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MEETING REVIEW

Advances in stem cells and regenerative medicine: single-cell dynamics, new models and translational perspectives

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ABSTRACT

An international cohort of over 300 stem cell biologists came together in Heidelberg, Germany in May 2017 as delegates of the 'Advances in Stem Cells and Regenerative Medicine' conference run through the European Molecular Biology Organization. This Meeting Review highlights the novel insights into stem cell regulation, new technologies aiding in discovery and exciting breakthroughs in the field of regenerative medicine that emerged from the meeting.

KEY WORDS: Stem cell, Regenerative medicine, Epigenetics, Metabolism, Regulation, Organogenesis

Introduction

One of the ultimate goals of stem cell biologists is to learn more about the development, homeostasis and regeneration of organisms, and to apply this knowledge to understand disease progression and eventually contribute to regenerative medicine. Conference organisers Clare Blackburn, Dónal O'Carroll (both University of Edinburgh, UK), Thomas Graf (Center for Genomic Regulation, Barcelona, Spain), Claus Nerlov (University of Oxford, UK), Oliver Pourquié (Harvard University, Cambridge, USA) and Shahragim Tajbakhsh (Pasteur Institute, Paris, France) provided a comprehensive and exciting program covering these themes and showcasing new perspectives on stem cells.

Emerging technologies such as gene editing, single-cell RNA sequencing and multidisciplinary approaches have led to new definitions of stem cells and their behaviours *in vitro* and *in vivo*. Moving away from the traditional view of a rigid stem cell hierarchy that only allows unidirectional differentiation, presentations at this meeting demonstrated that adult stem cells maintain enhanced plasticity and that less-plastic cells can acquire stem cell characteristics under certain conditions (Fig. 1). New aspects of stem cell regulation beyond signalling from fixed niches were considered, such as the effects of ageing, switches in metabolism and autoregulatory mechanisms. Novel techniques demonstrated how organoid models are becoming ever more sophisticated to allow for the non-invasive study of heterogeneous 3D cellular structures, paving the way for more complex *in vitro* disease modelling and regenerative medicine approaches. Effective translation of selected stem cell techniques into the clinic were also presented, providing hope for new tissue-replacement therapies to come.

Stem cell programming and reprogramming

The most potent stem cell arises early in embryonic development when an egg becomes fertilised and begins a complex process of transforming from a single cell into an intricate, well-developed organism. Cell cleavage and division lead to the creation of the blastocyst containing the trophoblast and inner cell mass (ICM), which consists of pluripotent stem cells (PSCs). Significant efforts in the field focus on examining how cells from the ICM differentiate into the three germinal layers (mesoderm, ectoderm and endoderm) and then into tissues of mature organs. Recent studies by the group of Maria-Elena Torres-Padilla (Institute of Epigenetics and Stem Cells, Munich, Germany) have identified cells resembling 2-cell-stage embryos (2C-like cells) that arise during culture of mouse embryonic stem cells (ESCs) upon downregulation of chromatin assembly activity (Ishiuchi et al., 2015). Delving deeper into the origins of these 2C-like cells using a combination of cell biology and molecular biology approaches, Torres-Padilla has set out to characterise transitional states to understand how 2C-like cells arise in culture. Mouse cells with the capacity to give rise to both embryonic and extraembryonic tissues were also described by Hongkui Deng (Peking University, China) and termed extended pluripotent stem cells (EPS cells) (Yang et al., 2017). Previous work had reported cells with similar properties using different culture media (Guo et al., 2009; Martin Gonzalez et al., 2016), but this work interestingly shows that human EPS cells contribute to both embryonic and extraembryonic tissue when transplanted into mice, which is not the case for human ESCs. Delineating the mechanisms behind the development of these highly plastic cells will provide important insight into the molecular underpinnings of stem cell reprogramming ability.

While studies on the developing embryo are commonly conducted by stem cell researchers with a view to better understand and manipulate PSCs in culture, Alfonso Martinez-Arias (University of Cambridge, UK) discussed his studies using ESCs to explore embryo development. Building on the work on embryonic neuromesodermal progenitors (NMPs) presented by Val Wilson (University of Edinburgh, UK), Martinez-Arias developed an *in vitro* protocol for ordered differentiation of mouse ESCs into NMPs that exhibit many of the characteristics of embryonic progenitors. This protocol aids in our understanding of the specific differentiation signals leading to the formation of the spinal cord and postoccipital somites in the embryo. Studies from Val Wilson found surprisingly large changes in Wnt, Notch and FGF signalling, as well as in Hox gene expression, in NMPs as axis elongation progressed. The importance of Notch signalling in the embryo was also discussed by Anna Bigas (Institut Hospital del Mar d'Investigacions Mediques, Barcelona, Spain), who showed that Notch1 regulates the specification of aortic (Notch-high) and hematopoetic (Notch-low) lineages from endothelial-like precursors. Together, these data provide a framework for better understanding embryonic signalling leading to stem cell differentiation *in vivo*.

Work presented by Lluc Mosteiro (National Cancer Research Centre CNIO, Madrid, Spain) aimed at investigating *in vivo* reprogramming in a more comprehensive manner, utilising a

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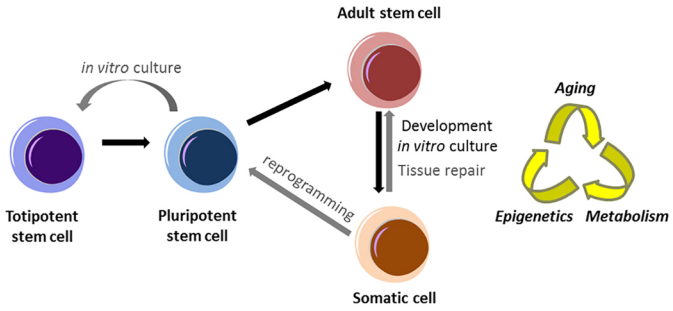

Fig. 1. An updated simplified model of stem cell regulation based on work presented at the meeting. The model illustrates the different conditions in which stem cells may differentiate or dedifferentiate *in vivo* and *in vitro*. The importance of aging, epigenetics and metabolism in stem cell regulation are being increasingly recognised.

doxycycline-inducible mouse model to express the key Yamanaka factors (Takahashi and Yamanaka, 2006) Oct4 (Pou5f1), Sox2, Klf4 and c-Myc in somatic cells from different organs (Mosteiro et al., 2016). As a result, a small number of cells formed teratomas – indicative of reprogramming – while surrounding cells underwent senescence mediated by the expression of the cytokine interleukin 6 (IL6). Intriguingly, this senescence promoted reprogramming and teratoma formation. Using the same mouse model, Han Li (Institut Pasteur, Paris, France) showed that after muscle injury senescent cells activate IL6, which is related to cells exhibiting a senescence-associated secretory phenotype *in vivo*. Again, senescence appears to promote reprogramming and cellular plasticity (Chiche et al., 2017).

Kim Jensen (University of Copenhagen, Denmark) and Ysbrand Nusse (University of California San Francisco, USA) found by independent methods of murine intestinal injury that cells involved in the repair process reprogram back to an embryonic-like phenotype to potentially stimulate regeneration. Triggered by chemical-induced inflammation or parasite-induced granuloma formation, cells in the repair phase expressed foetal epithelial markers and formed spheroid intestinal organoids more closely resembling the foetal rather than the budding adult phenotype. Similarly, evidence for reprogramming induced by tissue injury was presented by Fiona Watt (King's College London, UK), who demonstrated that, upon wounding, epithelial cells could be induced to a stem cell-like fate through GATA6 signalling. These studies suggest that injury-induced reprogramming to earlier stem cell-like states can facilitate tissue repair.

Organogenesis *in vitro*

One of the pioneers of organoid formation, Hans Clevers (Hubrecht Institute, Utrecht, The Netherlands) gave the first keynote presentation showing amazing animations of the cellular processes leading to the *in vitro* growth of intestinal stem cells. He also demonstrated the power of using patient-derived colon organoids to screen the efficacy and specificity of expensive cystic fibrosis drugs ahead of individualised treatment of patients. The use of organoids to determine patient-specific therapies is an important new application for precision medicine, as well as a powerful tool for dissecting organ development and disease. Organoids have been generated for many tissues and the protocols and technologies to facilitate their growth continue to advance rapidly.

Matthias Lutolf (Ecole Polytechnique Fédéral de Lausanne, Switzerland) has developed new strategies to improve existing organoid models by combining matrix engineering and microtechnology. For example, the Lutolf laboratory recently developed a microfluidic approach to generate intestinal crypt-derived tubes, with villus-like cellular structures that can be perfused with liquid to simulate the *in vivo* intestine more realistically. Another interesting technology developed by the Lutolf laboratory is a microchip that allows arrays of single organoids to be grown and studied in different wells under different conditions. This technology has been adopted by Anne Grapin-Botton (DanStem, University of Copenhagen, Denmark), who presented her work on growing mouse pancreatic organoids from early progenitors in Matrigel-overlaid hydrogel microwells. Importantly, Grapin-Botton demonstrated that the number and type of cells needed to create pancreatic organoids could be titrated in these microwells, finding that two cell types (Notch-positive and Notch-negative) were needed for organoid generation. Adaptations to current protocols such as these enable the fine-tuning of manipulations to harness organoid modelling to better understand development and test new strategies to deal with diseases.

Elegant models of organoid growth have also been developed by Christina Scheel (Helmholtz Center Munich, Germany) and Joseph Bonventre (Harvard Medical School, Boston, USA), who focus on the mammary gland and kidney, respectively. Christina Scheel investigates mechanical aspects of the mammary stroma and seeded human primary mammary adult cells in floating collagen 1 gels to produce complex branching mammary organoids that could be used to study mammary gland development and disease (Linnemann et al., 2015). By studying kidney embryogenesis *in vivo*, Joseph Bonventre determined the ideal conditions to direct the differentiation of kidney organoids utilising small molecules and drugs. Stepwise differentiation of human PSCs into nephron progenitor cells by low doses of fibroblast growth factor 9 (FGF9) followed by addition of the small molecule inhibitor CHIR99021 and then replating the cells into 3D culture creates kidney organoids with complex tubule formation and interconnected ducts (Morizane et al., 2015). Together, these studies highlight the importance of considering normal development and *in vivo* characteristics of both cellular as well as matrix components to model organs effectively *in vitro*.

Generating homogeneous cell populations and disease modelling

To derive cells of a specific lineage from a common progenitor requires fundamental knowledge of which signals lead to the decision of the cell to be directed towards one lineage over another. Clare Blackburn presented new data on the role that Notch signalling (through RBPJ) plays in directing the differentiation of medullary thymic epithelial cells over cortical thymic epithelial cells, providing an understanding of the mechanisms leading to successful thymus development.

The importance of deriving purified cardiac cell populations from human PSCs for future cell transplantation therapies was highlighted by Gordon Keller (McEwen Center for Regenerative Medicine, Toronto, Canada). His work focused on identifying the signalling pathways that promote the development of highly enriched ventricular cardiomyocyte populations that might be used in the future to repair the ventricular myocardium in patients who have suffered from a myocardial infarction. On the other hand, Christine Mummery (Leiden University Medical Centre, The Netherlands) showed that by manipulating MYC expression in cardiac progenitors derived from PSCs and transiently adding FGF, purified cardiomyocytes can be generated, and that they could be functionally tested by recording their electrical properties. She highlighted the differences in electrical properties between mouse

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and human cells, and between non-isogenic lines from a single species, warning cardiac biologists about interpreting functional tests when there are inherent differences between cells from different donors and genetic origins.

Olivier Pourquié presented new data on the efficiency of differentiating skeletal muscle fibres by functionally testing contraction force after stimulation. By modulating Wnt and BMP signalling pathways in human PSCs [both induced pluripotent stem cells (iPSCs) and ESCs] to direct skeletal muscle differentiation (Chal et al., 2016), he is taking his differentiation protocol one step further to study the phenotypes of cells mimicking Duchenne muscular dystrophy skeletal fibres to better understand the disease. Also studying disease modelling, Stuart Forbes (University of Edinburgh, UK) presented work examining the role that stem cells play in regeneration when hepatocyte regeneration is impaired using murine models of chronic liver injury and fibrosis. Through lineage-tracing studies he determined that ductular cells, which are characterised by phenotypic traits of intrahepatic biliary epithelium, are a significant source of hepatocyte regeneration at the site of injury. Lineage-tracing techniques were also employed by Cédric Blanpain (Université Libre de Bruxelles, Belgium), who presented work on mammary gland development. He pinpointed that bipotent stem cells switch to a unipotent state during the course of embryonic development, with potential lineage priming occurring ahead of commitment at the early stage of mammary gland morphogenesis. In his presentation, he proposed that oncogenes may reuse the same machinery that allows plasticity in normal development, which emphasises the importance of understanding physiological differentiation to better understand the disease state.

Multidisciplinary and computational approaches to stem cell biology

Many talks were highly interdisciplinary, integrating mathematical modelling, physical aspects and basic biology to derive algorithms that reveal modes of stem cell regulation, morphogenesis and other biological processes. Benjamin Simons (University of Cambridge, UK) presented a mathematical model of ductal branching in the pubertal mouse mammary gland. This allows prediction of whether the terminal end bud will bifurcate or terminate (Scheele et al., 2017), resulting in the proposal that this is related to adjacent ductal proximity. Currently, he is adapting the resulting algorithms to more complex structures such as the kidney, which will hold the key to further understanding stem cell fate decisions, differentiation and participation in morphogenetic processes. Shosei Yoshida (National Institute for Basic Biology, Okazaki, Japan) presented an unpublished story using a combination of experimental and computational approaches to address the role of FGF signalling in mouse spermatogenic stem cells.

Stefan Semrau (Leiden University, The Netherlands) examined the discordance between mRNA and protein expression in integrated RNA-sequencing and mass-spectrometry measurements of ESC differentiation (van den Berg et al., 2017 preprint). His kinetic model shows that most of the discrepancies are caused by a delay due to protein translation or degradation. The model also identifies genes that are dynamically regulated at the protein level and allows the prediction of protein levels in subpopulations using single-cell RNA-sequencing data. Other presentations by Zahra Karimaddini (ETH Zurich, Switzerland) and Meng Amy Li (University of Cambridge, UK) discussed how computational modelling and single-cell RNA-sequencing can aid in our understanding of stem cell characteristics and help define which factors are important in stem cell fate decisions. The power of single-cell sequencing was demonstrated in findings presented by Claus Nerlov, who showed that in the hematopoietic system a common granulocyte-macrophage progenitor does not exist but, instead, that committed myeloid progenitors consist of a Gata1-positive progenitor population capable of generating mast cells and eosinophils, and a Gata1-negative population that can generate neutrophils and monocytes.

Age, sex and metabolic influences on stem cell fates

Anne Brunet (Stanford University, USA) introduced the emerging importance of aging and metabolism in stem cell function in her keynote presentation in the context of neural stem cells (NSCs) (Brunet and Rando, 2017). NSCs provide a perfect example of the contrasting requirements of stem cells at different ages, such as the intensely proliferative versus slow-cycling statuses of NSCs in embryonic compared with adult states. However, studies presented by Yukiko Gotoh (The University of Tokyo, Japan) found that NSCs from the adult subependymal zone are set aside as slow-cycling subpopulations at an early stage, mediated through p57 (Cdkn1c) (Furutachi et al., 2015). Also focussing on age-dependent effects, Ruzhica Bogeska (German Cancer Research Center DKFZ, Heidelberg, Germany) presented data on the effects of chronic inflammation in a mouse model recapitulating human aging. This results in hematopoietic stem cell (HSC) depletion and age-associated decline in function.

The intrinsic sexual identity of stem cells also influences their ability to proliferate. Cell-specific reversal of sexual identity presented by Irene Miguel-Aliaga (Imperial College London, UK) demonstrates that masculinising intestinal stem cells *in vivo* in adult female *Drosophila* reduces their proliferation to levels comparable to those of males, resulting in an intestine of a smaller, male-like size that is also less likely to develop tumours (Hudry et al., 2016).

Metabolism is another key factor in stem cell differentiation fates. Toshio Suda (Cancer Science Institute of Singapore, National University of Singapore) demonstrated that HSCs with a high mitochondrial mass display a preference for megakaryocyte differentiation. Interestingly, data presented by Naomi Taylor (CNRS, Montpellier, France) demonstrated that HSC differentiation patterns are also influenced by utilisation of nutrient resources. In particular, she showed that erythropoiesis is dependent on glutamine metabolism (Oburoglu et al., 2014). A high density of Glut1 (Slc2a1) and ASCT2 (Slc1a5) on erythrocyte precursors allowed transport of glucose and glutamine, which is essential for optimal erythrocyte differentiation. Conversely, when ASCT2 was blocked, hematopoietic precursors were directed to a myelomonocytic fate.

Scaling down metabolomics to thousands of cells rather than millions, Sean Morrison (UTSW, Children's Research Institute, Dallas, USA) found that HSCs take up higher levels of ascorbate (vitamin C) as compared with restricted hematopoietic progenitors in mice and humans. Through studies in genetically modified mice unable to endogenously synthesise vitamin C (as is the case for humans), ascorbate was found to negatively regulate stem cell function, hematopoietic regeneration and leukaemia development, acting at least partly by promoting the function of ten-eleven translocation (TET) family DNA demethylases to maintain appropriate DNA hydroxymethylation levels in HSCs and other progenitors.

Interestingly, findings discussed by Andreas Trumpp (DKFZ/HI-STEM, Heidelberg, Germany) found another vitamin, vitamin A, to be important for the maintenance of dormant HSCs, which display high retinoic acid signalling. Lower levels of vitamin A inhibit HSC

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re-entry to the dormancy phase, ultimately resulting in HSC depletion in vitamin A-deficient mice over several months (Cabezas-Wallscheid et al., 2017). Trumpp's group also discovered overexpression of branched chain amino acid transaminase 1 (BCAT1) in primary human acute myeloid leukaemia stem cells, which leads to reduced levels of alpha-ketoglutarate (α-KG), a co-factor of TET family proteins. Lower levels of α-KG activity in these poor-prognosis patients led to hypermethylation patterns similar to those found in TET-mutated cells, providing a novel epigenetic mechanism affecting TET activity in leukaemic stem cells. Together, these presentations highlight the need to consider metabolic aspects and age in stem cell studies.

From the bench to the clinic

Work presented by Masayo Takahashi (RIKEN Center for Developmental Biology, Kobe, Japan) focused on translating research performed in the laboratory into surgical interventions aimed at reducing further degeneration and neovascularisation of the retina during macular degeneration. Her laboratory has targeted retinal pigment epithelial (RPE) cells, which are the supporting cells for photoreceptors in the eye. She has successfully developed a technique to derive RPE cells from autologous iPSCs and implant them into the retina (Mandai et al., 2017), where they were found to remain stable even after two and a half years. Takahashi provided important information for future clinical trials based on stem cells, including the tests they implemented for safe transplantation. Her group is now tackling the production of photoreceptor cells that could restore vision.

Discussions on taking stem cells into the clinic were also presented in the last keynote talk by Michele De Luca (Centre for Regenerative Medicine S. Ferrari, Modena, Italy), describing work of Graziella Pellegrini in which autologous cultured corneal epithelium restored visual acuity to patients with damaged corneal surfaces. Laws introduced in Europe in the early 2000s meant that De Luca's group was faced with a massive undertaking to build the Centre of Regenerative Medicine in order to continue his clinical translation work under Good Manufacturing Practice (GMP) conditions. Revisions to these laws have been made and were presented by Emmanuelle Rial-Sebbag (Inserm, Paris, France), who emphasised the importance of policy-makers working together with stem cell biologists to ensure the safe and rapid translation of stem cell therapies into the clinic. The latest work from the De Luca laboratory has been to grow sheets of gene-edited epithelial cells to be grafted onto patients devastatingly affected by junctional epidermolysis bullosa, restoring the patients' skin integrity. These studies demonstrate that through multiple efforts, slowly but surely, translation of stem cell research into clinical regenerative therapy is already making inroads into modern medicine, with more therapy protocols soon to enter clinical trials.

Conclusions

From the presentations at this meeting it is evident that the field of stem cell biology and regeneration has come a long way since the cultivation of the first human PSC in the 1990s (Thomson et al., 1998). Increasing evidence suggests that stem cell differentiation is not unidirectional and that events such as tissue injury can reprogram cells back for homeostatic recovery of function in various tissues. Other factors such as the age of the cell and metabolism influence the ability of a stem cell to differentiate to a desirable cell fate. Increasing knowledge of cell signals that direct differentiation at the single-cell level and the ability to mimic *in vivo* environments *in vitro* will improve stem cell differentiation and enable more accurate disease modelling in culture. It is important for stem cell biologists to adopt new perspectives and tools that rapidly advance the ability to translate research from the bench into the clinic in a safe and effective manner.

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Competing interests

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