Pluripotent and Somatic Stem Cells: From Basic Science to Utilization in Disease Modeling and Therapeutic Application

Meeting Report on the 7th International Meeting of the Stem Cell Network North Rhine Westphalia

Stefan Radtke and Peter A. Horn

IN APRIL OF 2013, the 7th International Meeting of the Stem Cell Network North Rhine Westphalia (NRW), organized by the Stem Cell Network NRW and the University of Cologne as a local host (see www.kongress.stammzellen.nrw.de), took place in the beautiful city of Cologne, Germany. This year's scientific program especially highlighted the outstanding breakthrough and well-deserved Nobel Prize in Physiology or Medicine for Shinya Yamanaka and Sir John B. Gurdon for their "discovery that mature cells can be reprogrammed to become pluripotent" (Gurdon et al., 1975; Takahashi and Yamanaka, 2006; Takahashi et al., 2007). Numerous researchers and speakers from various scientific fields presented their data on induced pluripotent stem cells (iPSCs) for basic research, regenerative medicine, disease modeling, and drug discovery. More than 700 participants presenting 180 posters were attracted by the scientific program, which provided an excellent and interdisciplinary platform for discussion and collaborations. This meeting report summarizes some of the most interesting presentations on various issues of stem cell research.

Numerous reports on the use of human pluripotent stem cells (hPSCs) and their functional derivatives in basic research and disease modeling and their applications for drug safety assessment have begun to appear in the scientific literature. These encouraging reports fuel further development of improved human cellular models. These models may increase the clinical relevance and reduce the need of experimental animals in preclinical drug discovery.

Petter Gunnar Björquist (Cellestic Stem Cells, Göteborg, Sweden) provided an overview on the emerging field of hPSC-based models for drug discovery and development. He focused on cardiac and hepatic cell models and how these approaches may improve current applications. Although there is still need for research to use hPSC-based models as routine or gold standard tools in the industry, he highlighted currently available opportunities using tissue preparations generated from hPSCs for the creation of complex *in vitro* models. These models aim to simulate the systemic drug response *in vivo*.

In line with this, **Christine L. Mummery** from the Department of Anatomy and Embryology at Leiden University Medical Centre (The Netherlands) presented her data on the generation and characterization of patient-derived iPSCs for

myocardial disease modeling (Bellin et al., 2012). The ability to differentiate iPSCs into functional cardiomyocytes provides an excellent tool for the analysis of complex drug and gene interactions in cardiac diseases. Using genetically manipulated mice with a mutation in the Na⁺ channel (Scn5a, 1798^{insD/+}) that causes a cardiac Na⁺ channel overlap syndrome, she was able to generate murine iPSCs recapitulating the characteristics of primary cardiomyocytes *in vitro* (Davis et al., 2012). Interestingly, cardiomyocytes generated from patient-derived iPSCs with an equivalent mutation (SCN5A, 1795^{insD/+}) showed similar changes in electrophysiological properties in comparison to healthy cardiomyocytes. This finding provides an excellent tool for basic research and drug discovery.

Bruce R. Conklin (Gladstone Institute of Cardiovascular Disease, University of California, San Francisco, CA) presented results showing how cardiomyocytes generated from patient-derived iPSCs can be used for isogenic disease models and genome engineering (Lahti et al., 2012). Life-threatening arrhythmias can be caused by a mutation in the protein BAG3 (BCL-2 associated athanogene 3). This protein is involved in protein synthesis, and degradation occurs under mechanical stress in cardiomyocytes (Villard et al., 2011). To identify new interaction partners and to study underlying signaling pathways causing cardiac disease, Conklin and his colleagues generated patient-derived induced pluripotent BAG3 knockout cell lines. For the knockout, he introduced a fluorescent marker protein into the coding sequence of BAG3 using the TALEN (Transcription Activator-Like Effectors Nucleases) technology (Mak et al., 2013). Whether TALEN genome engineering is also feasible for scarless gene correction of patient-derived iPSCs and the generation of gene-corrected healthy cardiomyocytes for clinical application needs to be analyzed in the future.

A promising alternative to gene correction using the TALEN technology in cardiac disease is the antisense-mediated exon skipping (Cirak et al., 2011; van Deutekom et al., 2007) presented by **Karl-Ludwig Laugwitz** (Cardiology, Technische Universität München, Germany). Truncating mutations in the *TTN* gene encoding Titin are the most common known genetic cause of dilated cardiomyopathy (DCM) (Herman et al., 2012). He previously characterized

the morphological and functional impairments that result from the DCM-causing *TTN* 2-bp insertion mutation (c.43628insAT in exon 26) in a Titin knockin mouse model mimicking the c.43628insAT allele (Gramlich et al., 2009). To investigate whether antisense-mediated exon skipping could rescue functional defects of the truncated Titin protein in both human patient-specific DCM-iPSC-derived cardiomyocytes *in vitro* and DCM knockin heterozygous mouse hearts *in vivo*, he generated iPSCs from patients carrying the c.43628insAT-*TTN* mutation. After antisense-induced skipping of exon 26, previously observed impaired sarcomere assembly and stability of DCM-iPSC-cardiomyocytes under basal and stress conditions were significantly improved. Typical features of DCM in knockin mice were almost abolished. These results suggest that RNA-based skipping of particular exons may be a potential therapeutic approach for DCM caused by truncating *TTN* mutations.

Gene-correction and gene transfer approaches dealing with Fanconi anemia, a severe genetic disease of the blood system characterized by progressive stem cell loss resulting in bone marrow failure (Kitao and Takata, 2011; Soulier, 2011), were presented by **David A. Williams** (Department of Pediatric Oncology, Dana Farber Cancer Institute, Boston, MA). So far, 15 individual genes involved in the DNA damage response have been identified as being responsible for this hematological disorder. A common strategy for patient treatment for those hypersensitive to chemotherapy and radiation is the application of unrelated donor hematopoietic stem cell transplants, increasing the survival from 20% to 60% (Wagner et al., 2007). Obviously, current treatment strategies are sub-optimal, and alternative strategies are necessary.

One possible approach is the genetic correction of autologous stem cells to eliminate the toxicity associated with allogeneic transplantation such as graft-versus-host disease (GvHD). However, gene correction or gene transfer into allogeneic cells is challenging due to significant depletion of hematopoietic stem cells in patients in combination with enhanced cell death and increased chromosomal instability of isolated cells *in vitro*. Furthermore, no suitable large animal model for Fanconi anemia is available for preclinical studies (Trobridge et al., 2012).

A suitable alternative is the gene correction of patient-derived iPSCs and the application of *in vitro*-generated hematopoietic stem or progenitor cells (HSPCs) (Raya et al., 2009). An impressive improvement of the reprogramming efficiency can be achieved under hypoxic conditions (5% O₂), leading to a reduction in double-strand breaks and reduced senescence of *in vitro*-differentiated hematopoietic progenitor cells (Muller et al., 2012).

An efficient and rapid strategy for generating functional neuronal cells for *in vitro* studies and prospective regenerative medicine without generating iPSCs before was presented by **Marius Wernig** (Institut for Stem Cell Biology and Regenerative Medicine, Stanford University, CA) (Vierbuchen and Wernig, 2012). Wernig and colleagues introduced three transcription factors, namely *Ascl1* (activating signal integrator 1), *Brn2* (also called *Pou3f2*; POU class 3 homeobox 2), and *Myt1l* (myelin transcription factor 1-like)—also termed BAM factors, into fibroblasts for direct reprogramming into neuronal-like cells (Vierbuchen et al., 2010). Conversion of fibroblasts took place within 8–12 days. The reprogrammed cells exhibited functional membrane channel proteins, were able to perform action potentials, and formed functional presynapses

expressing Synapsin. Still less is known about the underlying mechanisms during direct reprogramming leading to conversion of mature fibroblasts into neuronal like cells.

Focusing on the epigenetics of embryonic stem cells (ESCs), **Alexander Meissner** (Department of Cell and Developmental Biology, Penn Institute for Regenerative Medicine, Philadelphia, PA) presented his results on DNA methylation dynamics in early development and stem cell differentiation (Zhu et al., 2013). Meissner and colleagues could observe two specific DNA methylation mechanisms that take place during the differentiation of human ESCs. Regions that are highly methylated in the undifferentiated cells are decreasing their DNA methylation, gaining either H3K4me1 or H3K27me3 (Gifford et al., 2013). Most of these dynamics are not associated with changes in gene expression in the early populations, but many genes are activated in downstream cell types or found to be active in adult tissues. Changes from high DNA methylation to K27me3 may be required to move these cells from a repressed to a silent, but more accessible state, such that lineage-specific factors can bind their target genes.

Recent protocols for human ESC and (i)PSC culture commonly include the addition of bovine serum and/or the presence of murine feeder cells. These additives limit the clinical application because of xenograft ingredients. Therefore, stem cell researchers are ambitious to develop generic culture techniques suitable for clinical use and use under clinical-grade good manufacturing practice (GMP) standards. A promising approach for keeping pluripotent stem cells in suspension culture without feeder cells was presented by **Joseph Itskovitz-Eldor** (Department of Obstetrics and Gynecology, Rambam Medical Center, Haifa, Israel) (Amit et al., 2010; Amit et al., 2011). Single-cell suspension cultures showed an increased expansion rate in spinner flasks and culture bags that are necessary for the production and application of huge cell numbers, *i.e.*, for organ transplantation. Pluripotent cells cultured in suspension culture retained classical features of PSCs—expression of stemness-associated markers, a stable karyotype, ability to form embryoid bodies, and differentiation into all three germ layers as well as teratoma formation in mice. Not surprisingly pluripotent cells in suspension showed increased expression of cell–cell contact and downregulated levels of cell matrix-associated genes.

Focusing on the safety and carcinogenicity of iPSCs, **Peter Andrews** (Center for Stem Cell Biology and Dept. of Biomedical Science, University of Sheffield, UK) pointed out in his talk that any of the pluripotent stem cells, either ESCs or iPSCs, have three possible fates—self-renewal, differentiation, or death. Because both differentiation and death result in the loss of stem cells, any population of stem cells will be under continued selection for any variant cells that exhibit an increased probability of self-renewal over death or differentiation. Indeed, in the absence of any other regulatory system, a stem cell population would be expected to evolve over time to a nullipotent state in which the stem cells have lost their capacity for differentiation. Although nullipotent ESCs have not been reported yet, their malignant counterpart stem cells from teratocarcinomas, nullipotent embryonal carcinoma (EC) cells are well known (Knoepfler, 2009). In culture, human ESCs acquire analogous genetic changes similar to EC cells over time (Hyka-Nouspikel et al., 2012). Altered expression of an antiapoptotic gene, *BCL2L1*, has been recently identified as a possible candidate that drives a selective advantage of variant cells (Amps et al., 2011). Moreover, changed patterns of

differentiation have been detected in variant human ESCs that might be related to altered dynamics of lineage-primed sub-states within the undifferentiated stem cell compartment.

In summary, the 7th International Meeting of the Stem Cell Network North Rhine Westphalia in Cologne provided an excellent opportunity for researchers and students to listen to outstanding international speakers who presented important updates on the newest developments in stem cell research. As in previous years (Fleischmann and Horn, 2009; Radtke and Horn, 2011; Wurm and Horn, 2008), all participants enjoyed the well-organized meeting and the relaxed atmosphere for inspiring conversations with fellow researchers, collaboration partners, and the industry.

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Address correspondence to:
Prof. Dr. Peter A. Horn
Institute for Transfusion Medicine
University Hospital Essen
Virchowstr. 179
45147 Essen, Germany

E-mail: Peter.Horn@uk-essen.de; Stefan.Radtke@uk-essen.de