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Stem cell powwow in Squaw Valley

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Summary

The Keystone Symposium entitled 'The Life of a Stem Cell: from Birth to Death' was held at Squaw Valley, CA, USA in March 2012. The meeting brought together researchers from across the world and showcased the most recent developments in stem cell research. Here, we review the proceedings at this meeting and discuss the major advances in fundamental and applied stem cell biology that emerged.

Key words: Regeneration, Reprogramming, Stem cell niche

Introduction

In March 2012, a great gathering of many stem cell tribes took place in the Californian mountains. This gathering – the Keystone Symposium entitled 'The Life of a Stem Cell: from Birth to Death' – was organised by Margaret Goodell (Baylor College of Medicine, Houston, TX, USA), Ruth Lehmann (HHMI and Skirball Institute at NYU Medical Center, New York, NY, USA) and Thomas Rando (Stanford University, Stanford, CA, USA) and was held at a resort in Squaw Valley, close to the magnificent Lake Tahoe. Being the location of the 1960 Winter Olympiad, Squaw Valley was a fitting venue for discussions on a branch of biology of a similar age. The full diversity of stem cells (SCs) found in development and adult life, together with their relevance for physiology, disease and therapy, were on display at this meeting. As such, a wide variety of SCs and their niches from mammalian and non-mammalian model organisms were represented. Perhaps more than at previous Keystone meetings, this provided a broad overview of the diversity of SCs, as well as their commonalities, emerging themes and the requirements for their future study.

As expected, a number of the presentations centred on the genetic and intracellular signalling networks governing SC identity. However, in addition to intracellular molecular mechanisms, signalling pathways activated by extracellular cues from the microenvironment play a crucial role in regulating cell fate. Accordingly, important functions for the 'usual suspects' (cytokines, Notch, Hedgehog, bone morphogenetic proteins, integrins, etc.) were discussed for many systems, but Wnts seemed to pop up more than other signalling pathways during this meeting and more frequently than in the past. This might be due to ever improving methods for investigating this notoriously difficult signalling system. Finally, with the increasing resolution of current technologies, the heterogeneity of SC-derived systems is becoming increasingly apparent. Accordingly, the need for single-cell analyses, both for genome-wide quantification and for continuous analyses by imaging, was frequently discussed at the meeting as an important future requirement.

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Below, we highlight just some of the talks illustrating the diversity of SCs discussed at this meeting (Fig. 1).

Genome-wide analyses

Previous Keystone meetings have seen a strong focus on genome-wide chromatin studies in embryonic stem (ES) cells as a way to begin to unravel the mechanisms that control cell identity. Although this year's meeting was not as focused in this direction, several talks highlighted continuing progress in the field, both clarifying previous questions and raising novel questions about commonly accepted assumptions. Austin Smith (Stem Cell Institute, Cambridge, UK) kicked off this approach. His laboratory has championed the use of inhibitors of extracellular signal-regulated kinase (ERK) and glycogen synthase kinase-3β (GSK3β) signalling (termed dual inhibition, or '2i') for the derivation and maintenance of mouse ES cells (Ying et al., 2008). He has now compared the transcriptomes of ES cell lines cultured in foetal calf serum (FCS) with those cultured in 2i and finds that 1500-2000 genes differ by more than twofold (Marks et al., 2012). Whereas ectoderm and mesoderm genes show increased expression in FCS, germline and endoderm genes are similarly expressed in 2i and FCS, suggesting that 'lineage priming' of mesoderm/ectoderm but not endoderm is suppressed in 2i. This might mean that endoderm is open for population in 2i; an intriguing possibility given the developmental proximity of naïve pluripotency and primitive endoderm. Interestingly, in this study, comparative chromatin analysis showed that although no differences in histone H3 lysine 4 (H3K4me3) or lysine 36 (H3K36me3) trimethylation were seen in cells cultured in 2i or FCS, histone H3 lysine 27 trimethylation (H3K27me3) was reduced over promoters in 2i. Consequently, the number of bivalent domains observed was decreased in 2i. These results suggest that, at least in part, 'bivalency' is reflective of heterogeneity in the sample population. Smith and co-workers also investigated the transcription factors that act downstream of GSK3β and identified estrogen-related receptor beta (Esrrb) as a functional mediator of the effect of GSK3β inhibition. In a separate study, Ian Chambers (MRC Centre for Regenerative Medicine, Edinburgh, UK) identified *Esrrb* as a major direct transcriptional target of Nanog, indicating complex interconnections within the pluripotency gene regulatory network.

Alexander Meissner (Harvard University, Boston, MA, USA) also discussed genome-wide analyses that have been used by his group to examine the methylation changes occurring in the nuclei of gametes, from fertilisation to the blastocyst stage (Smith et al., 2012). Compared with sperm, the oocyte has significantly less methylation. A global decrease in DNA methylation in the zygote reflects a shift of some, but not all, loci to a decreased methylation state. The base-resolution view achieved in this study enabled a detailed dissection of the methylation dynamics occurring during this early embryonic phase. Given the sensitivity of the approach, Meissner and colleagues then compared normal fertilisation dynamics with the changes induced by the ooplasm after somatic cell nuclear transfer (SCNT). The importance of DNA methylation

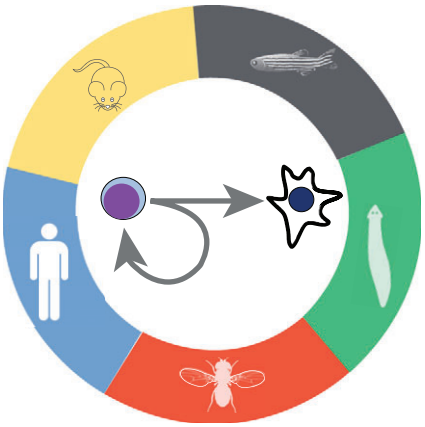

Fig. 1. A winning team in stem cell research. Stem cells (SCs), as defined by the ability to self-renew (circular arrow) and differentiate into more specialised cell types (straight arrow), were discussed at a former Olympic venue during the recent Keystone Symposium entitled 'The Life of a Stem Cell: from Birth to Death'. The use of a diverse team of organisms (mouse, zebrafish, planaria, *Drosophila* and human) has proved a winning combination for analysing the control of these defining SC features. The meeting synthesized aspects of SC behaviour and how these change as organisms develop or age.

for SC behaviour was further highlighted by Margaret Goodell (Baylor College of Medicine, Houston, TX, USA). The high expression of DNA methyltransferase 3A (Dnmt3a) in haematopoietic SCs (HSCs) led her to analyse the effects of deletion of Dnmt3-related proteins on HSC self-renewal. Serial transplantation studies showed increased long-term HSC self-renewal in the absence of Dnmt3a (Challen et al., 2011). Careful quantification of the output from individual HSCs demonstrated the amazing regenerative potential of SCs, which was further increased during serial transplantation. She estimated that a single normal HSC would produce ~10^4 HSCs after four rounds of transplantation, whereas a single Dnmt3a-deficient HSC would generate one billion HSCs by the same extrapolation. Although loss of Dnmt3b alone had a minimal effect, simultaneous deletion of Dnmt3a and Dnmt3b increased expansion even further. Genome-wide DNA methylation and transcriptome analyses showed that numerous genes are hyper- or hypomethylated upon Dnmt3a deletion, and that their expression is altered. Interestingly, however, modulation of individual gene transcription did not necessarily correlate with changing levels of methylation.

Another inspiring talk, from Joanna Wysocka (Stanford University, Stanford, CA, USA), used genome-wide analyses to study the changes occurring during neural crest (NC) development. NC gives rise to peripheral nervous system neurons and glia, mesenchymal progenitors and melanocytes. Many of these diverse cell types contribute to facial features. In fact, more than 100 different cell types, including cranial bone and cartilage, trace their origins to NC. Using methods similar to those developed by Lorenz Studer for differentiating human ES cells into NC (Lee et al., 2010), Wysocka used her previous findings on developmentally relevant chromatin enhancer signatures (Rada-Iglesias et al., 2011) to identify over 4000 putative NC enhancers. Strikingly, genes most closely associated with these active enhancers were implicated in craniofacial development and abnormalities. Examination of the motifs most enriched at NC enhancers identified a binding motif for transcription factor AP-2 (TFAP2A), a protein that is sufficient to transform *Xenopus laevis* neural progenitors into NC when ectopically expressed. Also highly enriched at NC enhancers are motifs for the nuclear receptors NR2F1 and NR2F2, the expression of which is strongly increased during differentiation to NC.

Pluripotency and the germ line

Pluripotent cells again featured strongly at this meeting. In addition to the above talks, many projects aimed to relate features of in vitro pluripotent cell lines to aspects of early development, and this included assessing the relationship between pluripotency and the germ line. Azim Surani (Gurdon Institute, Cambridge, UK) discussed his group's recent studies of reprogramming during primordial germ cell (PGC) development. Surani reminded us that during the reprogramming of epiblast stem cells (EpiSCs) to ES cells or toward the PGC lineage, certain alterations are common, including X reactivation and DNA demethylation. Genes activated during PGC specification include those encoding positive regulatory domain zinc-finger protein 1 (Prdm1, or Blimp1) and Prdm14. Together with their targets, such as Kruppel-like factor 2 (Klf2), Blimp1 and Prdm14 regulate reprogramming of the germ line. Surani reported that, during EpiSC to ES cell reprogramming, enforced expression of Prdm14 together with Klf2 enhances reprogramming efficiency (Gillich et al., 2012). During this reprogramming, PGC markers are also upregulated. Surani therefore asked whether the expression of PGC genes was required for the reprogramming of EpiSCs to ES cells by performing the same experiment in *Blimp1*$^{-/-}$ cells, in which PGC development is blocked at an early stage. Interestingly, it was still possible to reprogram the *Blimp1*-deficient EpiSCs to an ES cell state.

Continuing with the focus on germ cells, Ruth Lehmann (HHMI and Skirball Institute at NYU Medical Center, New York, NY, USA) described the role of gene repression during *Drosophila* germ cell development mediated by a germline-specific gene aptly named *polar granule component* (*pgc*). Pgc inhibits Positive transcription elongation factor b (P-TEFb; Cdk9 – FlyBase) function, which leads to a lack of phosphorylated RNA polymerase II. Pgc is expressed during the formation of repressive chromatin centres (RCCs), which act to block transposons in the germ line and *pgc* mutants show delayed RCC formation. Interestingly, mutations in the H3K9 methyltransferase dSETDB1 (Eggless – FlyBase) also show reduced levels of Piwi-interacting RNAs (piRNAs) and increased transposable element transcript expression. Yukiko Yamashita (University of Michigan, Ann Arbor, MI, USA) also presented studies of the *Drosophila* germ line, in this case analysing chromosome segregation during SC division. Although no asymmetric autosome segregation could be found (Yadlapalli et al., 2011), X and Y chromosomes segregate asymmetrically, with the SC retaining the original chromosome. Ongoing studies concern the role of centrosomes, cytoskeleton to chromosome linkage, and chromatin modification in the control of this surprising and potentially very basic mechanism of inheriting and preserving genetic information. In his talk, Shukry Habib (from the Nusse laboratory, Stanford University, Stanford, CA, USA) also noted an apparent asymmetric segregation of different molecules during ES cell division that could be induced by localised Wnt sources.

Regeneration, reprogramming and pluripotency

Peter Reddien (MIT, Whitehead Institute, Boston, MA, USA) described studies of pluripotent SCs ('neoblasts') in planaria, which are proving to be an interesting model system in tissue regeneration research. Radiation-sensitive neoblasts are widely distributed in the

organism and can rescue lethally irradiated recipients upon transplantation (Wagner et al., 2011). In fact, single cells from the neoblast population can completely take over the genetic composition of an irradiated host (though whether all neoblasts display such pluripotency remains unknown). Experimental blastema formation was used to examine whether subsets of neoblasts are specialised for particular functions. For example, eye regeneration occurs by activation of genes required for eye formation in neoblasts near wounds (Lapan and Reddien, 2011). Such cells can be seen trailing back from the forming eye to the site of wounding. Eye marker gene expression also suggests the presence of some specialised neoblasts in undamaged planaria. These studies suggest that, in this context, not all SCs are identical.

Using the chick embryo as a model, Claudio Stern (University College London, London, UK) discussed embryonic regulation, a unique property of amniote embryos (Stern, 2006). This form of regeneration can result in axis duplication and, in the extreme, the formation of identical twins. His previous studies showed that the transforming growth factor β (TGFβ) member vegetal 1 (Vg1), which can induce an ectopic axis when misexpressed, is normally expressed in the posterior marginal zone of the chick embryo. The Stern laboratory has now shown that when an embryo is cut into anterior and posterior halves, Vg1 becomes activated in the anterior portion at randomly left or right positions. Current efforts are focused on the functional analysis of microarray data to determine how Vg1 expression is activated and hence how patterning information is re-established during regeneration.

A number of studies have attempted to compare ES cells with induced pluripotent stem (iPS) cells. Konrad Hochedlinger (Massachusetts General Hospital, Boston, MA, USA) presented recent studies in which he and his colleagues have used a secondary reprogramming system [with Oct4 (Pou5f1)-Klf4-Sox2-myc expressed from *Col1a1*] to compare ES and iPS cells. Prior RNA expression analysis suggested that iPS and ES cells are very similar, with the exception of maternally expressed transcripts from the *Dlk1-Dio3* cluster, which are aberrantly silenced in many iPS cell lines. In work just published (Stadtfeld et al., 2012), Hochedlinger showed that aberrant silencing could be blocked using serum replacement media rather than FCS during reprogramming. The dominantly acting component was identified as ascorbic acid. The current model is that loss of an activating imprint at *Dlk1* recruits complexes including Dnmt3a that repress the locus. How ascorbate blocks this is presently unknown. Robert Blelloch (University of California, San Francisco, CA, USA) also focused on reprogramming. He has previously shown that the miRNAs let7 and miR-294 have opposing effects on ES cell self-renewal (Melton et al., 2010). His laboratory has now identified 20 miR-294 targets, which are being tested for their ability to recapitulate the co-operative effects of miR-294 on somatic cell reprogramming mediated by Oct4, Klf4, Sox2 and c-myc. Blelloch then described experiments that aim to determine whether these miRNAs play antagonistic roles during other developmental stages.

Turning to human SCs, Jamie Thomson (University of Wisconsin, Madison, WI, USA) introduced a new medium for human ES cell culture that improves cloning efficiencies. However, Thomson's main focus was on phosphorylation of the core pluripotency factors; using improved mass spectrometry, Thomson's group have identified 14 phosphorylation sites in Oct4 and six in Sox2 (Brumbaugh et al., 2012). Phosphorylation at two of the identified sites in Oct4, both of which lie within the homeodomain, decreases Oct4 DNA binding and transcriptional activity. Incubation of purified Oct4 with ERK identified sites outwith the DNA-binding domain as ERK targets, although other proline-directed kinases, including cyclin-dependent kinases, may act on the same residues. Nevertheless, it is interesting that ERK, a downstream effector of the fibroblast growth factor (FGF) signal required to maintain human ES cells, can act on one of the core transcriptional components. Dona Love (NIH, Bethesda, MD, USA) also described how pluripotency factors can be modified post-translationally. She described her studies of the addition of N-acetyl glucosamine (GlcNac) to chromatin components and speculated that this modification might link nutrient status to epigenetics. The pluripotency transcription factors Oct4, Sox2 and Nanog are proposed targets of this modification.

Potential therapeutics

Whereas the studies discussed above focused on undifferentiated pluripotent cells, others used such cells as a starting point to study more mature progenitors and differentiated cells of potential therapeutic relevance. Lorenz Studer (Sloan-Kettering Institute, New York, NY, USA) addressed problems of efficiency when generating therapeutically relevant neural cell types from iPS cells in vitro (Lee and Studer, 2011). As in many other studies that analyse directed differentiation of pluripotent cells, a thorough understanding of the differentiation steps occurring during embryonic development has helped to identify the inductive signals required for successful in vitro differentiation. As highlighted by Studer's research, mimicking the sequential signals that occur in vivo during the differentiation of a desired cell type has dramatically improved the efficiency of deriving such cell types in vitro and has also improved the purity of the resulting cells. This is an essential prerequisite for moving these approaches into clinical trials for cell therapy using iPS-derived cell types.

In line with this, Yoshiki Sasai (RIKEN Center for Developmental Biology, Kobe, Japan) provided an exciting summary of how spectacular and highly complex 3D eye structures can develop from ES cells in vitro (Eiraku et al., 2011). They challenge the assumption that proper development can only occur in the context of a developing embryo and indicate that differentiating ES cells are capable of non-chaotic self-organisation. Paul Gadue (Children's Hospital of Philadelphia, Philadelphia, PA, USA) described recently published work (Cheng et al., 2012) on multipotent endodermal progenitor (EP) cells that can be obtained from pluripotent human cells in serum-free medium. These EP cells can be cultured long term and upon transplantation to mice do not form tumours, in contrast to human ES cells. Importantly, EP cells can be differentiated in vitro into monohormonal beta-like cells that can secrete insulin in response to glucose stimulation. These approaches highlight both the potential therapeutic use of pluripotent cell derivatives and the many optimisation steps that are required for most cell types before their clinical application.

Adult tissue stem cells

Although some adult SCs appear to be closer to successful, safe clinical application, several talks demonstrated the varying degrees to which current knowledge limits the therapeutic application of different SCs. Leonard Zon (Children's Hospital of Boston, Boston, MA, USA) reviewed a screen that identified prostaglandin as an influence on the generation of haematopoietic stem/progenitor cells during early zebrafish development (North et al., 2007). Prostaglandins also affect adult mouse and human cord blood haematopoietic stem cells (HSCs) (Goessling et al., 2011); ex vivo treatment of HSCs with prostaglandin E2 before transplantation led to improved outcomes and shows promising results in an ongoing

phase 1 clinical trial. This is an excellent example of how basic research on a non-mammalian model organism can lead to promising clinical trials within a few years. It would be a great success if this bench – or rather fish tank – to bedside approach was able to yield patient benefit in less than a decade. However, it is worth noting that even with HSCs, as the most successfully clinically applied SC system, this transfer takes many years of intensive effort. Zon also discussed in vivo imaging approaches that identified specific migrational behaviour of nascent haematopoietic cells in zebrafish embryos. Unexpectedly, reciprocal signalling between HSCs and endothelial cells might lead to endothelial reorganisation, potentially creating novel and transient haematopoietic niches.

Landmark experiments from Hans Clevers (Hubrecht Institute, Utrecht, The Netherlands) have identified Lgr5 as a marker of intestinal SCs or crypt base columnar cells (CBCs) located at the base of the intestinal crypt. A key finding from the Clevers group is that individual Lgr5-postive cells can form 'organoids' in vitro. Toshio Sato and Clevers noticed that the cells that went on to form organoids rapidly divided to produce a Paneth cell, which is a differentiated cell found intimately interspersed among the CBCs in vivo. Sato then examined the efficiency of organoid formation using FACS-isolated cell doublets in which one cell is a CBC and the other a Paneth cell. Amazingly, the efficiency of organoid formation was radically improved, indicating that although the CBCs are the organoid-initiating cell, they perform this function most effectively when in communication with a Paneth cell (Sato et al., 2011). Moreover, FACS-sorted populations of single CBCs and Paneth cells replated at high density are able to self-assemble to form organoids. The functionality of such organoids was assessed by transferring fluorescently marked organoids into the gut of a mouse with a lesioned colon. Remarkably, several days post-injection the lesion was repaired and fluorescent cells were found at the lesion sites.

Clevers then addressed the issue of the functionality of cells at positions further up the crypt wall. He noted that delta-like 1 (Dll1) was expressed at position +5 and upwards. When these cells divide they either become Notch-expressing enterocytes or Dll1-positive cells. Interestingly, Dll4 is expressed in the CBCs, with Paneth cells expressing Notch. If the CBCs are damaged, then Dll1-expressing cells are hypothesised to divide and drop down into positions previously occupied by CBCs, where they take on CBC function, indicating that they are facultative SCs.

Environmental effects

In addition to cell or tissue replacement as a potential therapeutic SC application, the rejuvenation of aged SCs to reverse degeneration was the focus of several exciting talks. Thomas Rando (Stanford University, Stanford, CA, USA) summarised numerous published studies that used parabiosis or specific molecular manipulations to alter the molecular state of old and young SCs in muscle, skin, blood, brain and liver (Rando and Chang, 2012). These studies suggest that the aged state of SCs is not an accumulation of damage or the loss of properties of these cells but rather is induced and actively maintained by the cells' environment and is, therefore, reversible. Currently, it seems more likely that aged microenvironments suppress young SC phenotypes that are supported by young niches. This opens the possibility of interfering with the aging signals from the old niches, which is typically easier than activating specific 'youthful' signals. Analysis of the molecular mechanisms underlying these changes could allow the development of therapies for rejuvenating old SCs, thus enhancing the maintenance or regeneration of aged tissues. Although examples exist of numerous different signalling pathways (including, for example, Notch, NFκB, chemokines and mTOR), genome-wide studies suggest that Wnt signalling and chromatin modifications play a central role. Preliminary studies presented by Rando confirmed this by demonstrating the aging effect of Wnts as well as chromatin-modifying enzymes in both in vitro and in vivo models. Manipulation of Wnt signalling might thus be a promising pathway to alter and potentially reverse aged phenotypes. Obviously, targeting of therapeutics to the appropriate cell type in vivo will be challenging, and the prevention or reversion of SC aging in vitro could be a promising approach.

Christa Muller-Sieburg (Sanford-Burnham Institute for Medical Research, La Jolla, CA, USA) discussed careful quantitative analyses of haematopoietic regeneration, demonstrating predetermined and inheritable bias of repopulation kinetics, life span, proliferation potential and lineage generation of individual HSCs (Muller-Sieburg et al., 2012). These lead to changes in the pool sizes of different HSC types over time, explaining some of the changing properties of aging haematopoietic systems. Anne Brunet (Stanford University, Stanford, CA, USA) also focused on aging and discussed the epigenetic control of lifespan and neural SC fate in *C. elegans*. In addition to alterations in longevity due to genetic and environmental influences, interesting transgenerational epigenetic effects could be observed: wild-type offspring of mutants for modifiers of specific histone marks showed increased lifespan for up to three generations (Greer et al., 2011). Although the molecular mechanism underlying this surprising finding is not yet understood, it illustrates the many layers of regulation that still remain to be elucidated before a comprehensive understanding of longevity and cell fate control is achieved.

These studies, which demonstrate the importance of the niche in the aging organism, were complemented by studies highlighting the niche and its influence on SC metabolomics. Celeste Simon (Abramson Family Cancer Research Institute, University of Pennsylvania, Pennsylvania, PA, USA) reviewed the strong influence of oxygen levels and hypoxia-inducible factor (HIF) signalling on numerous developmental processes and SCs. She discussed how multiple signalling pathways, including Wnt and phosphatidyl inositol 3-kinase (PI3K) signalling, are influenced by hypoxia to regulate different SC fates. Also focusing on the importance of hypoxia for HSC self-renewal, Toshio Suda (Keio University, Tokyo, Japan) demonstrated that use of the glycolytic pathway can help to reduce oxidative stress and increase self-renewal of HSCs. Detailed molecular analyses then allowed the identification of small molecules that induce glycolysis and improve the maintenance of HSCs ex vivo.

Finally, Benjamin Ohlstein (Columbia University Medical Center, New York, NY, USA) addressed the important question of how the number of SCs in a tissue can be controlled. Careful enumeration in the *Drosophila* gut demonstrated the reversible decline of intestinal SCs after dietary restriction. Lineage-tracing analyses and live imaging then revealed that, remarkably, diploid intestinal SCs are regenerated from polyploid enterocytes. This surprising change in ploidy during regeneration is reminiscent of the somatic divisions that reduce polyploidy during regenerative scenarios in the mammalian liver (Duncan et al., 2009).

Imaging stem cells

As with many of the studies presented at this meeting, the studies from the Ohlstein group nicely illustrated how careful quantitative microscopy can lead to unexpected novel insights even in well-

defined tissues. The well-recognised importance of imaging approaches in developmental biology was further emphasised by several talks that centred on quantitative, live, single-cell imaging.

The importance of continuous single-cell analysis was highlighted by Timm Schroeder (Helmholtz Center, Munich, Germany). He introduced bioimaging approaches for long-term SC observation at the single-cell level (Schroeder, 2011). Recent developments allow the continuous quantification not only of cell fates, but also of the expression of multiple transcription factor proteins in living SCs. He discussed how this novel type of quantitative time-resolved data challenges long-standing paradigms about the role of transcription factor networks in SC fate control. Valentina Greco (Yale School of Medicine, New Haven, CT, USA) also showed impressive two-photon imaging approaches to follow cell dynamics during hair follicle growth in the mouse in vivo. Furthermore, Shosei Yoshida (National Institute for Basic Biology, Okazaki, Japan) discussed detailed time-lapse microscopy studies that reveal that mouse spermatogonia are highly motile. This led to the proposition that individual spermatogonial SCs might be able to take over large areas of the testes through competition based on this enhanced mobility (Klein et al., 2010). This concept of 'neutral competition' was also picked up by Hans Clevers to describe the colonisation of intestinal crypts by individual CBC progeny.

Conclusions

This year's Keystone Symposium on SCs once again surpassed expectations, with presentations covering a wide range of interesting systems. One of the unexpected aspects of the meeting, however, was just how ignorant we still are about many aspects of SC biology and biochemistry. Much remains to be learned, and it was clear from this meeting that careful and quantitative in-depth analyses of even long-studied tissues and cell systems promise to yield novel, surprising and important insights into the life of an SC.

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Competing interests statement

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References

Brumbaugh, J., Hou, Z., Russell, J. D., Howden, S. E., Yu, P., Ledvina, A. R., Coon, J. J. and Thomson, J. A. (2012). Phosphorylation regulates human OCT4. *Proc. Natl. Acad. Sci. USA* **109**, 7162-7168.

Challen, G. A., Sun, D., Jeong, M., Luo, M., Jelinek, J., Berg, J. S., Bock, C., Vasanthakumar, A., Gu, H., Xi, Y. et al. (2011). Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat. Genet.* **44**, 23-31.

Cheng, X., Ying, L., Lu, L., Galvao, A. M., Mills, J. A., Lin, H. C., Kotton, D. N., Shen, S. S., Nostro, M. C., Choi, J. K. et al. (2012). Self-renewing endodermal progenitor lines generated from human pluripotent stem cells. *Cell Stem Cell* **10**, 371-384.

Duncan, A. W., Hickey, R. D., Paulk, N. K., Culberson, A. J., Olson, S. B., Finegold, M. J. and Grompe, M. (2009). Ploidy reductions in murine fusion-derived hepatocytes. *PLoS Genet.* **5**, e1000385.

Eiraku, M., Takata, N., Ishibashi, H., Kawada, M., Sakakura, E., Okuda, S., Sekiguchi, K., Adachi, T. and Sasai, Y. (2011). Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* **472**, 51-56.

Gillich, A., Bao, S., Grabole, N., Hayashi, K., Trotter, M. W., Pasque, V., Magnusdottir, E. and Surani, M. A. (2012). Epiblast stem cell-based system reveals reprogramming synergy of germline factors. *Cell Stem Cell* **10**, 425-439.

Goessling, W., Allen, R. S., Guan, X., Jin, P., Uchida, N., Dovey, M., Harris, J. M., Metzger, M. E., Bonifacino, A. C., Stroncek, D. et al. (2011). Prostaglandin E2 enhances human cord blood stem cell xenotransplants and shows long-term safety in preclinical nonhuman primate transplant models. *Cell Stem Cell* **8**, 445-458.

Greer, E. L., Maures, T. J., Ucar, D., Hauswirth, A. G., Mancini, E., Lim, J. P., Benayoun, B. A., Shi, Y. and Brunet, A. (2011). Transgenerational epigenetic inheritance of longevity in Caenorhabditis elegans. *Nature* **479**, 365-371.

Klein, A. M., Nakagawa, T., Ichikawa, R., Yoshida, S. and Simons, B. D. (2010). Mouse germ line stem cells undergo rapid and stochastic turnover. *Cell Stem Cell* **7**, 214-224.

Lapan, S. W. and Reddien, P. W. (2011). dlx and sp6-9 Control optic cup regeneration in a prototypic eye. *PLoS Genet.* **7**, e1002226.

Lee, G. and Studer, L. (2011). Modelling familial dysautonomia in human induced pluripotent stem cells. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **366**, 2286-2296.

Lee, G., Chambers, S. M., Tomishima, M. J. and Studer, L. (2010). Derivation of neural crest cells from human pluripotent stem cells. *Nat. Protoc.* **5**, 688-701.

Marks, H., Kalkan, T., Menafra, R., Denissov, S., Jones, K., Hofemeister, H., Nichols, J., Kranz, A., Stewart, A. F., Smith, A. et al. (2012). The transcriptional and epigenomic foundations of ground state pluripotency. *Cell* **149**, 1-15.

Melton, C., Judson, R. L. and Blelloch, R. (2010). Opposing microRNA families regulate self-renewal in mouse embryonic stem cells. *Nature* **463**, 621-626.

Muller-Sieburg, C., Sieburg, H. B., Bernitz, J. M. and Cattarossi, G. (2012). Stem cell heterogeneity: implications for aging and regenerative medicine. *Blood* **119**, 3900-3907.

North, T. E., Goessling, W., Walkley, C. R., Lengerke, C., Kopani, K. R., Lord, A. M., Weber, G. J., Bowman, T. V., Jang, I. H., Grosser, T. et al. (2007). Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. *Nature* **447**, 1007-1011.

Rada-Iglesias, A., Bajpai, R., Swigut, T., Brugmann, S. A., Flynn, R. A. and Wysocka, J. (2011). A unique chromatin signature uncovers early developmental enhancers in humans. *Nature* **470**, 279-283.

Rando, T. A. and Chang, H. Y. (2012). Aging, rejuvenation, and epigenetic reprogramming: resetting the aging clock. *Cell* **148**, 46-57.

Sato, T., van Es, J. H., Snippert, H. J., Stange, D. E., Vries, R. G., van den Born, M., Barker, N., Shroyer, N. F., van de Wetering, M. and Clevers, H. (2011). Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature* **469**, 415-418.

Schroeder, T. (2011). Long-term single-cell imaging of mammalian stem cells. *Nat. Methods* **8**, S30-S35.

Smith, Z. D., Chan, M. M., Mikkelsen, T. S., Gu, H., Gnirke, A., Regev, A. and Meissner, A. (2012). A unique regulatory phase of DNA methylation in the early mammalian embryo. *Nature* **484**, 339-344.

Stadtfeld, M., Apostolou, E., Ferrari, F., Choi, J., Walsh, R. M., Chen, T., Ooi, S. S., Kim, S. Y., Bestor, T. H., Shioda, T. et al. (2012). Ascorbic acid prevents loss of Dlk1-Dio3 imprinting and facilitates generation of all-iPS cell mice from terminally differentiated B cells. *Nat. Genet.* **44**, 398-405.

Stern, C. D. (2006). Evolution of the mechanisms that establish the embryonic axes. *Curr. Opin. Genet. Dev.* **16**, 413-418.

Wagner, D. E., Wang, I. E. and Reddien, P. W. (2011). Clonogenic neoblasts are pluripotent adult stem cells that underlie planarian regeneration. *Science* **332**, 811-816.

Yadlapalli, S., Cheng, J. and Yamashita, Y. M. (2011). Drosophila male germline stem cells do not asymmetrically segregate chromosome strands. *J. Cell Sci.* **124**, 933-939.

Ying, Q. L., Wray, J., Nichols, J., Batlle-Morera, L., Doble, B., Woodgett, J., Cohen, P. and Smith, A. (2008). The ground state of embryonic stem cell self-renewal. *Nature* **453**, 519-523.