CellPress

Overcoming iPSC Obstacles

We asked six leaders in the reprogramming field to share their views on remaining gaps in our scientific understanding of iPSCs that will need to be addressed to achieve their clinical promise. Their insights echo many issues raised in the Review by Tapia and Schöler in this issue.

Line-to-Line Variability

Marius Wernig
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When we began to develop iPSC-based approaches to treat the monogenic skin disease epidermolysis bullosa 8 years ago, we believed that reprogramming, gene targeting, and differentiation would be the main hurdles that limit clinical translation. However, the field has made enormous progress in these areas and—while they are still important considerations—they do not represent the principal roadblocks anymore. A multitude of more effective gene targeting, reprogramming, and differentiation methods have been developed for making functional, clinically relevant cell types in culture.

A somewhat underappreciated issue that has evolved into one of the main problems in our hands is the variability between lines from different and even the same donor for differentiation endpoints. The current state of the art requires adjusting differentiation protocols for essentially every line. While this may be achievable for allogeneic approaches, it is less feasible for autologous repair, which is a main advantage of iPSCs and of importance for tissues such as skin that are highly immunosensitive and may require autologous grafts for long-term repair.

How will we overcome this problem? It is conceivable that more selective growth conditions may exist that force cells to become more similar, such as conditions supporting naive pluripotency. Alternatively, direct lineage conversion approaches could be developed that bypass the pluripotent state and potentially the line-to-line variability altogether.

Reprogramming for Neural Repair

Magdalena Götz
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It feels like a long time ago that the concern of new neurons disturbing the complex brain networks was taken as an argument against adult neurogenesis. While we now understand all the positive aspects that new neurons can bring to an established network, it is still not known if and how new neurons also integrate into regions lacking endogenous adult neurogenesis. This question is not only pertinent for the entire field of neural repair, but also very important in the field of reprogramming, as it will tell us about the quality of the neurons that we generate. Would neurons differentiated from induced pluripotent stem cells be better than those obtained by direct reprogramming from various cell types in vitro or local glial cells in vivo? The challenge now is to move toward analyzing the in vivo integration and function of such neurons to learn about the precise identity induced by reprogramming as well as the plasticity of the pre-existing circuitry connecting to these newcomers. Indeed, generating the correct type of 10,000 or more different neuronal subtypes with their appropriate projections is a daunting task. Reprogramming shows us how well we really understand the specification of this multitude of subtypes and how well they can match the endogenous champions, young neurons from the developing brain. The exciting times ahead of us will finally connect neural repair and neuronal reprogramming with the fascinating area of neuronal connectomics and network function.

Cell Evaluation for Therapy

Koji Eto
CiRA Kyoto University

The transition from iPSC research to iPSC application will require a significant leap. Where suboptimal standards are acceptable for research, any experiments short of satisfying the strictest criteria will not proceed to the ultimate goal of innovative patient care. Masayo Takahashi and her team excited the field by conducting the first iPSC-based therapy in humans. At the same time, that breakthrough project has also demonstrated a need for consensus on the evaluation of iPSC-derived cells. Uncertainty in this matter resulted in the indefinite postponement of the next trial and will likely cause similar delays in future clinical research. To achieve this consensus, I believe the field should aim to attract scientific minds outside the usual medical and biological backgrounds. After all, the evaluation of cell quality for human care will require objective metrics. Including people with more mathematical acumen will not automatically provide us with a robust evaluation scheme, but researchers trained to study systems abstractly should help provide a framework for predicting the associated risks and therapeutic impact of genome or transcriptome modifications. The new opportunities provided by iPSCs have already expanded the demographic of stem cell researchers well beyond developmental biologists. Emphasizing the mathematic components will make stem cells into a complete science.

The Safety of Snowflakes?

Kristin Baldwin
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After a decade of research, it is clear that iPSCs can functionally mimic key properties of other pluripotent stem cells, offering a powerful new clinical cell source. Yet, because each iPSC line is genomically unique, appropriate safety tests for iPSC-based therapeutics must differ from those used for small molecules or biologics with defined invariant compositions. Unlike these treatments, therapeutics derived from iPSCs are likely to each derive initially from a different differentiated cell of a patient. Thus they will contain all the stochastically arising somatic mutations found in that cell. We are just beginning to appreciate that every skin, blood, or brain cell harbors a unique genome, peppered with hundreds or thousands of somatic mutations that likely accumulate with age. While these mutations may appear benign in their original context, we know little about their impact on iPSC-derived therapeutics. Mutations also accumulate in iPSC lines during reprogramming and culture. The threat of these may be underestimated at present due to the difficulty in detecting them in bulk iPSCs versus clonally derived cells. Finally, while most epigenetic changes are reset during reprogramming, iPSCs harbor residual epigenetic diversity that also may be stochastic. Together, these snowflake-like properties of iPSCs call for improved estimates of iPSC variation and genome-wide monitoring of iPSC-based therapeutics that takes this variation into account.

Chemical Reprogramming

Hongkui Deng
Peking University

Over the past decade, the breakthroughs in the iPSC field have revolutionized regenerative medicine. However, there are still some obstacles that need to be removed to fulfill the clinical potential of iPSCs, such as tumorigenicity, genetic abnormities, and manufacturing complexity. Small-molecule-based strategies for generating iPSCs may help overcome multiple manufacturing challenges because they are non-integrative, easy to standardize, and cost-effective. These advantages may enable large-scale applications and easier approval with GMP standards. Moreover, chemical compounds offer greater spatiotemporal flexibility in regulating multiple signaling pathways and epigenetic status, which potentially enables instant fine-tuning for lineage reprogramming and generation of high-quality iPSCs. Previous studies have shown that mouse somatic cells can be reprogrammed into iPSCs by pure small-molecule combinations. Excitingly, the generation of human iPSCs by small molecules should be available in the near future and will open the door to broad applications of this technology. To ensure this, it will be important to optimize robust methods for generating chemical-only hiPSCs with high efficiency and fast kinetics. When combined with direct lineage differentiation by small molecules in the future, the chemical reprogramming platform could be a powerful and safe platform for applications in therapeutic practice and may offer new insights into manipulating cell fate in vitro and in vivo.

Better Disease Models

Guo-li Ming
Johns Hopkins University

Human iPSCs (hiPSCs) offer immense opportunities for deciphering mechanisms of human diseases. To make an impact in the clinic, however, a better recapitulation of the complexity of human tissue is a prerequisite. The ability to grow brain organoids from hiPSCs that recapitulate the architecture and unique properties of developing human brains, such as the presence of human-enriched outer radial glial stem cells (oRGs) and stratified cortical layers, exemplifies how advances in iPSC technology may revolutionize our understanding of early human brain development. For example, by lineage-tracing of oRGs and monitoring brain organoid development, we can address previously intractable questions about the origin and destiny of oRGs and their contribution to cortical expansion and human brain evolution. Beyond these basic insights, organoid technology provides unprecedented opportunities to dissect the pathogenesis of developmental brain disorders, including congenital brain abnormalities and psychiatric disorders. Using brain organoids generated from patient iPSCs combined with modern techniques in genome-editing and "omics" studies, we can start to address causal relationships between specific genetic or environmental insults and pathophysiology and investigate underlying mechanisms. When these models better approximate in vivo tissue complexity and recapitulate neuronal circuitry, they will enable development of rational therapeutics based on gained mechanistic insights.