Sean Morrison reported the development of a new method that permits detection of metabolites in rare populations and found that ascorbic acid levels are high in hematopoietic stem cells and decline during differentiation. Ascorbate is a cofactor for the dioxygenase Tet2, whose mutations have been associated with blood cell malignancies. Interestingly, using a mouse model of oncogene-induced myeloid leukemia, they could show that low levels of ascorbate facilitate leukemogenesis by outcompeting normal hematopoietic stem cells (Agathocleous et al., 2017). This predicts that maintaining high levels of dietary vitamin C could help prevent certain blood malignancies.

Stem Cells and Aging

A surprising new mechanism for hair loss during aging of male mice was reviewed by Emi Nishimura, who discovered the crucial involvement of the hemi-desmosome component collagen 17A. This collagen, which is expressed in the bulge area of hair follicles, is gradually degraded by proteolysis in response to DNA damage. As a result, stem cells differentiate, creating ever smaller hair follicles with subsequent hair loss, an effect also observed during aging of humans (Matsumura et al., 2016). Strikingly, this could be prevented by the forced expression of COL17A, giving hope that at some point in the future bald men will become a thing of the past.

Men lose their hair as they age, but women are not spared when it comes to skeletal muscle loss, a topic discussed by

Tom Rando. In earlier work, his group found that satellite stem cells exhibit diminished regenerative capacity in older animals. Examining aged satellite stem cells with an "epigenetic" lens revealed substantial alterations in the profile of activating and repressive histone modifications that explain, in large part, the changes in the cells' phenotype. He also reviewed experiments with conjoined old and young mice, showing that aged muscle can be rejuvenated and that this is due to the activation of satellite cells. However, in spite of observing multiple epigenetic and transcriptional changes, which can last for several months, it is still not clear which signaling pathways are responsible for calling satellite stem cells into action (Brunet and Rando, 2017).

Margaret Goodell talked about the role of DNA methylation during aging of the hematopoietic system. It has been known for a while that a reduced number of hematopoietic stem cell (HSC) clones accumulate in older people and that these often have mutations in enzymes that are required for DNA methylation or de-methylation, most prominently in the *de novo* methyltransferase DNMT3A and the deoxygenase TET2. Modeling these findings in mice, her group showed that although HSCs with reduced *Dnmt3a* are defective in differentiation, they outcompete wild-type cells in competitive transplantation experiments. Investigating the methylation pattern of young and old HSCs, she focused on so-called "methylation canyons," typically seen within the regulatory sites of genes encoding transcription factors involved in lineage decisions. Here, she discovered significant differences in methylation at the edges of the canyons, even when the relevant genes are not expressed. She went on to show that Dnmt3a competes with TET2 for the methylation of these edges (Zhang et al., 2016) and that in ESCs they often demarcate topologically associating domains (TADs). This shows that aging of HSCs is accompanied by very specific changes in methylation and suggests a link between methylation and 3D genome structure.

Epigenetics of Evolution, X Inactivation, and Cell Reprogramming

Since the work of Nicole Le Douarin in the 1970s with avian chimeras, it has been known that neural crest cells generate craniofacial structures. To study the genes that are responsible for cranial features that distinguish us from other primates, Joanna Wysocka used iPSC-derived human and chimp neural crest cells. Comparing the cells' gene regulatory regions, she found major differences in enhancers encoded in the DNA sequence. Using a face-recognition program (facial phenotyping), she recently extended these analyses to differences among human individuals by studying regions marked with active chromatin marks in neural crest cells. Combining these data with GWAS data, she identified clusters of poised and primed enhancers that could be linked to genes associated with facial variation (Prescott et al., 2015). These studies are a beautiful example of how molecular epigenetics is providing inroads into areas that until recently seemed inaccessible. Darwin would surely have smiled if he had lived to see Wysocka's cellular anthropology approach.

Edith Heard, the newly appointed EMBL director, reported work on the mechanisms of X chromosome silencing in female cells during dosage compensation. Using an inducible form of Xist RNA, she generated data detailing time kinetics of gene expression using allele-specific GroSeq to map the location of early and late silenced genes. This revealed that early silenced genes encode certain chromatin-binding proteins such as the polycomb repressive complex 1 gene RING1b as well as TAF1 and HDAC2. She also described that another silencing protein, SPEN, is largely dependent on its association with HDAC3 and that its binding leads to the loss of H3K27Ac, an active enhancer mark, before loss of the promoter mark H3K4me3 (da Rocha and Heard, 2017). In short, the X chromosome inactivation model keeps revealing new secrets of how genes are silenced during development.

Alexander Meissner, examining the DNA methylation profile of early mouse epiblasts, found a remarkable difference between the embryo proper and the extraembryonic ectoderm. While the pattern of the embryo proper resembled that seen in most somatic tissues—high methylation of gene bodies, low methylation of regulatory regions—the extraembryonic ectoderm largely exhibited the reverse pattern—low gene body methylation and elevated methylation of regulatory regions. Interestingly, the latter pattern was remarkably similar to that seen in chronic lymphocytic leukemia and other blood cell malignancies, suggesting a nexus between the regulation of methylation in development and cancer, perhaps guided by shared signaling pathway(s) (Smith et al., 2017).

Staying within the topic of epigenetics, Konrad Hochedlinger reported that male ESCs grown for prolonged periods of time in medium containing MEK and GSK3 inhibitors (2i) plus LIF lose their developmental potential and exhibit chromosomal abnormalities, as do female ESCs grown long term in serum. He traced the cause of these effects to genome-wide hypomethylation. A central effector is the MAP kinase pathway, as the MEK inhibitor contained in the 2i formula widely used to generate naive PSCs leads to the erosion of DNA methylation at most imprinted regions. This seems to be mediated, in part, by the downregulation of DNA methyl transferases, although the exact mechanism by which MAP kinases prevent genome instability remains to be shown (Choi et al., 2017). The deleterious effects of hypomethylation in long-term PSC cultures recalls the fleeting nature of the PSC state in the embryo

and are a warning about the use of long-term PSC cultures under 2i medium conditions.

Stem Cells and Cancer

In her earlier work, Valentina Greco showed that after ablation of stem cells contained in the bulge of the hair follicle, surrounding epithelial cells can acquire stem cell properties and participate in hair regeneration, in a similar fashion as the one discussed for the lung and the gut. She now presented work showing that the skin epithelium has a surveillance system ensuring that tissue deformations induced by cells that contain oncogenic mutations are corrected (Brown et al., 2017). These findings contribute to mounting evidence about the importance of niche cells not only for cell regeneration but also for the control of cancer cells.

Elaine Fuchs, the 2017 ISSCR McEwen Award winner, eloquently reviewed her studies on stem cells of the skin. One of several vignettes she presented was about the discovery that, after wounding of the skin, hair follicle stem cells show a period of lineage infidelity in which they transiently express both lineage- and progenitor-restricted genes. Surprisingly, after oncogenic H-RAS mutations, the cells activate enhancers of stress-related genes that lock the cells in a permanent stage of lineage infidelity, a process that can culminate in the development of squamous cell carcinoma (Ge et al., 2017).

A direct connection between diet and metastasis-inducing potential was presented by Salvador Aznar-Benitah. His group made the remarkable discovery that the metastasis-inducing subpopulation of human oral squamous cell carcinomas is enriched for the expression of genes associated with lipid metabolism. Furthermore, a diet containing high levels of palmitic acid boosted the cells' metastasis-forming potential, following transplantation into immunodeficient mice. They identified the fatty acid receptor CD36 as a critical target. Its inhibition essentially eliminated the metastatic potential not only of squamous cell carcinomas but also of tumor cell lines derived from luminal breast cancer and melanoma (Pascual et al., 2017). This makes the cell-surface antigen CD36 an attractive therapeutic target for cancer and calls for the elimination of palmitic acid as a food additive.

A long-standing question in cancer is whether, in patients with a relapse, the outgrowth of the tumor cells is caused by the development of new mutations or by pre-existing mutations. This question was tackled by John Dick, the 2017 ISSCR Tobias Award winner, who studied the progression of acute myeloid leukemia by RNA sequencing patient-derived tumor cells transplanted into immune-deficient mice. They identified therapy-resistant cells already present at diagnosis, with mutations in either rare leukemia stem cells within the hematopoietic stem/progenitor cell pool or in committed leukemia cells with stem cell-related transcriptional signatures (Shlush et al., 2017).

Pluripotent Stem Cells and Reprogramming

Several talks covered recent advances in cell reprogramming, including that of Hongkui Deng, who reported the development of a new culture medium, containing 4 inhibitors of distinct signaling pathways, which permits maintaining and expanding both human and mouse ESCs in a novel cell state that differs from both naive and primed cells. Most astonishing, these cells, if injected singly into blastocysts, contributed to the formation of not only embryonic but also of extraembryonic tissues (Yang et al., 2017). The newly described 4i culture conditions might well turn into the medium of choice for a wide variety of applications, including the culture of ESCs and iPSCs for applications in regenerative medicine and for disease modeling.

Kathrin Plath reported that the reprogramming of mouse embryo fibroblasts into iPSCs entails a surprising collaboration between the Yamanaka transcription factors with lineage-restricted factors. Thus, early after expression in fibroblasts of the reprogramming factors OCT4, SOX2, and KLF4 (OSK), these bind transiently to lineage-specific enhancers primed by AP1 (JUN, FRA1), RUNX1, and C/EBPa/b. This in turn induces the redistribution of the lineage regulators to enhancers engaged by OSK factors, resulting in the silencing of the somatic and activation of the pluripotency gene expression program. The displacement of the lineage factors appears to be a prerequisite for the activation of the pluripotency program, as overexpression of somatic factors inhibits reprogramming (Chronis et al., 2017). These findings were echoed by findings from the laboratories of Duanqing Pei and Yuin Han Loh, highlighting the role of AP1 factors as critical somatic regulators that antagonize reprogramming of mouse and human fibroblasts.

Shangqin Guo, while imaging somatic cells during reprogramming, observed that the most rapidly changing cells showed a reduced nuclear size and altered morphology. Transcriptional profiling of these cells identified MKL1 (also known as myocardin like 1 or MRTa) as a transcriptional regulator that, together with the serum response factor SRF, controls nuclear compactness involving actin polymerization. MKL1, if overexpressed, blocks reprogramming and accelerates reprogramming when knocked down. These findings suggest that the regulation of nuclear compactness is a so-far unappreciated cellular feature relevant for cell fate transitions.

One of the important players for reprogramming is the dioxygenase Tet2, which can initiate demethylation of cytosines but exhibits no DNA-binding capacity on its own. Diana Guallar from Jianlong Wang's laboratory reported

an unexpected mechanism by which Tet2 can be recruited to DNA. They discovered that it interacts with the RNA-binding protein PSCP1 and that this recruits Tet2 to the RNA (not DNA!) of retrotransposons (such as MERVL). This results in the destabilization of MERVL RNAs (not DNAs!) through hydroxymethylation of cytosines and recruitment of HDAC repressors. Interestingly, Pscp1 becomes upregulated after the 2-cell stage of embryo development, the time at which many retrotransposons become silenced, supporting a role in their repression. These findings reveal a novel function of Tet2 that may well be relevant not only for development but also for cancer.

Stem Cells and Regeneration

Several talks covered recent efforts to bring the regenerative potential of iPSC derivatives into the clinic. Shinya Yamanaka has worked toward establishing a bank of iPSCs recovered from the most prevalent HLA-haplotypes in Japan, with the idea to facilitate a more cost-effective application of the cells in regenerative medicine than would be possible with autologous isolates. In a first round of experiments, he concentrated on two iPSC clones derived from a single patient that were propagated long term under GMP conditions in various facilities around the world. Surprisingly, one of the clones accumulated a mutation in the proto-oncogene BCL1, thus making it unsuitable as a source of donor cells. Curiously, this mutation arose only in some but not other culture facilities, suggesting that subtle environmental factors are at play that lead to the selective outgrowth of mutant iPSCs. Perhaps most importantly, this is a strong warning about the clinical use of iPSCs that have not been extensively characterized.

Masayo Takahashi reported her efforts to cure age-related macular degeneration by the transplantation of iPSC-derived retinal pigment epithelial cells. After an initial success with a patient transplanted with autologous cells, she is now studying the suitability of allogenic donor cells (Mandai et al., 2017). These experiments by the pioneer of the use of iPSCs for regenerative purposes are followed with great interest by clinicians around the world.

In a landmark study reported by Michele De Luca, his team successfully combined adult stem cell transplantation with genetic engineering. In earlier work, together with Graziella Pellegrini, he developed the *ex vivo* expansion of limbal stem cells from the eye of patients with an injured cornea and transplanting them back, with high therapeutic success rates. He now reported the cure of a severe form of epidermolysis bulbosa in a boy whose skin was delaminating from the whole body, due to a mutation in the laminin B3 gene that is necessary for the attachment of the skin to the dermis. They expanded epithelial cells from the patient, introduced the LAMB3 gene with a retroviral vector, and retransplanted the cells, eventually covering all areas of the body with healthy skin. Strikingly, retroviral tracing experiments showed that the regenerated epidermis was sustained by only a few transgenic epidermal stem cell clones with extensive self-renewal potential. With the rapid improvement of CRISPR-mediated gene editing, this approach promises to find applications in therapies using other types of adult stem cells.

In conclusion, at the recent ISSCR meeting in Boston, we could watch how the continuing explosion of stem cell research is reaching all areas of biology and biomedicine, fueled in no small part by the iPSC revolution. Unquestionably, the annual ISSCR conferences remain the premier forum for stem cell researchers from all corners of the globe to present their most exciting work. Congratulations to the organizers of the 2017 meeting, led by Elaine Fuchs, for the breadth and quality of the program and for choosing so many great female speakers! We all now look forward to the next ISSCR meeting, to be held in Melbourne in June of 2018.

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