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meeting point

A blizzard of stem cells in Santa Fe

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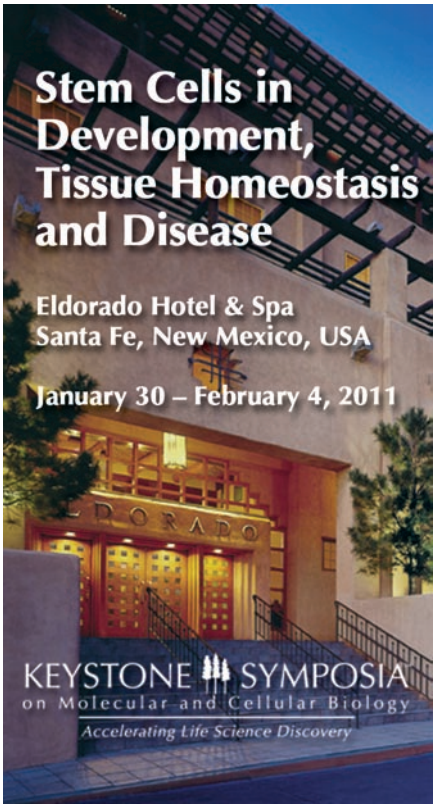
The Keystone Symposium 'Stem Cells in Development, Tissue Homeostasis and Disease' was held between 30th January and 4th February 2011 in Santa Fe, New Mexico, USA. The organizers gathered together an impressive panel of speakers to discuss various aspects of stem-cell biology from early development to adult homeostasis, as well as the implications of stem cells for human diseases.

Each year, Keystone hosts a meeting that highlights recent advances in the rapidly moving field of stem-cell biology. This year, Thea Tisty (U. California San Francisco, USA), Elaine Fuchs (HHMI/Rockefeller U., USA) and Ron McKay (NIH, USA) organized a meeting in Santa Fe, New Mexico, that covered many aspects of developmental and stem-cell biology and explored how stem-cell research might lead to better understanding and treatment of human diseases. Despite the snowstorm that paralysed some important airports in North America, most of the attendees made it to the meeting, and the warm atmosphere overcame the freezing weather outside. Instead of covering every talk presented at the meeting, we highlight some emerging and recurrent topics that were presented by speakers and at the poster sessions.

Significance of stem-cell heterogeneity

It has been proposed that stem cells do not represent a homogeneous population of cells. Austin Smith (U. Cambridge, UK) and Ian Chambers (U. Edinburgh, UK) have shown that Nanog expression is heterogeneous, dynamic, fluctuant and reversible in embryonic stem cells (ESCs; Chambers *et al*, 2007). Similar results have been reported for other genes including *Stella* and *Rex1*. At the meeting, Smith showed that heterogeneity of ESCs is lost when cultured in the presence of Erk and GSK3 inhibitors, which anchor ESCs in their naive state.

Peggy Goodell (Baylor College of Medicine, USA) showed that haematopoietic stem cells (HSCs), as defined by the co-expression of various cell-surface markers, are heterogeneous in regard to their side population, which is defined by the ability of those cells to extrude fluorescent dye. She showed that HSCs with the strongest side-population characteristics display higher self-renewal ability and are multipotent, but are biased toward myeloid differentiation (Challen *et al*, 2010).



Signals regulating stem-cell fate

Liz Robertson (U. Oxford, UK), presented evidence in her Keynote lecture that Eomes, a transcription factor of the TBX family, has a key role in specifying two cell fates: the definitive endoderm and the cardiac mesoderm. Eomes loss-of-function embryos have defects in early gastrulation, and no cells exit the primitive streak in Eomes-null embryos, similar to what is observed in Mesp1/2-null embryos. Eomes fate-mapping revealed the contribution of Eomes-derived cells to heart and gut development. Robertson proposed a model in which Eomes expression in the prospective cardiogenic mesoderm induces the specification of cardiovascular progenitors through the upregulation of Mesp1. This promotes the expression of downstream cardiovascular and epithelial-to-mesenchymal transcription factors and represses the expression of endoderm genes, thereby inhibiting the positive effect of Eomes in promoting endoderm formation in cardiac mesoderm. In prospective endoderm, Eomes does not induce Mesp1 expression—although it is a mechanism that might involve Nodal/Activin signalling—and only promotes endoderm cell fate.

Tudorita Tumbar (Cornell U., USA) showed that Runx1—a transcription factor expressed in embryonic hair-follicle progenitors—and its underlying mesenchyme regulate Wnt/β-catenin signalling in an opposing manner between the epidermis and the dermis. Deletion of Runx1 in the epidermis decreases Wnt/β-catenin signalling and delays hair-follicle morphogenesis and regeneration, suggesting a role for Runx1 in the activation of hair-follicle stem cells. Conversely, dermal deletion results in enhanced Wnt/β-catenin signalling and loss of hair-follicle stem cells, suggesting that Runx1 is also important in the maintenance of hair-follicle stem cells.

Gordon Keller (University Health Network, Toronto, Canada) presented data showing that the same signalling pathway can have opposing roles during the differentiation of ESC into different cell lineages.

He showed that Activin signalling stimulates cardiovascular progenitor specification during the first days of ESC differentiation, but inhibits cardiac differentiation a couple of days later.

> **…the regulation of Pol II pausing has an important function in regulating lineage commitment during HSC differentiation**

Wnt/β-catenin signalling regulates the renewal and differentiation of many types of stem cell, including ESCs. The way in which Wnt/β-catenin signalling regulates pluripotency is unclear. Two independent groups proposed a model in which Wnt/β-catenin signalling might inhibit active repression of pluripotency genes mediated by Tcf3. Austin Smith showed that Tcf3 inhibition mimics the effect of GSK3 inhibition in promoting pluripotency, and that a mutant form of β-catenin that is unable to stimulate transcription can rescue the defect associated with β-catenin loss of function. Brad Merrill (U. Illinois, USA) showed that Tcf3 acts as a transcriptional repressor of pluripotency genes in a manner similar to that which Smith presented. He found that Wnt still stimulates renewal, albeit at a reduced level, in ESCs expressing mutant Tcf3 that cannot bind to β-catenin. Merrill's group found that Tcf1 is also expressed in ESCs and mediates Tcf3–β-catenin-independent effects of Wnt in promoting self renewal. This suggests that two mechanisms mediate derepression of Tcf3 on pluripotency genes, which accounts for the Wnt/β-catenin stimulation of self renewal. Sergei Sokol (Mount Sinai School of Medicine, USA) proposed that during *Xenopus* gastrulation and in mouse ESCs, Tcf3 is phosphorylated by a mechanism that depends on β-catenin and leads to the dissociation of Tcf3 from its target genes, releasing its repressor function.

Kathryn Anderson (Memorial Sloan–Kettering Cancer Center, USA) found an important new role for Wnt inhibition in regulating early mouse development. By using a forward recessive genetic screen in mice, Anderson and colleagues found a new mutant they called Canopus, which is characterized by several defects suggestive of altered Wnt signalling. They identified that a mutation in Axin2, a negative regulator of Wnt signalling, is responsible for the Canopus phenotype and that this mutation promotes Axin2 stabilization, thereby enhancing Axin2-mediated inhibition on Wnt signalling *in vivo*.

Point of no return in stem cells

Although the ground-state of pluripotency might represent the epiblast component of the blastocyst stage *in vivo*, cells isolated from post-implantation embryos are also pluripotent. Ian Chambers presented data obtained in collaboration with Val Wilson (U. Edinburgh, UK) that examine the loss of pluripotency *in vivo*, assayed by the establishment of epiblast stem-cell lines and teratocarcinoma formation. Pluripotency is lost throughout the embryo at the onset of somatogenesis, correlating with the loss of Nanog messenger RNA expression and epigenetic modifications of the *Nanog* and *Oct4* loci. The sole re-expression of Oct4 after this point of no return allows cells to re-acquire pluripotency, which was paralleled by the re-opening of regulatory elements in chromatin around pluripotency transcriptional regulators, indicating that Oct4 expression regulates this developmental switch.

Elaine Fuchs discussed her analysis of the point at which hair-follicle bulge stem cells become irreversibly committed to a differentiation lineage. During hair-follicle regeneration, bulge stem cells are activated and proliferate to sustain hair growth. Fuchs's team showed that early bulge stem-cell descendants can return to their niche and act as a reservoir of stem cells to initiate the next round of hair-follicle regeneration. Other bulge stem-cell progeny further along the lineage can also return to the niche, although they will no longer contribute directly to hair-follicle regeneration. Rather, these non-stem-cell residents in the bulge anchor the hair and regulate bulge-stem-cell quiescence through the secretion of soluble growth-inhibitory proteins. This study demonstrates that the long-term ability of bulge stem cells to renew is not lost as they exit their niche. Furthermore, it suggests an interesting concept in which more-committed cells along a lineage can return to and regulate the stem-cell niche that they came from (Hsu *et al*, 2011).

Transcriptional regulation in stem cells

Three speakers presented evidence of the way in which transcription pausing regulates stem-cell renewal, differentiation and asymmetrical cell division. A forward genetic screen in zebrafish has identified a mutant called moonshine that displays a profound anaemia, owing to a mutation in the *Tif1g* gene. Leo Zon (Children's Hospital, Boston, USA) presented work that used a genetic modifier screen to look for mutants that could rescue the erythroid defect in moonshine mutants. His team identified one suppressor mutant, called sunrise, with a mutation in the *cdc73* gene, which encodes a subunit of the elongation factor complex. Zon presented evidence that *Tif1g* promotes the transcription elongation of erythroid genes by counteracting Pol II pausing. This study provides a clear genetic demonstration that the regulation of Pol II pausing has an important function in regulating lineage commitment during HSC differentiation (Bai *et al*, 2010).

> **…many subunits of the mediator and cohesin complexes, as well as the cohesin-loading factor Nipbl regulate Oct4 expression**

Rich Young and colleagues (Whitehead Institute, USA) performed a screen for regulators of Oct4 expression in ESCs. They found that many subunits of the mediator and cohesin complexes, as well as the cohesin-loading factor Nipbl regulate Oct4 expression. Mediator, cohesin and Nipbl together occupied enhancer and core promoter regions of active genes in ESCs. Young found evidence that the mediator–cohesin complex mediates enhancer–promoter looping that facilitates transcriptional elongation (Kagey *et al*, 2010).

During brain development in flies, neuroblasts divide asymmetrically. Alteration in the balance of asymmetrical cell division can lead to stem-cell expansion and brain tumour formation or stem-cell depletion. Jürgen Knoblich and colleagues (Institute of Molecular Biotechnology, Austria) performed a genome-wide RNA interference screen to identify new regulators of asymmetrical cell division during brain development. The results of their phenotypic classifications and bioinformatic analyses have led them to propose a gene network that regulates asymmetrical cell division in fly neuroblasts. The screen identified both genes that are known to control asymmetrical cell division and new classes of gene. Among the newly identified genes were several genes known to control transcriptional elongation, shown here to also affect asymmetrical cell division.

Model organisms of stem-cell function

Several presentations illustrated the use of model organisms to study stem cells. In contrast to mammals, planarians are able to regenerate heads or tails. Peter Reddien (Whitehead Institute, USA) presented evidence that the cells—called neoblasts—that mediate this regeneration process are multipotent stem cells that are scattered throughout the body and are able, at the clonal level, to differentiate into several cell lineages. This model can now be applied to screen for new regulators of this regenerative process. Elly Tanaka (Centre for Regenerative Therapies, Dresden, Germany) illustrated that some concepts derived from studying neural regeneration in salamanders can be applied to the study of mammalian neural stem cells. An early event in spinal cord regeneration in salamanders is the re-expression of EP-cadherin, suggesting that cells de-differentiate into early stages of neurogenesis. By differentiating Sox1-GFP ESCs in matrigel and medium that allows neuronal differentiation, Tanaka and colleagues were able to form a polarized cystic structure expressing GFP—reminiscent of the expression of Sox1 in the neural plate during embryonic development—which could further differentiate into neurons and neuroepithelium able to respond to patterning cues. By optimizing the culture conditions for human ESCs, they were able to form cysts expressing retina markers that can differentiate into functional retinal pigment epithelium.

Stem cells and ageing

Sean Morrison (HHMI/U. Michigan, USA) presented evidence that the response of stem cells to an oncogenic event depends on their developmental stage, providing a possible explanation for the change in the spectrum of mutations that lead to leukaemia that occurs with age. PTEN deletion has been found in various forms of acute leukaemia. Morrison showed that PTEN deletion leads to a detectable activation of PI3K pathways in adult HSCs, but not in young mice. As PTEN deletion in mice stimulates HSC proliferation but only induces leukaemia in adult mice, it seems that only adult HSCs are sensitive to this oncogenic signal. He also showed that different genes downstream from PTEN regulate physiological HSC self-renewal and progression of leukaemogenesis (Lee *et al*, 2010). Ron DePinho (Dana Farber Cancer Institute, USA) highlighted the importance of telomerase in controlling the long-term proliferation of stem cells from rapidly renewing tissues, as well as in ensuring the maintenance of post-mitotic tissues, such as the liver or the heart. He provided genetic and biochemical evidence that loss of telomerase is associated with mitochondrial dysfunction in cardiac, liver and haematopoietic stem cells. He also showed that activated p53 represses the master regulators of mitochondrial biogenesis and function—PGC co-activators—thereby linking genotoxic stress and mitochondrial theories of ageing (Sahin *et al*, 2011). By using knock-in mice that allow the reactivation of telomerase reverse transcriptase by TAM administration, DePinho's group have shown that telomere reactivation in late generation of telomerase-deficient mice eliminates the entrenched degenerative phenotypes across many organs, and can reverse neurodegeneration and its behavioural consequences (Jaskelioff *et al*, 2011).

> **…the administration of CD47-blocking antibodies [targets] cancer stem cells and, in combination with other therapies, provides a survival advantage in pre-clinical models**

Stem cells and diseases

Talks and posters highlighted the importance of establishing reliable and reproducible protocols to obtain differentiated cells during ESC differentiation. Gordon Keller presented a protocol involving the sequential addition of instructive factors that guide the differentiation of ESCs into endocrine pancreatic cells that are able to produce insulin. Although this protocol generates cells that produce large amounts of insulin—similar to levels observed in the adult pancreas—the ESC-derived cells are not glucose responsive, suggesting a problem with their final maturation (Nostro *et al*, 2011).

Lorenz Studer (Memorial Sloan–Kettering Cancer Center, USA) showed the way in which induced pluripotent stem cells (iPSCs) can be used to model human diseases and discover new drugs that target these diseases. His group derived iPSCs from patients with familial dysautonomia, a syndrome characterized by the degeneration of sensory and sympathetic neurons. Neural crest precursors derived from familial dysautonomia iPSCs present defects similar to those seen during human disease. In a screen of US Food and Drug Administration-approved drugs for their ability to correct the pathological phenotype in familial dysautonomia iPSCs, Studer's team found that administration of the plant extract kinetin during ESC-induced neuronal differentiation resulted in a marked increase in the generation of peripheral neurons. This suggests that kinetin might be useful for the prevention of disease progression in patients with familial dysautonomia (Lee *et al*, 2009).

Irve Weissman (Stanford U., USA) presented another remarkable medical application of stem-cell research. His group recently found that CD47 is expressed by HSCs and leukaemic stem cells to inhibit their phagocytosis by macrophages—the so-called 'don't eat me' signal. CD47 expression is predictive of poor clinical outcomes in acute myeloid leukaemia. The administration of CD47-blocking antibodies increases the phagocytosis of leukaemic stem cells (Jaiswal *et al*, 2010). At the meeting, Weissman presented evidence that CD47 is expressed in cancer stem cells from different human neoplasia, including acute lymphoblastic leukaemia, non-Hodgkin lymphoma and bladder cancer, as well as other epithelial cancers. He showed that the administration of CD47-blocking antibodies targets these cancer stem cells and, in combination with other therapies, provides a survival advantage in pre-clinical models.

This meeting highlighted new, exciting aspects of stem-cell and developmental biology, as well as their potential implications for understanding and treating human diseases. We hope that this conference inspired and stimulated the participants to continue their restless efforts in opening new horizons in stem-cell research.

REFERENCES

Bai X *et al* (2010) *Cell* **142:** 133–143
Challen GA *et al* (2010) *Cell Stem Cell* **6:** 265–278
Chambers I *et al* (2007) *Nature* **450:** 1230–1234
Hsu YC, Pasolli HA, Fuchs E (2011) *Cell* **144:** 92–105
Jaiswal S *et al* (2010) *Trends Immunol* **31:** 212–219
Jaskelioff M *et al* (2011) *Nature* **469:** 102–106
Kagey MH *et al* (2010) *Nature* **467:** 430–435
Lee G *et al* (2009) *Nature* **461:** 402–406
Lee JY *et al* (2010) *Cell Stem Cell* **7:** 593–605
Nostro MC *et al* (2011) *Development* **138:** 861–871
Sahin E *et al* (2011) *Nature* **470:** 359–365

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