Viewpoint

Progress and challenges in stem cell biology

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Since stem cells were first discovered, researchers have identified distinct stem cell populations in different organs and with various functions, converging on the unique abilities of self-renewal and differentiation toward diverse cell types. These abilities make stem cells an incredibly promising tool in therapeutics and have turned stem cell biology into a fast-evolving field. Here, stem cell biologists express their view on the most striking advances and current challenges in their field.

Effie Apostolou: induced pluripotency – the continued reprogramming revolution

The seminal discovery of pluripotency induction achieved by means of transcription factors or chemical cocktails has revolutionized multiple biomedical fields and shed light on processes including development, aging, regeneration and cancer. Over the past 15 years, many burning questions around reprogramming mechanisms, trajectories and translational limitations have been addressed.

High-throughput functional screens identified critical regulators and barriers of reprogramming, while multimodal omics studies helped with constructing four-dimensional (4D) roadmaps of the complex transcriptional, epigenetic, topological, proteomic and metabolic changes that somatic cells undergo upon loss of their initial identity and acquisition of pluripotency. Parallel studies have also identified potentially detrimental, long-lasting aberrations that are introduced along the way. Moreover, single-cell technologies during cellular reprogramming captured intriguing intermediate and refractory states, reminiscent of early embryonic fates, senescence response, regeneration or tumorigenesis.

Despite this progress, important gaps remain and new questions continually arise. What are the cause-and-effect relationships during the multi-layered molecular chain reaction of reprogramming, and which factors lie at the top of the regulatory hierarchy? How can we reproducibly and deterministically reprogram cell identity, if we know the start and end points, to enable efficient and safe generation of any therapeutically relevant cell type from easily accessible tissues? How can we either avoid or rationally exploit the epigenetic variability of induced pluripotent stem cells? Can we capture, and propagate in vitro, transient intermediate cell states of biomedical relevance? Future studies using advanced engineering approaches for acute and reversible perturbations in defined time windows will be critical to address the functional interconnections of various reprogramming regulators and enable fine-tuning toward end states of interest. Moreover, ongoing single-cell efforts to map the continuum of cell states in early embryos and tissues or synthetic structures will determine more definitively the degree to which reprogramming intermediates recapitulate physiological or pathological transitions. Together with continuously improved computational approaches and modelling, these efforts will enable accurate predictions of critical conditions and cocktails for precise, reproducible and error-free cell fate engineering. These engineered fates can ultimately expand the toolbox for generating complex tissues and organoids for disease modelling and drug screening and for understanding and ameliorating hallmarks of ageing and cancer.
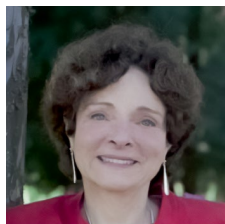
Helen Blau: multiple strategies to augment muscle regeneration and increase strength

Mobility is a major determinant of quality of life. Elderly patients with sarcopenia or patients with heritable muscle-wasting disorders suffer from a debilitating loss of muscle strength for which there is no approved treatment. COVID-19 highlighted the need for strategies to strengthen atrophied diaphragm muscles after ventilator support. Although our knowledge of stem cell function in regeneration has markedly increased, major knowledge gaps and challenges remain.

First, muscle stem cells (MuSCs) are a heterogeneous population that diverges over time and in response to disease or ageing. Targeting the functional subset of MuSCs is an unmet challenge. Second, understanding the role of the microenvironment and the muscle stem cell niche in muscle stem cell behaviour is key. Data are emerging showing that MuSCs respond not only to biochemical but also to biomechanical cues and that the elasticity of the niche matters. This suggests that stiffer fibrotic muscles, characteristic of muscular dystrophy or ageing, will harbour stem cells with impaired regenerative function. The development of hydrogels that can stiffen or soften on demand, while maintaining stem cells in a viable state, could provide new molecular and signalling insights into stem cell mechanosensing mechanisms, how they change with ageing and how they can be overcome. Third, from advances in single-cell and single-nuclei RNA sequencing, we are gaining knowledge of the gene expression patterns of the complex, diverse array of cell types that populate the niche. However, these technologies entail tissue destruction and therefore do not provide spatial information regarding cell–cell interactions that are crucial to maintaining stem cell quiescence and inducing stem cell activation and efficacious regeneration. There is a great need for spatial proteomics and multiplexed imaging modalities that preserve information about cell location and the dynamics of cell–cell interactions characteristic of regeneration, disease and ageing. Finally, inflammation has beneficial roles in wound healing, but is deleterious when chronic, as in aged muscles. Finding ways to rejuvenate muscle form and function remains a major challenge. The discovery of the prostaglandin degrading enzyme 15-PGDH, an immune modulator, as a pivotal molecular determinant of muscle ageing is a

notable step in that direction. Remarkably, overexpression of 15-PGDH for one month in young adult mouse muscles induces atrophy and weakness, whereas inhibition of 15-PGDH in aged mouse muscles results in a 15% increase in muscle mass, strength and exercise performance. Solving these challenges will pave the way for new, effective stem cell-targeted therapeutic agents to regenerate and rejuvenate muscle.

Kenneth Chien: heart progenitors rebuild cardiac muscle

Rebuilding the failing human heart with working muscle is the holy grail of regenerative medicine. Although initial therapeutic attempts with non-cardiac cells have proven unfruitful, mouse studies have shown the potential to create de novo cardiomyocyte-like cells in situ by direct reprogramming via gene transfer. A novel class of adult claudin-6$^+$ epicardial progenitors can convert to muscle, contributing to regeneration of the injured vertebrate heart. The studies point to a key role of tight junction proteins in the formation of a honeycomb-like regenerative structure. Although the adult human epicardium lacks these specific progenitors, uncovering their regenerative molecular pathways could identify new signals that can restore the myogenic potential of non-human epicardial cells via conversion to a progenitor state.

Thus far, the most advanced stem cell therapeutic agents are based on human embryonic stem (ES) cells for the generation of either cardiomyocytes or human ventricular progenitors (HVPs) for transplantation in large animals following cardiac injury. Issues of scalability, efficacy, clear evidence of working ventricular muscle grafts, lack of teratoma formation and tissue integration have all been largely addressed, moving both ES-cell-derived cell types toward the clinic with large pharma partners. However, additional issues remain, including safety (arrhythmias), durability (rejection) and the development of clinically tractable in vivo delivery systems. Our work on cardiogenesis over two decades recently led to the discovery of HVPs, which can migrate toward the injury site, prevent fibrosis via fibroblast repulsion, and proliferate to form large human ventricular muscle grafts to improve function in failing pig hearts. Additional work is ongoing, but early returns support the therapeutic potential of HVPs with minimal major side effects, with a two-year projected timeline for a first-time-in-human study. Prevention of rejection with optimal drug regimens, hypoimmune ES cell lines and new tolerization strategies, as well as novel catheters for in vivo delivery, are on the horizon. With these advances, HVPs might eventually provide new hope for patients with near-end-stage heart failure and no other options.

Madeline A. Lancaster: next-generation human neural stem cell models

The field of neural stem cell biology has made great strides in the past decade. What started out with neural stem cells that were cultured ex vivo to generate neurons and glia has evolved into a diverse field of ever-more-complex tools to model not just individual cells, but whole 3D neural tissues in a dish called neural organoids. Such organoids mimic not only the cellular makeup of the developing brain, but also local tissue architecture, with recent methods even demonstrating morphogenetic movements of neurulation.

Organoids and other in vitro models of the nervous system are becoming increasingly complex, for example through the use of so-called assembloids to combine different regions and examine their integration. Neural organoids also enable extensive neuronal maturation, even reaching hallmarks seen in the postnatal brain. However, as these models increase in complexity, so too do the challenges. With increasing size and maturity, the lack of vasculature becomes problematic. Although promising results have come from in vivo transplantation and integration of endothelial cells, vascularization leading to more advanced tissue development remains to be demonstrated. This challenge will likely represent one of the most difficult hurdles not just for the neural organoid community, but for the field of organoids as a whole, and creative approaches will be needed.

Brain organoids are already paving the way to fundamental discoveries in human neurobiology and are providing new understanding of disease pathogenesis. The future will hold new insight into why the human brain is unique, as well as how to prevent and treat various neurological conditions. Organoids may hold the key to these insights, but they cannot be the only tool, and it will be important to use them as complementary approaches alongside more established methods. Marrying in vitro and in vivo approaches will be the key to uncovering fundamental processes of neurobiology and answer age-old questions such as how genetics influence connectivity, how networks of neurons compute and how information is stored in the brain. The brain is still a largely uncharted territory, and powerful techniques combined with creative minds are needed to untangle its mysteries.

Purushothama Rao Tata: phenotypic and functional interrogation of lung biology at single-cell resolution

Lung tissues are relatively quiescent at homeostasis, but they respond rapidly to regenerate lost cells after injury. Early lineage tracing studies in animal models showed that this regeneration is driven predominantly by several 'professional' and facultative stem and progenitor cells in different regions of the lung, including basal and secretory cells in the airways and type 2 pneumocytes in the alveoli. These studies also uncovered a remarkable plasticity of some differentiated cell populations that contribute to regeneration following severe injury. More recently, multiple groups have used single-cell omics approaches to catalogue lung cells and their associated molecular signatures in great detail. Remarkably, in the case of the human lung, these efforts have identified previously unknown and uncharacterized cell types located in discrete regions. These cell populations are often quite heterogeneous, and include transitory states enriched in lungs from patients with respiratory disease. Significantly, these cell types are not found in the mouse, the animal model most commonly used for lung research. Consequently, there is an urgent need to develop new experimental tools to test their normal in vivo function and role in regeneration and disease.

To address this problem, efforts are underway by several groups, including our own, to develop genetically engineered ferrets and pigs as new animal models. Similarly, analytical tools are being optimized to infer cell lineages in human lungs based on clonally

amplified genetic variants (single-nucleotide polymorphisms or mitochondrial heteroplasmy). In the case of ex vivo organotypic cultures, such as those derived from human induced pluripotent stem cells or primary foetal or adult lung progenitors, there remain many challenges. These include attaining or retaining mature cell types in the correct ratios to match those in normal in vivo lung tissue. To overcome this challenge, collaborative efforts are underway between lung stem cell biologists and bioengineers to generate new scaffolds to reassemble and mimic the cell–cell interactions found in native lung tissue niches. Taken together, these new approaches have the potential to identify the genetic circuits that regulate normal and disease-associated human lung cell states, establish scalable disease models and, ultimately, develop cell-based therapies to treat degenerative lung diseases.

Eirini Trompouki: the time journey of blood stem cells

Haematopoietic stem and progenitor cells (HSPCs) are critical for sustaining lifelong haematopoiesis via their extensive self-renewal and multilineage differentiation capacities. The secrets to how HSPCs acquire these capacities reside in the enigmatic process through which they are generated during an embryonic endothelial-to-haematopoietic transition (EHT). On the other end of the spectrum, age alters HSPCs, resulting in defective haematopoiesis. The most critical problems in HSPC biology relate to these lifetime bookends. Recently, human HSPC development was addressed in a spatial and single-cell manner, revealing that a haematopoietic stem cell (HSC) transcriptional signature is established after the emergence of HSCs along with continuously evolving cell surface markers, while haematopoietic heterogeneity already starts to be established at the haemogenic endothelium stage. Single-cell transcriptomics also led to the identification of a progenitor population that is responsive to retinoic acid and gives rise to haemogenic endothelial cells. Our group and others pinpointed the importance of DNA and RNA sensors in EHT. We and others found that transposable elements and R-loops trigger innate immune sensors to induce sterile inflammation that enhances EHT. Another layer of regulation lies in the interaction between HPSCs and other cells, such as macrophages or T cells, that are proposed to perform quality control of HSPCs during development and adulthood, respectively. Despite this progress, however, we still cannot faithfully recapitulate EHT in vitro and produce the massive quantities of HPSCs required for transplantations and gene therapy. Therefore, I think one of the most important aspects of haematology in the near future will be generating and maintaining good quality and quantity of HSPCs in vitro.

Ageing of HSPCs, on the other hand, is especially relevant because the population of the Earth is continuously ageing. An interesting feature of ageing that is lately gaining more and more attention is clonal haematopoiesis, which has been linked to haematological (and other) diseases. Inflammation, chemotherapy and irradiation have been shown by many groups to be advantageous for mutated clones. It is interesting to speculate that a collection of stressful moments experienced during life are 'memorized' by HSPCs and aided by clonality to instigate ageing. It was recently demonstrated that epigenetic memory is a feature not only of immune cells but also of HSCs. Further research needs to show whether every stress in life could be depicted in our genome as 'memory' and finally constitute the intricate mechanism of ageing.

Fiona M. Watt: understanding epidermal stem cell biology through data integration

Although mammalian skin contains many different cell types, the best-characterized stem cell population is in the epidermis, the multilayered epithelium that forms the skin surface. Autologous sheets of cultured epidermis were one of the first cell therapies involving ex vivo expansion of stem cells to be validated clinically, dating back to the early 1980s. That approach has been refined over the years, and the life-saving effects of combining cell and gene therapy to treat blistering skin disorders have been demonstrated unequivocally. In parallel with the development of techniques to culture human epidermis, the mouse became a key model for stem cell studies because of the availability of tools to target the different epidermal layers and the demonstration that genetic lesions in humans could be phenocopied in the mouse. With the advent of extensive single-cell RNA sequencing (scRNA-seq) databases for healthy and diseased human skin, it is essential that stem cell researchers use these resources both to validate their experimental models and to design new experiments. We need to look hard at the extent to which mouse models are still appropriate for modelling healthy and diseased human skin.

A very exciting challenge we face is data integration. There are many different axes along which integration can be achieved. One is spatiotemporal – the ability to correlate changes in cell types and states as a function of time and distribution within the skin. I am particularly intrigued by the possibility of correlating macroscopic skin features that are captured by optical coherence tomography with features obtained via spatial transcriptomics. Another example is integrating epidermal datasets from transcriptomics, proteomics, lipidomics and glycomics to gain a more holistic understanding of the nature of the stem cell state. In our enthusiasm for scRNA-seq, we risk ignoring the central dogma that DNA makes RNA that makes protein, and failing to remember the importance of protein modifications and turnover. I believe that by integrating epidermal stem cell responses to different extracellular cues, whether physical or biochemical, we will gain new insights into stem cell function and find switches between cell states that are conserved between tissues.

Yi Arial Zeng: the journey to islet regeneration

The islets of Langerhans are endocrine regions of the pancreas containing hormone-producing cells. β-cells produce and secrete insulin – the hormone that lowers blood glucose levels. Insufficient numbers of functional β-cells are associated with both type 1 and late-stage type 2 diabetes. With 1 in 11 people being diabetic, there is a great need to understand how the adult islet mass is maintained and how β-cells are regenerated to guide new therapies.

Stimulation of in situ islet regeneration is one approach for replenishing β-cells, through the formation of new progenitor-derived β-cells and enhanced proliferation of existing β-cells. Although the existence of islet progenitors in postnatal life has long been debated, recent work using mouse models has reported their existence in adults, leading

to exciting opportunities for dissecting the activation mechanisms of these progenitors during homeostasis, regeneration and aging. It is noteworthy that neogenesis from progenitors and β-cell replication are not mutually exclusive: the proliferative β-cell subpopulation could possibly be the progeny of the progenitors, or there could be parallel proliferative pathways. Considering that relatively few insulin-secreting cells are needed to ameliorate hyperglycaemia, in vivo transdifferentiation represents another promising route. It has been reported that pancreatic exocrine cells and gut cells can be transdifferentiated into insulin-secreting cells. Collectively, these approaches aim to offer therapeutic strategies to stimulate in situ regeneration.

Pancreatic islet transplantation from donors is a recognized approach for replacing lost or damaged β-cells. Because of the shortage of donors, ongoing efforts aim to identify a renewable supply of human β-cells. A promising idea involves the differentiation of human pluripotent stem cells into β-like cells, and clinical trials using these β-like cells are underway. However, one may ask whether transplanting only mature β-cells is optimal, as proper glucose regulation requires coordination between various islet cell types. Will it be advantageous to produce whole islets in vitro rather than differentiating cells solely into β-like cells? Murine adult islet progenitors can generate organoids that contain all endocrine cell types of the intact islet and are proven to ameliorate diabetes in murine models. More work will be needed to establish the identity of these progenitors in the human pancreas and to translate the organoid culture system to human cells. As our understanding of islet regeneration matures, therapeutic transplant options will continue to emerge.

Magdalena Zernicka-Goetz: stem cells in modelling embryology

ES cells, derived from the pluripotent epiblast, can host transgenes and be reintroduced back into the embryo to generate a chimeric animal and a pure breeding line in future generations. A stunning application of ES cells in recent years has been their use to generate embryo-like structures in vitro. Several approaches have advanced our quest to recapitulate embryogenesis.

A 2D method using exclusively ES cells cultured as micropatterns offered a powerful route toward understanding how different cell types are established and signal between themselves. A second model, in which large aggregates of ES cells are treated with chemicals and growth factors, generated 3D structures developing many aspects of the segmental body plan, although still lacking body regions, particularly those required for forebrain development.

The importance of extraembryonic signalling was recognized through a series of whole-embryo models. The first such model, built from ES cells alone, pointed to the role of signals normally provided by the extraembryonic primitive endoderm, which can be replaced by the extracellular matrix to polarize ES cells to form a rosette-like structure that undertakes lumenogenesis. The second model, built from ES cells and trophectoderm stem cells, taught us that this interaction alone is sufficient to establish amniotic cavity and posterior embryo identity to induce mesoderm and germ cells. By incorporating a third stem cell type, extraembryonic endoderm cells, we achieved the formation of the anterior signalling centre and anterior–posterior patterning. Recently, additional approaches we and others undertook led to the generation of embryo models that were capable of developing much further to establish brain and heart structures and initiate organogenesis. Such whole-embryo-like models have brought insight into the biophysical and biochemical factors mediating stem cell self-organization and defining the cellular constituents, the chemical environment and the physical context required for embryo assembly.

Despite this progress, challenges remain. Cell fate specification relies on chemical cross-talk within and between lineages. Cell fate decisions must be spatiotemporally coordinated by establishing and interpreting gradients of numerous diffusible signalling proteins. We have much to learn about these combinatorial effects and about how to improve the efficiency with which different cell types combine to form embryo-like structures. A deep understanding of the components of cellular, biochemical and biophysical networks will be crucial to reaching this goal. Computational modelling will allow us to predict and guide self-organizational outcomes through exploitation of the capacity of cell communication to promote self-organization in vivo. It would also be powerful to advance our abilities to culture model embryos and replicate the maternal environment by delivering suitable nutrients to the circulatory system of the developing structure. These problems are also inherent to the assembly of synthetic organs, and I am certain that we will see a cross-talk between these different disciplines of synthetic biology for mutual benefit.

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