Cells, Niche, Fate: Meeting Report on the 6th International Meeting of the Stem Cell Network North Rhine Westphalia

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In April 2011, leading stem cell researchers from all over the world came together for the 6th International Meeting of the Stem Cell Network North Rhine Westphalia (www.kongress.stammzellen.nrw.de), organized by Hans Schöler (Münster), Oliver Brüstle (Bonn), and Peter Horn (Essen). Notably, more than 600 participants, presenting 136 posters, joined the congress in Essen attracted by the scientific program focussing on “Homing and migration,” “Cell Fate Specification,” and “Disease Modeling.” In addition to these well-established topics new sessions dealing with “Nonhuman Primate Pluripotent Stem Cells” and “Niche, Hypoxia, and Metabolism” were integrated into the congress program. This report highlights a few of the most interesting presentations from the broad spectrum of stem cell research.

The hematopoietic component of marrow consists predominantly of developing erythrocytes and neutrophils: two lineages that start off as proliferating blasts with a high nuclear-to-cytoplasmic ratio, but go on to develop widely different metabolic characteristics and requirements. The idea presented by Michael Cross (Department of Hematology/Oncology IZKF, University of Leipzig, Germany) is that hematopoiesis may be organized into metabolic regions of the bone marrow, influenced on the one hand by the relative proximity to arterial or sinusoidal vessels, and on the other hand by the metabolism of the intervening cells.

It is now generally accepted that stem cells are maintained in areas in which oxygen tension (and with it oxidative damage) is low. In more active multipotent progenitors, however, hypoxia activates glycolysis and results in differentiation. In comparison, high osmolarity maintains the undifferentiated state while reducing glycolysis and increasing the use of glutamine, consistent with a metabolism biased toward nucleic acid synthesis. In this way, the spatial separation of metabolic pathways in distinct compartments may tailor metabolism to function and avoid the accumulation of potentially toxic products (Cross et al., 2008).

Working with hematopoietic stem cells (HSC), Norman Iscove (The Ontario Cancer Institute, University Health Network, Toronto, Canada) presented his data on Gata3, a transcription factor and significant regulator of HSC fate. He could identify strong overexpression of Gata3 transcripts and protein in murine marrow cell fractions comprising 30–85% long-term hematopoietic stem cells (LT-HSC) relative to purified intermediate-term (IT-) HSC (Benveniste et al., 2010). Gata3 transcripts were also most highly expressed in fractions of human cord blood cells comprising 5–10% of cells able to stably reconstitute immunodeficient mice. In Gata3-knockin mice expressing eGFP from the endogenous Gata3 promoter, all LT-HSC were located in eGFP+ cells, whereas all IT-HSC were isolated in nonfluorescent fractions, confirming directly the transcriptional activity of the Gata3 locus in LT- but not in IT-HSC. Gata3 protein was cytoplasmic in freshly isolated LT-HSC in the quiescent state, but translocated to the nucleus when he induced the cells to cycle actively in culture. In mice engineered to delete Gata3 conditionally after induction of Cre recombinase, deletion in the steady state had no significant early or late effects on hematopoiesis, as expected, because Gata3 should remain cytoplasmic in quiescent HSC. In marked contrast he could show, when deleted cells from such mice were injected into irradiated recipients they strongly outcompeted control cells expressing Gata3, and this advantage expanded when marrow cells from these primary recipients were used to reconstitute secondary recipients. The HSC autonomy of the regenerative advantage was confirmed in experiments in which Gata3 deletion was induced after injection of conditionally deletable cells together with wild-type cells into wild-type hosts prior to induction of Cre recombinase followed by a test for competitive advantage in secondary hosts. These experiments establish Gata3 acting to constrain the extent of self-renewal/expansion in dividing LT-HSC.

The presentation from Denis Corbeil (Biotec, Technische Universität Dresden, Germany) was focused on the role of prominin-1, a cholesterol-binding protein (alias CD133), that is widely used as a stem and cancer stem cell marker. He and others previously showed that in both stem cell types, prominin-1 is either symmetrically/asymmetrically distributed during cell division (Fonseca et al., 2008; Giebel et al., 2004) or released into the extracellular milieu in association with small membrane vesicles during the process of cellular differentiation. However, molecular mechanisms underlying these phenomena seem to differ according to the origin of the stem/progenitor cells. In neuroepithelial cells, the segregation of the prominin-1-containing apical plasma membrane that is determined by the orientation of the cleavage plane appears to be the essential cause of its symmetrically/
asymmetrically distribution in the nascent daughter cells (Kosodo et al., 2004), whereas in dividing hematopoietic cells the endosomal compartment seems to play a major role in such prominin-1 distribution (Fonseca et al., 2008). Likewise, the release of prominin-1-containing membrane vesicles involves a budding mechanism from plasma membrane protrusions in neural progenitors (Marzesco et al., 2005) and an exosome-mediated one in hematopoietic cells (Bauer et al., 2011). Dr. Corbeil suggests that, nevertheless, in both stem/progenitor cell types, protein–lipid assemblies might be the structural determinant in prominin-1’s releasing process. The presented data support the concept that prominin-1-containing lipid rafts may host key determinants necessary to maintain stem cell properties and their quantitative reduction (or loss) may result in cellular differentiation.

Adult stem cells modulate their output by varying between symmetric and asymmetric divisions, but have rarely been observed in living, intact tissues. Germline stem cells (GSCs) in the Drosophila testis are anchored to somatic hub cells and were thought to exclusively undergo oriented asymmetric divisions, producing one stem cell that remains hub-anchored, and one daughter cell displaced out of the stem cell-maintaining microenvironment (niches). Erika Matunis (Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, USA) developed extended live imaging of the Drosophila testis niche and found that the mechanism by which new wild type GSCs are incorporated into the niche during steady-state tissue is a previously undetected event she terms ‘symmetric renewal’, where interconnected GSC-daughter cell pairs swivel such that both cells contact the hub. Together with her team she also captured GSCs undergoing the opposite event (symmetric differentiation) by detaching from the niche. They conclude that symmetric renewals are the mechanism by which GSC numbers are restored following starvation-induced GSC loss. Furthermore, upon more severe (genetically-induced) GSC loss, both symmetric renewal and dedifferentiation (where interconnected spermatogonia fragment into pairs while moving toward then establishing contact with the hub) replenish the GSC pool. Thus, stereotypically oriented stem cell divisions are not always correlated with an asymmetric outcome in cell fate, and changes in stem cell output are governed by altered signals in response to tissue requirements (de Cuevas and Matunis, 2011).

Research with hiPSC is often propagated because it respects the moral objections against hESC-research based on certain concepts of embryo rights. Ludwig Siep (Philosophical Institute, University Münster, Germany) pointed out that the avoidance of ethical controversies may, however, deprive society of common experiences and “moral learning.” In addition, hiPSC research is open to ethical questions as well. Because their source is the donation of cells, questions of informed consent (especially from vulnerable patients) and the sharing of possible profits from derivatives arise. The second group of questions relates to the risks of therapies, especially regarding the “memory” of reprogrammed cells. A special set of problems concerns the possibility of deriving gametes from pluripotent cells and their possible use (or abuse) in new ways of human reproduction. Another controversy regards the replacement of animal testing by the use of hiPSC-derived tissue. Finally, the legal concept of “totipotency” is questioned in view of the new possibilities of changing the genetic potential of cells. The main ethical criteria dealing with these questions are the autonomy and benefits of patients, parents, and future children. A shift from genetic potential to the justification of actions in view of a gradual development with increasing and irreversible rights may help to meet them.

Continuing the ethical discussion, Douglas Sipp (Riken Kobe Institute, Center for Dev. Biology, Kobe, Japan) focused on the efforts in the formulation and enforcement of appropriate regulations, combined with better communication with prospective patients, and the necessity to protect the future of legitimate stem cell research from pseudomedical businesses. The unregulated commercialization of stem cell products and applications that have not been tested for safety and efficacy is a growing problem worldwide. Hundreds of companies, some in leading research nations, advertise treatments for a wide range of serious medical conditions online, luring patients to travel overseas to receive injections of stem cells of no demonstrated efficacy. This phenomenon has triggered responses on the part of legislators, regulators, patient groups, and the scientific and medical communities, but medical law and custom in some jurisdictions have made enforcement problematic, and this combination of regulatory uncertainty and economic incentives has emboldened some companies that previously outsourced clinical practice to increase their domestic activities. Additionally, a number of unregulated companies now portray stem cells not as a purely scientific approach to medicine, but rather as a naturalistic form of unorthodox or “alternative” healthcare.

The talk from Juan Carlos Izpisúa Belmonte (Salk Institute for Biological Studies, La Jolla, CA) presented an IPS-based model to study the pathogenesis of human premature and physiological vascular aging. For this aim he generated induced pluripotent stem cells (iPSCs) from fibroblasts obtained from patients with Hutchinson–Gilford progeria syndrome (HGPS), a rare and fatal human premature aging disease (Burner and Kennedy 2010; Davies et al., 2009; Kudlow et al., 2007; Merideth et al., 2008; Worman et al., 2010), characterized by premature arteriosclerosis and degeneration of vascular smooth muscle cells (SMCs) (Olive et al., 2010; Ragnauth et al., 2010; Varga et al., 2006). The HGPS disease is caused by a single-point mutation in the lamin A (LMNA) gene, resulting in the generation of progerin, a truncated splicing mutant of lamin A. Accumulation of progerin leads to various aging-associated nuclear defects including disorganization of nuclear lamina and loss of heterochromatin (Dechat et al., 2008; Scaffidi and Misteli 2005, 2006). HGPS–iPSCs show absence of progerin, and more importantly, lack the nuclear envelope and epigenetic alterations normally associated with premature aging. Upon differentiation of HGPS–iPSCs, progerin and its aging-associated phenotypic consequences are restored. Specifically, directed differentiation of HGPS–iPSCs to SMCs leads to the appearance of premature senescence phenotypes associated with vascular aging. Within his studies he was able to identify a DNA-dependent protein kinase catalytic subunit (DNAPKcs, also known as PRKDC) as a downstream target of progerin. The absence of nuclear DNA PK holoenzyme correlates with premature as well as physiological aging.

Shoukhrat Mitalipov (Oregon National Primate Research Center, La Jolla, CA) presented his initial efforts on reprogramming somatic cells to the pluripotency by somatic cell
nuclear transfer (SCNT). Much of the progress in deriving and characterization of human embryonic stem cells (ESCs) can be attributed to the prior pioneering studies made in a nonhuman primate model (Thomson et al., 1995, 1996). He demonstrated that experimental pluripotent stem cells can be efficiently derived by SCNT-based reprogramming using adult and aged rhesus monkey somatic cell nuclei (Byrne et al., 2007). He could further improve the efficacy of this technique and demonstrated that only 10 or less eggs would be required to produce one ESC line by SCNT (Sparrman et al., 2009). Moreover, their latest unpublished studies suggest that SCNT delivers more complete transcriptional, epigenetic, and developmental reprogramming of aged somatic cells compared to the iPS approach. These remarkable advances have the potential for development of human autologous ESCs and cures for many human diseases.

Erika Sasaki (Central Institute for Experimental Animals, Department of Applied Developmental Biology, Kawasaki, Japan) talked about the prospect for the future of use of transgenic nonhuman primates in biomedical science. Their group is attempting to establish a preclinical study system for regenerative medicine using the common marmoset (Callithrix jacchus). Because the genetic and physiological differences between primates and mice including their physiologic functions hamper the extrapolation of results from mouse disease models to direct clinical applications, the development of nonhuman primate models that mimic various human systems would accelerate the advance of biomedical research (reviewed in Horn et al., 2006). In particular, genetically modified primates would be a powerful human disease model for preclinical studies for developing new therapies or drugs. The common marmoset is a small nonhuman primate that has attracted considerable attention as a potentially useful biomedical research animal in fields such as neuroscience and regenerative medicine. Recently, Sasaki’s group has produced transgenic marmosets using a lentiviral vector system. Applying this technique, they have produced human disease models. The transgenic marmosets expressed the transgene in neonatal tissues, and showed germ-line transmission of the transgene. Further, to enable advanced magnetic resonance (MR) neuroimaging analysis techniques, they have developed a tissue-segmented, population-averaged standard template of the common marmoset brain. The human disease transgenic models were validated by the population-averaged standard template (Sasaki et al., 2009).

Sasaki’s group also has established marmoset embryonic stem (ES) cell lines (Muller et al., 2009; Sasaki et al., 2005) and induced pluripotent stem (iPS) cell lines (Tomioaka et al., 2010). Especially, marmoset iPS cells were established by SCNT-based reprogramming using adult and aged rhesus monkey somatic cell nuclei (Byrne et al., 2007). They have developed a tissue-segmented, population-averaged standard template of the common marmoset brain. The axolotl is able to regenerate the spinal cord after injury. In recent years, Elly Tanaka’s group (Max Planck Institute of Molecular Cell Biology, Dresden, Germany) has shown that this occurs by reverting the resident stem cells to a more epithelial state that is found during development of the embryonic spinal cord. These injured cells then organize into a neuroepithelial tube that replays embryonic development to regenerate the missing portion of the spinal cord. To understand how to engineer such a regenerative neuroepithelial tube from mammalian stem cells the group subjected different stem cell populations to three-dimensional epithelial cyst cell cultures. They found that mouse and human ES cells placed in epithelial cyst culture conditions coupled with neural induction efficiently formed neuroepithelial cysts. Interestingly, the “default” identity of the human ES cell-derived neuroepithelial cysts was the eyefield. Further differentiation of these eyefield cysts under previously described conditions for retinal pigment epithelium resulted in essentially conversion of all cells into a uniform layer of retinal pigment epithelial cells within 3 weeks. These results show the power of using three-dimensional cultures to induce the structure and function of the neuroepithelium.

Concluding, the 6th International Meeting of the Stem Cell Network North Rhine Westphalia in Essen was very well organized and one of the largest meetings in the history of this congress series (Fleischmann and Horn 2009; Wurm and Horn 2008). It gave all participants a great opportunity to obtain up-to-date information on a great variety of topics in stem cell research. Everyone had the opportunity to discuss important issues and find new collaborations in a comfortable and nice atmosphere.

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References


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