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MEETING REVIEW

On human development: lessons from stem cell systems

Alexander Medvinsky^{1,*} and Frederick J. Livesey²

ABSTRACT

In September 2014, over 100 scientists from around the globe gathered at Wotton House near London for the Company of Biologists' workshop 'From Stem Cells to Human Development'. The workshop covered diverse aspects of human development, from the earliest stages of embryogenesis to differentiation of mature cell types of all three germ layers from pluripotent cells. In this Meeting Review, we summarise some of the exciting data presented at the workshop and draw together the main themes that emerged.

KEY WORDS: Directed differentiation, Embryogenesis, Embryonic stem cells, Human development, Pluripotent stem cells

Introduction

For four days in late September 2014, the beautiful venue of Wotton House, Dorking, UK, played host to the Company of Biologists' workshop 'From Stem Cells to Human Development'. For this exciting workshop, the organisers Olivier Pourquié, Benoit Bruneau and Austin Smith (all editors of this journal) brought together leading scientists from around the world with a common interest in understanding human development using stem cell systems. The objective of the meeting was to explore how the early human embryo is organised, how diverse cell lineages emerge and mature to form organs and tissues, and how we can carry out mechanistic studies of these processes using stem cell systems. The workshop also considered what happens when these developmental processes go wrong in disease states and how they can be corrected. Ethical questions posed by work with human stem cells and their derivatives were an important theme of the meeting, detailed elsewhere in this issue (Hermerén, 2015; Hyun, 2015). Tributes were paid to a pioneer in field, Yoshiki Sasai, who was scheduled to speak at the meeting, and whose recent loss has been deeply felt by his friends and colleagues.

The programme of the meeting was carefully crafted to explore how understanding embryonic development and pluripotent stem cell (PSC) biology have mutually benefited each other. Many presentations demonstrated a remarkable convergence of these two research topics in recent years, stimulated both by fundamental biological and applied, translational research questions. In this Meeting Review, we describe exciting findings presented by the speakers and summarise some of the main themes that emerged from the various presentations. We apologise that due to space limitations not all talks are mentioned directly.

Stem cells: pluripotent states and preimplantation human development

The meeting started with discussions of the earliest stages of human and mouse preimplantation development, and their relationship

with different types of embryonic stem cells (ESCs). Pluripotency is a key property of ESCs. However, it is becoming increasingly clear that pluripotency is not a single state: stem cells persist in the inner cell mass from the preimplantation stage through to gastrulation, which lasts for 3 days in mouse and much longer in human. Naïve stem cells change their properties and become primed for differentiation when they are closer to exit from pluripotency. Human ESCs derived from blastocysts are closer to primed than naïve mouse ESCs and it is important to understand why this is. To address this issue, Austin Smith (Cambridge Stem Cell Institute, UK) and colleagues expressed NANOG and KLF2, two factors that define naïve status in mouse ESCs, in human ESCs, and treated them with a PKC inhibitor (known to block mouse ESC differentiation). These cells showed stable naïve-like status (Takashima et al., 2014). Such 'reset' pluripotent cell lines maintain normal karyotype and can make teratomas; metabolically, transcriptionally and by genome-wide hypomethylation, they closely resemble mouse naïve ESCs. In agreement with these findings, Jennifer Nichols (Cambridge Stem Cell Institute, UK) described human ESCs as slowly growing, poorly clonogenic cells, which express markers of postimplantation embryos. She emphasised how our understanding of the human naïve pluripotent state can be informed by mouse studies: given that in the human embryo the early segregation of naïve epiblast and primitive endoderm occurs in a similar fashion as in the mouse, why is it so difficult to derive naïve cells from human?

Susan Fisher (University of California San Francisco, USA) described considerable morphological diversity of blastomeres from very early stages of human development and successful derivation of human ESCs from individual blastomeres. Blastomeres of a single eight-cell embryo yielded karyotypically normal, highly hypomethylated pluripotent cell lines. Bioinformatics analysis showed that these cell lines clustered away from conventional human ESCs and differed from each other, even if derived from the same embryo. The capacity of these cells to make teratomas and to differentiate to trophoblast cells suggests that these cells are totipotent.

The importance of intercellular signalling in blastocyst development and its relevance for ES cell derivation was also a theme of Janet Rossant's (Hospital for Sick Children, Toronto, Canada) presentation: using live imaging of fluorescent reporter mice, her lab has studied the timing of commitment to pluripotency versus trophectoderm formation and has shown the importance of components of the Hippo signalling pathway in this process. The timing of lineage commitment in the human blastocyst appears to be later than in the mouse and it is currently unclear whether the same signalling pathways are involved. Joanna Wysocka (Stanford University, USA) also emphasised the need for a greater understanding of the biology of human preimplantation development, given the striking activation of primate- and human-specific endogenous retroviral elements that she and her colleagues are studying at those stages of development.

A powerful approach for mechanistic studies of the earliest steps in human preimplantation development was described by Ali Brivanlou (Rockefeller University, New York, USA) in his

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presentation on self-organisation and patterning of human ESCs in culture. By using micropatterned substrates to standardise ESC colony size and shape, his lab, in collaboration with Eric Siggia (Rockefeller University, New York, USA), has demonstrated highly reproducible spatial patterns of differentiation in response to BMP4 (Warmflash et al., 2014). The process of establishing patterns can be tracked in real time using reporter lines, and gastrulation-like movements could be observed, providing high-content data for computational biology studies.

Haematopoiesis: from mouse to human and back again

A number of presentations addressed different aspects of the challenging problem of studying human haematopoiesis *in vitro*, and how we can learn from work in model systems. Alexander Medvinsky (MRC Centre for Regenerative Medicine, Edinburgh, UK) discussed the development of adult haematopoietic stem cells (HSC), emphasising that differentiation of ESCs can generate various haematopoietic cell types but not HSCs. His lab is studying mechanisms of HSC formation in the mouse embryo and models this process in culture. Analysis of mouse allowed testing main principles of HSC development in human embryos (Ivanovs et al., 2014). In a related talk, Elaine Dzierzak (MRC Centre for Inflammation Research, Edinburgh, UK) provided insights into the organisation of intra-aortic clusters and their role in the production of HSCs. Combining specific reporter mice to visualise HSC formation in a model culture system, with tracking of populations preceding HSC formation, her lab has analysed their gene expression profiles to identify transcription factors potentially involved in early developmental specification of HSCs.

Gordon Keller (McEwen Centre for Regenerative Medicine, Toronto, Canada) argued that, if we want to recapitulate development of different mesodermal derivatives *in vitro* from pluripotent cells, manipulation of duration and concentration of factors is likely to be required. He addressed the difficulties in producing true, clinically relevant HSCs from human ESCs and described his strategy to achieve this goal (Kennedy et al., 2012; Sturgeon et al., 2014). Keller underscored that the haematopoietic lineage is segregated from the mesoderm at very early stages of embryogenesis, and that this must be taken into account for *in vitro* ESC differentiation protocols. His lab has made notable progress in manipulating signalling during ESC differentiation down the HSC route and in identifying cell fractions that give rise to primitive and definitive types of haematopoiesis.

Extending the haematopoiesis theme, Cedric Ghevaert (University of Cambridge, UK) discussed approaches to generate significant amounts of platelets from induced pluripotent stem cells (iPSCs) for transfusion in humans under Good Manufacturing Practice-compatible conditions. Two main issues have to be addressed: efficient production of megakaryocytes (MKs) and efficient platelet release from MKs. He showed that enforced expression in iPSCs of three transcription factors (TAL1, GATA1, FLI1) generates a population of MK precursors that can be maintained in culture for a long period of time (120 days) and that produce mature MKs similar to their primary counterparts. His group is actively working on the problem of platelet release in functionalised three-dimensional (3D) collagen-based scaffolds.

Building brains

One session at the meeting dealt with the challenges of studying human brain development *in vitro*. A common theme from these talks was the need for systems to study human-specific aspects of brain development (particularly cortical development), including

differences in brain size and complexity. Although the mouse remains a powerful model system, it differs in several important aspects from human, in a manner that is clinically important when studying neurodevelopmental disorders.

Arnold Kriegstein (University of California San Francisco, USA) discussed how he and his colleagues have approached the problem of how to carry out mechanistic studies of human brain development by using explant culture to analyse the complexity of human stem and progenitor types in the developing neocortex. Using a labelling strategy, they have thoroughly characterised the proliferation and cell division kinetics of different types of cerebral cortex progenitor cells by live imaging in this *ex vivo* system (Ostrem et al., 2014). Rick Livesey (University of Cambridge, UK) described his lab's use of directed differentiation of human PSCs to replay forebrain neurogenesis for comparative studies of human and non-human primate brain development. Jürgen Knoblich (Institute of Molecular Biotechnology, Vienna, Austria) presented recent advances using organoids/embryoid bodies generated from iPSCs to study brain development in 3D culture (Lancaster et al., 2013). In this system, he and his colleagues have observed the development of multiple brain regions over several months, including the retina, cerebral cortex and ventral forebrain. He reported exciting applications of this system to study the role of spindle orientation in neurogenesis, using genome engineering for targeted knockout of genes of interest.

Muscle and fat cells

In addition to the talks that centred on the haematopoietic and nervous systems, a recurring theme of the meeting was how to exploit developmental principles to replay development for the production of defined cell types. For example, Olivier Pourquié (Harvard Medical School, Boston, USA) discussed his lab's development of a successful strategy for differentiation of mouse and human ESCs into skeletal muscle cells. Using their understanding of muscle development, they recapitulated successive stages differentiation *in vitro* to generate paraxial mesoderm and ultimately contractile skeletal muscle fibres. Remarkably, these cultures also developed satellite cells. Sanjay Sinha (University of Cambridge, UK) focused on vascular smooth muscle cells (SMCs), which originate from three different compartments of the embryo. Using defined chemical culture conditions based on their knowledge of development, he modelled development of the three principal types of SMCs from human iPSCs and ESCs, and used this to model several aspects of the pathogenesis of the developmental disorder Marfan syndrome.

Christian Dani (Institute of Biology Valrose, Nice, France) described two types of adipocytes that co-exist in human: brown adipocytes that spend energy, generate heat and are abundant in babies but remain mainly in deep organs in adults; and white adipocytes that store energy and are associated with obesity. Human iPSCs can generate both types of adipocytes in a lengthy protocol but with low efficiency. Dani's lab has used a variety of approaches to optimise brown and white adipocyte production from iPSCs (Mohsen-Kanson et al., 2014).

Back in an *in vivo* system, Benoit Bruneau's lab (Gladstone Institute of Cardiovascular Disease, San Francisco, USA) has mapped the origin of heart compartments in the developing mouse embryo using complex lineage analyses. A major finding from their work is that there are no common precursors that give rise to left and right ventricles (Devine et al., 2014). They are now applying this work to disease modelling of human cardiac developmental disorders.

Islet cells in health and disease

Given the increasing incidence of diabetes mellitus worldwide, there has been a good deal of attention paid to the challenges of creating human endocrine and exocrine pancreatic tissue *in vitro*. Danwei Huangfu (Memorial Sloan Kettering Cancer Center, New York, USA) introduced a powerful CRISPR/Cas9-based system that enables fast concurrent mutation of several genes (González et al., 2014), a system she is using for studying the functions of multiple genes in islet cell development. Ray Dunn (Institute of Medical Biology, Singapore) has focused on the regulation of the transcription factor PDX1, essential for pancreas development, as a way to understand the mechanisms controlling pancreatic specification. In a human ESC differentiation model, his lab has found that PDX1 might both promote expression of a pancreas-specific genetic program and suppress liver-specific genes, through cooperation with co-factors. Henrik Semb (University of Copenhagen, Denmark) reported on his group's studies of pancreas organogenesis *in vivo* (in mouse) and asked whether tissue architecture governs cell fate decisions. To this end, he has explored the roles of the small GTPases Cdc42, RhoA or Rac1, which are involved in actin polymerisation, cell movement and epithelial cell polarisation. Genetic manipulation of these genes enabled shifts to various pancreatic exocrine and endocrine cell phenotypes: acinar, ductal and endocrine (Kesavan et al., 2014). His lab is delineating the cellular pathways involved, as a means to better control human islet cell production from ESCs and iPSCs.

From 2D to 3D: human development in the round

Although there is a good deal of effort in a number of fields to generate specific, individual cell types from PSCs (as discussed above), a related challenge is harnessing developmental biology to generate complex cell assemblies, tissues and organs. A number of talks, including that of Jürgen Knoblich mentioned above, dealt with the problem of generating 3D human tissues.

Hiro Nakauchi (Stanford University, USA, and University of Tokyo, Japan) focused on the generation of entire organs *in vivo* from iPSCs by blastocyst complementation to generate chimeras. Using Pdx1-null mice, which completely lack a pancreas, he successfully generated a normal adult pancreas by injecting wild-type cells into the mutant blastocyst. Importantly, this was effective between species: mouse ESCs successfully integrated and differentiated in the rat and vice versa, providing proof-of-principle that inter-specific chimeras are feasible (Kobayashi et al., 2010). Interestingly, the size of the chimeric organ corresponded to size of the host species. Thus, if we want to generate human organs we should choose a host animal of appropriate size. Nakauchi went on to describe pioneering work his lab is doing in pig (Matsunari et al., 2013) to create human organs in non-human species. To address the ethical concerns over neural and gamete contribution, his team developed a method of 'targeted organ generation'. By conditional expression of Mixl1 during the first phase of embryonic development, they could direct differentiation of PSC-derived cells selectively toward the endodermal lineage (Kobayashi et al., 2014).

A number of talks approached the problem of organogenesis *ex vivo*. Hans Snoeck (Columbia University, New York, USA) aims to generate airway epithelia and complete airways from human PSCs. He reported a number of studies in which he and his colleagues have generated different parts of the human respiratory system *in vitro* (Huang et al., 2014), including recapitulating branching morphogenesis of alveoli. Clare Blackburn (MRC Centre

for Regenerative Medicine, Edinburgh, UK) described her lab's work on thymus development and regeneration. They found that overexpression of a single transcription factor, Foxn1, in a mouse model is sufficient to increase the regeneration and juvenilisation of the thymus, through expansion of thymic epithelium progenitors (Bredenkamp et al., 2014a). Furthermore, overexpression of Foxn1 in mouse embryonic fibroblasts in culture was sufficient to convert them into thymic epithelium that, when grafted under the kidney capsule in combination with foetal thymus mesenchyme, could build a thymus capable of maintaining lymphoid differentiation in nude mice (Bredenkamp et al., 2014b).

Finally, Jim Wells (Cincinnati Children's Hospital Medical Center, USA) described a series of experiments on the differentiation of human PSCs into 3D organoids of different regions of the gastrointestinal tract (McCracken et al., 2014). Their operating principle is to control early embryonic morphogenesis by manipulating signalling pathways that are known from embryonic development to induce definitive endoderm and subsequently gut tube morphogenesis. Manipulation of extracellular signalling promotes further specialisation into specific organoids of different regions of the gastrointestinal tract. Transplantation of such organoids under the kidney capsule of immunocompromised mice results in the formation of tissues with mature gut architecture, including the various muscle and epithelial layers (Watson et al., 2014). These organoids are now being used for studying a number of gut diseases.

Concluding remarks

The main theme to emerge from this meeting was the striking advances that have been made in using mouse and human stem cell systems to study human development in health and disease. The field has clearly advanced a great deal from the early days of the production of individual cell types. The potential of stem cell systems, combined with genome engineering and the information emerging from the different genome projects, is beginning to be realised. Current applications range from mechanistic studies of human development, to disease modelling and tissue engineering. The participants left the meeting energised about the possibilities for the field and committed to establishing a biannual meeting to share progress and findings in what is a fast-moving area. The meeting closed, as it began, with an acknowledgement of Yoshiki Sasai's legacy, and the meeting was dedicated to his memory.

Competing interests

The authors declare no competing financial interests.

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