© 2017. Published by The Company of Biologists Ltd | Development (2017) 144, 12-16 doi:10.1242/dev.142778

MEETING REVIEW

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

April M. Craft* and Matthew Johnson

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.

Boston Children's Hospital, Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115, USA.

*Author for correspondence (april.craft@childrens.harvard.edu)

A.M.C., 0000-0002-4423-8008; M.J., 0000-0001-6909-5712

Early events in human embryonic development

The pre-implantation embryo is the fundamental embodiment of pluripotency. Developmental biologists have long sought to understand the process by which a single cell, the fertilized egg, can give rise to a complex organism, and decades of research in animals have led us to draw conclusions in the humans that they 'model'. However, as our understanding of these processes improves and experimental techniques are refined, it is now time to ask directly how these processes occur in humans. What does it mean to be naïve, primed, pluripotent or self-renewing? By what criteria can we define these stages? To address these questions, the editors of Development invited investigators at the forefront of human pre-implantation biology and the pluripotent state to discuss their work.

The reactivation of the silenced X chromosome is thought to define one aspect of successful reprogramming or pluripotentency. In pre-implantation mouse embryos, the paternal X chromosome becomes reactivated in cells within the inner cell mass (ICM), yet the lack of X-chromosome inactivation after the pluripotent stage is embryonic lethal. In humans, however, the X chromosome does not become completely silenced during early development, according to Edith Heard (Institut Curie, Paris, France), who showed that cells in the human pre-implantation embryo express the long non-coding RNA (lncRNA) *XIST* from both the paternal and maternal alleles (Okamoto et al., 2011). Fredrik Lanner (Karolinka Institute, Solna, Sweden) independently confirmed this phenomenon when he observed a double dose of X-linked gene expression in female cells in the pre-implantation embryo compared with male cells. The dose was reduced to levels observed in male cells by day 7. Interestingly, this was not the result of X-inactivation but rather reduced expression, or dosage compensation, from both X alleles in human cells – a phenomenon that is not observed in mice.

Building on the culture conditions and transcriptional networks that support the 'resetting' of mouse embryonic stem cells (ESCs) from their gastrulating epiblast-like state to a naïve state, Austin Smith has applied this strategy to generate karyotypically stable naïve-like human pluripotent stem cells (PSCs) from both dissociated human ICM cells (Guo et al., 2016) and existing human ESC lines without the use of transgenes. He discussed the challenges that remain towards achieving a 'ground state' in human: some transcription factors found in the mouse are absent, heterogeneity is observed, responsiveness to LIF is diminished and imprints are eroded. Smith commented that arresting human pre-implantation cells in an artificial *in vitro* self-renewing state is less robust than in mouse, and suggested that the naïve phase of pluripotency may be intrinsically less stable in the human embryo.

Fredrik Lanner spoke about his group's efforts to understand the earliest lineage-commitment stages of pre-implantation development. Lanner presented his work on single-cell RNA sequencing

Development (2017) 144, 12-16 doi:10.1242/dev.142778

Fig. 1 . Participants at The Company of Biologists' 'From Stem Cells to Human Development' meeting, held in Southbridge, MA, USA.

(scRNA-seq) in developing human embryos, which has revealed a relatively homogenous population of cells that co-express both ICM and trophectoderm genes at the morula stage. During the transition into the blastocyst, these two transcriptional programs begin to resolve and become distinct, at the same time as the ICM cells begin to diversify into epiblast and primitive endoderm lineages (Petropoulos et al., 2016). These data suggest that lineage choices in human embryos may occur later than in mice. Combined with Ali Brivanlou's (The Rockefeller University, New York, USA) approach to investigate the post-implantation embryo (Deglincerti et al., 2016), improved molecular labeling and amazing imaging technologies now permit the resolution and visualization of individual lineage-commitment steps during early human development.

One of the earliest lineage-commitment decisions in the embryo is the specification of primordial germ cells (PGCs) during gastrulation. Azim Surani (Wellcome Trust/CRUK Gurdon Institute, Cambridge, UK) discussed how he and his group sought to determine whether PGC-like cells derived from human ESCs (hPGCLCs) are equivalent to authentic human PGCs from the embryo around 2-7 weeks after fertilization. Surani discovered, beginning with the *in vitro* cell model, that *SOX17* was the initiating transcriptional regulator of hPGCs, rather than *PRDM14* and *PRDM1*, as had previously been shown in the mouse. Interestingly, conservation of the human transcriptional hierarchy was observed by Ramiro Alberio (University of Nottingham, UK) in the porcine embryo, which develops as a bilaminar disk similar to human embryos. Studying these early developmental stages *in vitro* using human ESCs and porcine embryos enables us to investigate the dynamic competence of specific cell populations.

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 Gladstone 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 organiods (HLOs) derived from fetal tissue to delineate the signals responsible for maintenance of human distal epithelial progenitors

Development (2017) 144, 12-16 doi:10.1242/dev.142778

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 heterotypic 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 Pourquié and April Craft (Harvard Medical School, Boston Children's Hospital, MA, USA). Through the conserved mechanisms of somite development between mouse and human, Pourquié's group has successfully generated skeletal myocytes from human PSCs that retain the ability to generate regenerative satellite cells *in vitro* and *in vivo* in mice (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 Pourquié 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 dysautonomia. Although the iPSC lines could generate many lineages, peripheral neurons could only be efficiently generated from IPSCs from individuals with mild but not severe dysautonomia. By contrast, peripheral neurons from both severe and mild dysautonomia did not survive efficiently post-differentiation. Strikingly, despite harboring

Development (2017) 144, 12-16 doi:10.1242/dev.142778

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

Development (2017) 144, 12-16 doi:10.1242/dev.142778

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 organ systems 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.

Acknowledgements

The authors thank all of the speakers and workshop participants, and Fredrik Lanner and Sally Temple for kindly reviewing the Meeting Review.

Competing interests

The authors declare no competing or financial interests.

References

Benveniste, P., Frelin, C., Janmohamed, S., Barbara, M., Herrington, R., Hyam, D. and Iscove, N. N. (2010). Intermediate-term hematopoietic stem cells with extended but time-limited reconstitution potential. *Cell Stem Cell* **6**, 48-58.

Bordeleau, M.-E., Aucagne, R., Chagraoui, J., Girard, S., Mayotte, N., Bonneil, E., Thibault, P., Pabst, C., Bergeron, A., Barabe, F. et al. (2014). UBAP2L is a novel BMI1-interacting protein essential for hematopoietic stem cell activity. *Blood* **124**, 2362-2369.

Chal, J., Oginuma, M., Al Tanoury, Z., Gobert, B., Sumara, O., Hick, A., Bousson, F., Zidouni, Y., Mursch, C., Moncuquet, P. et al. (2015). Differentiation of pluripotent stem cells to muscle fiber to model Duchenne muscular dystrophy. *Nat. Biotechnol.* **33**, 962-969.

Chal, J., Al Tanoury, Z., Hestin, M., Gobert, B., Aivio, S., Hick, A., Cherrier, T., Nesmith, A. P., Parker, K. K. and Pourquie, O. (2016). Generation of human muscle fibers and satellite-like cells from human pluripotent stem cells in vitro. *Nat. Protoc.* **11**, 1833-1850.

Craft, A. M., Rockel, J. S., Nartiss, Y., Kandel, R. A., Alman, B. A. and Keller, G. M. (2015). Generation of articular chondrocytes from human pluripotent stem cells. *Nat. Biotechnol.* **33**, 638-645.

Davis, R. J., Blenkinsop, T. A., Campbell, M., Borden, S. M., Charniga, C. J., Lederman, P. L., Frye, A. M., Aguilar, V., Zhao, C., Naimark, M. et al. (2016). Human RPE stem cell-derived rpe preserves photoreceptors in the royal college of surgeons rat: method for quantifying the area of photoreceptor sparing. *J. ocul. Pharmacol. Ther.* **32**, 304-309.

Deglincerti, A., Croft, G. F., Pietila, L. N., Zernicka-Goetz, M., Siggia, E. D. and Brivanlou, A. H. (2016). Self-organization of the in vitro attached human embryo. *Nature* **533**, 251-254.

Dye, B. R., Dedhia, P. H., Miller, A. J., Nagy, M. S., White, E. S., Shea, L. D. and Spence, J. R. (2016). A bioengineered niche promotes *in vivo* engraftment and maturation of pluripotent stem cell derived human lung organoids. *Elife* **5**, e19732.

Fares, I., Chagraoui, J., Gareau, Y., Gingras, S., Ruel, R., Mayotte, N., Csaszar, E., Knapp, D. J., Miller, P., Ngom, M. et al. (2014). Cord blood expansion. Pyrimidoindole derivatives are agonists of human hematopoietic stem cell self-renewal. *Science* **345**, 1509-1512.

Fares, I., Rivest-Khan, L., Cohen, S. and Sauvageau, G. (2015). Small molecule regulation of normal and leukemic stem cells. *Curr. Opin Hematol.* **22**, 309-316.

Gao, X., Bali, A. S., Randell, S. H. and Hogan, B. L. (2015). GRHL2 coordinates regeneration of a polarized mucociliary epithelium from basal stem cells. *J. Cell Biol.* **211**, 669-682.

Grote, D., Moison, C., Duhamel, S., Chagraoui, J., Girard, S., Yang, J., Mayotte, N., Coulombe, Y., Masson, J.-Y., Brown, G. W. et al. (2015). E4F1 is a master regulator of CHK1-mediated functions. *Cell Rep.* **11**, 210-219.

Guo, G., von Meyenn, F., Santos, F., Chen, Y., Reik, W., Bertone, P., Smith, A. and Nichols, J. (2016). Naive pluripotent stem cells derived directly from isolated cells of the human inner cell mass. *Stem Cell Rep.* **6**, 437-446.

Kriks, S., Shim, J. W., Piao, J., Ganat, Y. M., Wakeman, D. R., Xie, Z., Carrillo-Reid, L., Auyeung, G., Antonacci, C., Buch, A. et al. (2011). Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. **Nature 480**, 547-551.

O'Rahilly, R., Gardner, E. and Gray, D. J. (1956). The ectodermal thickening and ridge in the limbs of staged human embryos. *J. Embryol. Exp. Morphol.* **4**, 254-264.

Okamoto, I., Patrat, C., Thepot, D., Peynot, N., Fauque, P., Daniel, N., Diabangouaya, P., Wolf, J. P., Renard, J. P., Duranthon, V. et al. (2011). Eutherian mammals use diverse strategies to initiate X-chromosome inactivation during development. *Nature* **472**, 370-374.

Petropoulos, S., Edsgard, D., Reinius, B., Deng, Q., Panula, S. P., Codeluppi, S., Reyes, A. P., Linnarsson, S., Sandberg, R. and Lanner, F. (2016). Single-cell RNA-seq reveals lineage and X chromosome dynamics in human preimplantation embryos. *Cell* **167**, 285.

Pulvers, J. N., Bryk, J., Fish, J. L., Wilsch-Brauninger, M., Arai, Y., Schreier, D., Naumann, R., Helppi, J., Habermann, B., Vogt, J. et al. (2010). Mutations in mouse Aspm (abnormal spindle-like microcephaly associated) cause not only microcephaly but also major defects in the germline. *Proc. Natl. Acad. Sci. USA* **107**, 16595-16600.

Xue, Z., Hennelly, S., Doyle, B., Gulati, A. A., Novikova, I. V., Sanbonmatsu, K. Y. and Boyer, L. A. (2016). A G-rich motif in the lncRNA braveheart interacts with a zinc-finger transcription factor to specify the cardiovascular lineage. *Mol. Cell* **64**, 37-50.