MEETING REVIEW

From stem cells to human development: a distinctly human perspective on early embryology, cellular differentiation and translational research

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ABSTRACT

Over 100 scientists with common interests in human development, disease and regeneration gathered in late September 2016 for The Company of Biologists’ second ‘From Stem Cells to Human Development’ meeting held in historic Southbridge. In this Meeting Review, we highlight some of the exciting new findings that were presented, and discuss emerging themes and convergences in human development and disease that arose during these discussions.

KEY WORDS: Development, Embryonic stem cell, Human, Organoid, Pluripotency, Translation

Introduction

Inspired by the success of the first meeting two years ago, Development and The Company of Biologists held the second ‘From Stem Cells to Human Development’ meeting in September 2016 in historic Southbridge (Massachusetts, USA). From the first human embryology paper published in Development in 1956 (O’Rahilly et al., 1956), the journal has supported pioneering works of both human development and stem cell biology for over half a century. Olivier Pourquié (Harvard Medical School, Brigham and Women’s Hospital, Boston, MA, USA), Benoit Bruneau (The Gladstone Institute of Cardiovascular Disease, San Francisco, CA, USA), Gordon Keller (University Health Network, Toronto, Canada) and Austin Smith (WT-MRC Cambridge Stem Cell Institute, Cambridge, UK), all of whom are editors on the journal, organized and invited leading scientists and young investigators from around the world (Fig. 1) to discuss our evolving understanding of human development. Executive Editor Katherine Brown (The Company of Biologists, Cambridge, UK) warmly welcomed meeting participants, and dedicated the workshop to the memory of our esteemed colleague Professor Yoshiki Sasai.

The workshop brought together basic developmental biologists, stem cell scientists and tissue engineers, all with a common interest in how cells within the human embryo differentiate into complex tissues and organ systems. Decades of developmental studies in model organisms combined with advances in ex vivo and in vitro cell culture systems have dually inspired and enabled nearly all work that was presented at the conference. Here, we highlight some of the exciting new findings that were presented and discuss the major themes that emerged from the meeting.

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Development in a dish: using in vitro differentiation to
understand developmental processes

Understanding cellular responses to biochemical cues used to
stimulate proliferation, differentiation and maturation can be
challenging in complex tissue systems. Works presented by Eric
Siggia (The Rockefeller University, New York, USA) and Silvia
Santos (MRC-Imperial College, London, UK) have broken down
the complexity of the system to identify changes at the cellular level.
Eric Siggia and Ali Brivanlou developed a method to culture human
PSCs on a membrane filter, which allows for growth factor
stimulation from either the apical or basal side of the cultured cell
layer. Using this model, Siggia discovered that the ability of the cells
to detect spatial boundaries and respond differentially to signaling
molecules is, in part, related to the subcellular localization of the
receptors for activin and BMP, which can be affected by cell
density. In a complementary approach, Santos discovered that
irreversible commitment of human PSCs to the mesoderm lineage
occurs in an unexpectedly short time frame. Her data demonstrated
that cells can respond at the molecular level specifically and
differentially to spatially or temporally distinct signals, and suggest
that in vitro differentiation protocols might be improved if these
differential cellular responses can be harnessed.

Advances in basic developmental biology and refined culture
techniques contribute to the continued success of in vitro-directed
differentiation methods, and three-dimensional (3D) organoid
cultures are increasingly used to study complex tissues of all three
germ layers. The diversity of 3D organoid systems was a topic
introduced broadly and effectively by Todd McDevitt (The
Glade Institute of Cardiovascular Disease, San Francisco, CA,
USA). His work provided insight into modulating the cellular
cytoskeleton and defining the cellular composition of organoids in
order to provide instructive heterotypic interactions. In his thought-
provoking talk, McDevitt suggested that developmental and stem
cell biologists should consider and take advantage of tissue
engineering-related parameters such as fabrication strategies,
chosen source of cells and incorporation of biomaterials in order
to generate successful three-dimensional environments for in vitro
organogenesis.

Endoderm-derived lineages

Both Brigid Hogan (Duke University, Durham, NC, USA) and
Jason Spence (University of Michigan, Ann Arbor, MI, USA) used
organoids derived from primary lung cells as model systems in
which new points in lineage commitment and PSC differentiation
strategies could be discovered. Hogan wanted to better understand
the mechanism by which unpolarized lung basal cells differentiate
into polarized cells, such as the ciliated and secretory cells of the
lung airway epithelium. Building on the role of the transcription
factor grainyhead-like 2 in the repair of epithelial-like tissues in
Drosophila, Hogan’s group used Cas9-based gene editing and
conditional mutational approaches in primary human and mouse
lung organoids to show that grainyhead-like 2 plays an important
role in the commitment of a basal cell towards the ciliated cell
program (Gao et al., 2015). Spence used primary human lung
organoids (HLOs) derived from fetal tissue to delineate the signals
responsible for maintenance of human distal epithelial progenitors
in vitro (Dye et al., 2016). Ex vivo primary fetal lung bud cultures allowed Spence to identify several signaling pathways that promoted long-term in vitro growth of distal epithelial progenitors. His approach also led to the observation that there are significant differences between human and mouse in the expression patterns of SOX-family transcription factors. This information facilitated the generation of HLOs from human PSCs that more closely reflect in vivo structures, including the generation of well-defined proximal and distal lung domains with budding structures.

Anne Grapin-Botton (DanStem, University of Copenhagen, Denmark) spoke about her work, which aims to better understand the self-organization process of a pancreas organoid system by decoding the required cellular composition parameters. In this case, a 3D culture system was implemented in an effort to maintain the expression of PDX1, a marker of pancreatic progenitor fate, in the flat cells of sub-confluent 2D cultures. Notch signaling was necessary for organoid formation from mouse pancreatic progenitors and, interestingly, through cell-dosing experiments she discovered that at least one cell expressing the Notch-induced transcriptional repressor Hes1 (hairy and enhancer of split 1) and one cell that lacks Hes1 expression were required to successfully form an organoid. Human PSC-derived pancreatic progenitors maintained PDX1 expression in this organoid culture system, yet could be induced to generate acinar and endocrine cells. Although morphogenesis of human PSC-derived pancreatic organoids is not yet as complex and organized as those derived from primary mouse cells, this model will prove very useful for studies of pancreas development.

Takanori Takebe (Yokohama City University, Japan and Cincinnati Children’s Hospital, OH, USA) discussed his group’s approach to organoid formation, which has evolved from using relatively homogeneous aggregates towards increased heterogeneity and complexity. With human liver development as a model, Takebe generated the multiple cell types found in the developing human liver, such as hepatic progenitors, endothelial cells and mesenchymal cells, and combined them in vitro to generate a condensed and more mature organoid that he showed could then engraft and function in vivo. Molecular analyses demonstrated that hetertotypic mixing can activate new signaling pathways that are not observed in monolayer culture. One might venture forward and take this approach to model interactions between organs in the body, such as the multi-organ bud systems in the developing gut tube.

**Cardiac and musculoskeletal development**

During the directed differentiation of hPSCs into cardiomyocytes, various cell types of the heart are generated. Using a well-established differentiation approach, Gordon Keller and colleagues reported the emergence of a SIRPa-positive Nkx2.5-negative population resembling sinoatrial node (SAN) pacemaker cells. The ability to monitor SAN pacemaker cells by flow cytometry allowed them to optimize their differentiation protocol and enrich for these cells by providing specific signals (bone morphogenetic protein and retinoic acid, and fibroblast growth factor inhibition) that the cells would normally experience in the developing heart. Functionally, they demonstrated that purified SAN cells can ‘pace’ a rat heart ex vivo. The ability to specify, enrich for and isolate individual cellular components of the heart will enable investigators to generate clinically relevant engineered heart tissues, i.e. in the form of a cell-based biological pacemaker that, in the future, could replace electronic pacemaker devices. In keeping with the cardiac theme, Laurie Boyer (Massachusetts Institute of Technology, Cambridge, MA, USA) presented her group’s work in determining the secondary structure of a long non-coding (lnc) RNA called Braveheart (Bvht) that is necessary for cardiac differentiation of mouse ESCs (Xue et al., 2016). Targeted disruption of a small G-rich internal loop (AGIL) motif of this lncRNA impaired cardiomyocyte differentiation and interaction with cellular nucleic acid-binding protein (CNBP, also known as ZNF9), a zinc-finger protein whose deletion partially rescued the Bvht mutant phenotype. These data open the door for exploring broader roles of RNA structures and lncRNAs in development and disease.

The musculoskeletal lineages have also been the focus of directed differentiation efforts by Olivier Pourqué and April Craft (Harvard Medical School, Boston Children’s Hospital, MA, USA). Through the conserved mechanisms of somite development between mouse and human, Pourqué’s group has successfully generated skeletal myocytes from human PSCs that retain the ability to generate regenerative satellite cells in vitro and in vivo (Chal et al., 2016, 2015). Interestingly, paraxial mesoderm-staged cell aggregates develop into epithelial rosettes that are similar in structure and marker expression to somites. When transplanted into the chick embryo – by Pourqué himself – these ‘somitoids’ integrated and contributed to myotome structures. April Craft has identified many of the developmental signals that effectively direct progenitor cells towards the generation of two functionally distinct cartilage tissues. Whereas the growth plate-like cartilage tissue initiates new bone formation in vivo, similar to the way in which long bones form during embryonic development, the articular cartilage cells derived from human PSCs generate and maintain stable cartilage in mice (Craft et al., 2015). Moving towards clinical applications, Craft demonstrated the ability of articular cartilage derived from human PSCs to repair damaged cartilage in the rat knee and in large animal models. Both human PSC-derived skeletal muscle satellite cells and articular cartilage are ideal candidates for cell therapy because they have the profound potential to improve the quality of life for those who suffer from musculoskeletal diseases.

**Neural development and disease**

Nervous system development, and particularly that of the cerebral cortex, is an especially compelling application for human PSC-derived culture systems. The human forebrain is more complex in its neuronal diversity, circuitry and structure compared with that of the mouse, and is also generated by a greater diversity of progenitor cells; 3D organoid culture systems may be the most versatile yet for modeling human brain development. This was highlighted by Paola Arlotta (Harvard University, Boston, MA, USA) who demonstrated the ability to generate multiple neuronal lineages from human PSCs in a self-assembling, self-directed organoid culture system. Directed differentiation protocols continue to be refined for modeling diseases with cell type-specific deficits. Elena Cattaneo (University of Milan, Italy) presented her work modeling Huntington’s disease using a directed differentiation protocol that produces all of the major striatal cell types. Applying this protocol to induced pluripotent stem cells (iPSCs) from individuals with Huntington’s disease, she showed that iPSCs harboring CAG repeats in the disease range strongly affected aspects of neuronal differentiation. Lorenz Studer (Memorial Sloan Kettering Cancer Center, New York, USA) presented a compelling analysis comparing iPSCs from individuals with mild or severe familial dystautonomia. Although the iPSC lines could generate many lineages, peripheral neurons could only be efficiently generated from iPSCs from individuals with mild but not severe dystautonomia. By contrast, peripheral neurons from both severe and mild dystautonomia did not survive efficiently post-differentiation. Strikingly, despite harboring...
the exact same mutation, individuals differ in showing neuro-developmental versus neurodegenerative phenotypes. Whole-exome sequencing identified promising candidate ‘second hit’ mutations that might underlie the disease heterogeneity.

Microcephaly is a compelling condition to model in human cerebral organoids, given that several mouse models fail to recapitulate the dramatic reduction in brain size that is seen in humans with this disease (Pulvers et al., 2010). Pierre Vanderhaeghen (Université Libre de Bruxelles, Brussels, Belgium) presented his group’s work using cell lines from five individuals with microcephaly with mutations in the abnormal spindle microtubule assembly (ASPM) gene, which are the most common genetic cause of microcephaly. Using a 3D model that mimics the earliest stages of corticogenesis, he showed that cortical-like neuroepithelial progenitors derived from these lines differentiate prematurely. Disruption of the initial expansion of these progenitors could constitute a specific pathogenic mechanism for the human microcephaly by reducing the tangential expansion of the early developing cortex, which is particularly prominent in primate species.

Finally, Matthew Johnson (Harvard Medical School, Boston Children’s Hospital, MA, USA) presented transcriptomic studies of primary human, ferret and mouse neural progenitor cells, providing molecular insights into species differences in the duration and output of forebrain neurogenesis. Using single-cell data to reconstruct the transcriptional dynamics of cortical progenitors, he showed that significantly more individual human cells displayed concurrent expression of proliferative and differentiating factors. Interestingly, one of the genes frequently expressed in these transitioning cells, MLLT3, was also discussed by Vincenzo Calvanese (The Mikkola Lab, UCLA, Los Angeles, CA, USA) as promoting self-renewal of a subset of hematopoietic stem cells (see below).

Basic biology leads to translation
Bone marrow transplants are the most widely used stem cell therapy in the clinic to this day. Yet limitations, such as donor availability and the delayed production of lymphocytes needed to protect against infection following transplantation, motivate researchers to increase our understanding of the hematopoietic stem cell (HSC) and its niche, in order to improve the treatments available to patients. Yann Barrandon (Swiss Federal Institute of Technology, Lausanne, Switzerland) discussed the development of the human thymic epithelium, a tissue that is crucial for proper maturation of T cells. The visualization of extraordinarily long cytoplasmic processes of a subpopulation of thymic epithelial cells suggested that these structures function to provide interactive niches for T cell maturation. Vincenzo Calvanese discussed factors required for the self-renewal of fetal liver HSCs. The transcriptional elongation factor MLLT3 was found to be highly enriched in self-renewing HSCs, and could be used to continuously expand these therapeutic progenitors in culture without inducing a transforming phenotype. Guy Sauvageau (University of Montreal, Canada) discussed two ways that his lab has approached the therapeutically crucial self-renewal property of HSCs. Sauvageau’s group observed that the 3- to 6-month lifespan of mice lacking Bmi1, a member of the polycomb group proteins, correlated with that of sub-lethally irradiated wild-type mice that had received an injection of ‘intermediate-term’ HSCs (IT-HSCs) (Benveniste et al., 2010). This observation suggested that Bmi1 may be essential for the maintenance of long term HSCs (Bordeleau et al., 2014; Grote et al., 2015). Concurrently, his translational work has identified and optimized a small molecule that supports the expansion of cryopreserved HSCs in vitro (Fares et al., 2014, 2015). A multifaceted approach combining efforts of Sauvageau’s basic biology group, the engineering group of Peter Zandstra (University of Toronto, Canada) and federal support has led to the translation of this therapy into patients this year.

Like Sauvageau, Sally Temple (Neural Stem Cell Institute, Rensselaer, NY, USA) and Lorenz Studer provided inspirational examples of how studies in basic developmental biology and stem cell differentiation can lead to new regenerative medicine treatments. Age-related macular degeneration (AMD), a vision-related condition that steals independence from a significant number of aging individuals, is initiated by a loss of retinal pigmented epithelial (RPE) cells. Sally Temple’s recent work has focused on a small subset of normally dormant adult human RPE stem cells, which she found had the capacity to proliferate in vitro in response to growth factors and could restore the ability to preserve photoreceptors in a rat retinal degeneration model (Davis et al., 2016). Through the development of large-scale production processes to eventually treat individuals with AMD, Temple identified additional factors that maintain the potential of the cells to regenerate the RPE, as well as factors involved in RPE pathogenesis. Studer’s experience in translating methods to produce dopaminergic neurons from human PSCs to treat Parkinson’s disease (Kriks et al., 2011) provided insights into the processes by which safety concerns are addressed. Studies are geared towards a first-in-human clinical trial and include detailed validations of cryopreserved batches of dopaminergic neurons and preclinical in vivo assessments. These points, as well as the challenges associated with funding and staffing translational studies, and maintaining metrics of academic success such as publications, were highlighted in a lively panel discussion about translation of developmental and stem cell biology to the clinic.

Conclusions and emerging themes
In recent decades, major advances in our understanding of pre-implantation development and lineage commitment have been gained from using mice and other model organisms. However, many of the talks at this meeting highlighted a perhaps formerly underappreciated level of species specificity, particularly in naïve cells, PGCs and forebrain development. Furthermore, it is often the case that human developmental disorders do not have direct correlates in rodents, and thus modeling the molecular mechanisms of such diseases in human cells is undeniably of great value. Given the recent technological advances in gene editing, single cell ‘omics’ approaches and our ever-improving in vitro culture systems, it would seem that now, more than ever before, we are finally able to directly address questions in human developmental biology, and that by doing so we are gaining insight into some distinctly human traits.

In addition to highlighting some unique aspects of human biology, the meeting also showcased how successful research programs in developmental biology can lead to encouraging translational results when researchers are inspired by both basic and clinical problems. Chemically defined culture conditions have opened the door to novel ways to model developmental processes, and we can now choose whether ‘to direct’ or ‘not to direct’ cell differentiation. In vitro culture systems have evolved to support progenitor cells that self-organize, such that their endogenous lineage potential can be observed. Remarkably, cells grown in vitro can recapitulate aspects of their bona fide in vivo counterparts, albeit in a sometimes less organized manner. Ultimately, we have learned that exploring the use of these different culture systems, whether 2D or 3D, whether directed or self-organizing, will translate to
improved protocols for generating specific cell types, and to a better understanding of specific human diseases. Knowing how to control the differentiation of stem cells is fundamentally a question of developmental biology, and one that is crucially important for the generation of cells, tissues and organs that can be used for regenerative medicine purposes. As such, developmental biology has been and will continue to be crucial for the generation of new cell therapies, and with the ability to investigate human developmental biology specifically, it is clear that many exciting discoveries await the field in the years to come.

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Competing interests

The authors declare no competing or financial interests.

References


