BioSkryb GENOMICS

WhitePaper

Comprehensive, Single-Cell Multiomic Analysis Is Needed for Safer Cell and Gene Therapies Based on Induced Pluripotent Stem Cells

Key Takeaways:

- **Induced pluripotent stem cell (iPSC)-based regenerative medicine therapies hold promise for restoring tissue function.**
- **Genomic and transcriptomic variation in iPSC culture can lead to unintended consequences, especially when coupled with gene editing technologies like lentivirus, adeno-associated virus (AAV) or CRISPR/Cas9.**
- **Precise and accurate single-cell whole genome sequencing, enabled by primary template-directed amplification (PTA) found in ResolveDNA® kits, can accurately detect on-and off-target mutations following CRISPR/Cas9 gene editing.**
- **ResolveOME™, a single-cell whole genome and transcriptome workflow, further enables investigators to draw novel links between genomic edits and gene expression, improving understanding of events that may impact safety and efficacy of iPSC-based therapies.**

To talk to a BioSkryb Genomics scientist email:

science@bioskryb.com

The public health impact of cell and gene therapies is likely to be measured on the same scale as the great medical advances of the previous three centuries: antibiotics, anesthesia, and vaccines. However, significant challenges remain with today's cell and gene therapy technologies, foremost the potential for inducing unintended and variable genomic edits and gene dysregulation, which can reduce the safety and efficacy of these therapies. With more sensitive single-cell and low input DNA analysis methods, cell and gene therapy developers will have the tools to detect off-target genomic edits and transcriptomic expression changes in single cells and population subsets, increasing confidence in therapeutic safety and efficacy.

Induced pluripotent stem cell (iPSC)-based regenerative medicine therapies hold promise for restoring tissue function

iPSC-based therapies, which involve the harvest, reprogramming, and re-introduction of patient cells, have shown great potential for regenerative medicine. Reprogramming involves the use of transcription factors that induce pluripotency, returning differentiated cells to a pluripotent state. They are then capable of unlimited, self-renewing growth, and possess the potential to

mature into an array of differentiated cell types (**Figure 1**).[1]

In vitro maturation permits the generation of unlimited quantities of differentiated cells in culture, which are re-introduced into the patient as a transplant, graft, or transfusion. *In vivo* maturation of iPSCs occurs within body tissues according to the signals in the tissue microenvironment. Therapies employing this method may restore cells that have been lost or damaged due to injury and disease.[1]

While iPSC therapies are still in an early stage, multiple Phase I trials have demonstrated the safety of iPSCs in limited applications, and more Phase I/II trials are ongoing. Conditions currently under study include macular degeneration, congenital heart disease, respiratory failure, ALS, multiple myeloma, and cystic fibrosis.[2]

Genomic and transcriptomic variation in iPSC culture can lead to unintended consequences

The nature of iPSC production makes these cells prone to oncogenesis. Factors used to induce pluripotency play critical roles in cell growth, differentiation, and apoptosis. Unsurprisingly, these same factors are frequently upregulated in cancer, and associated with metastatic potential and poor prognosis.[3] The oncogenic potential of iPSCs is often associated with the silencing of tumor suppressor genes.[3]

Detection and characterization of oncogenic potential in iPSC cell populations is complicated by their heterogeneous nature. While the majority of such heterogeneity in iPSC cells is related to genetic variation between – and within – the individuals from whom cells are harvested, including variances in both coding

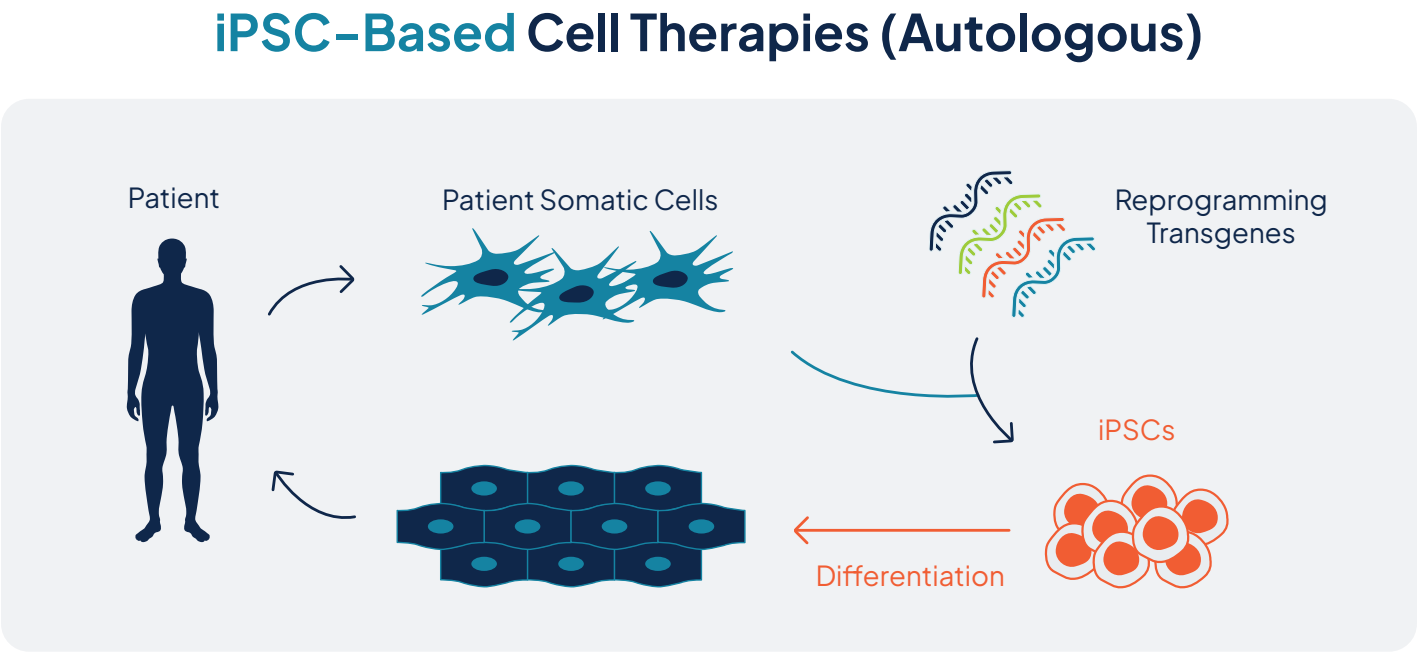

Figure 1. Induced pluripotent stem cells (iPSCs) hold therapeutic potential to restore lost or damaged cells. For autologous iPSC-based therapies, a patient's differentiated somatic cells could be induced to take on a pluripotent stem cell state using transgenic reprogramming factors, then re-differentiated to the cell type of interest using the right culture conditions. Genomic and transcriptomic variability may originate from mutations in the somatic cells used, transgenic insertional mutagenesis, and/or incomplete differentiation.

and noncoding regions, genomic variants may also be introduced during cell culture.[4] Observed genomic variations in iPSC lines frequently involve genes related to stem cell maintenance and differentiation, and may confer selective advantages that result in clonal expansion of these lines in culture.[4] iPSC heterogeneity may also be caused by gene expression differences unrelated to genotype, which may be induced by culture conditions, culture duration, environmental factors, or incomplete reprogramming.[4] Regardless of the root cause, these variations in cell identity can lead to a mixed population of cell phenotypes, even in lines which are, or appear to be, fully differentiated. The issue of incompletely differentiated cells existing within populations of apparently mature or terminally differentiated cells is a particular concern due to these cells' inherent potential for limitless growth.[5]

Additionally, when inducing pluripotency by transient adeno-associated virus (AAV) transduction of transcription factors, random integration events may result in persistent expression of these transcription factors.[6] Transduction techniques for generating iPSCs also have the same potential for insertional mutation as their respective vector.

Characterizing iPSC-based cell and gene therapy products with single-cell genomics and transcriptomics

iPSC-based therapies hold immense promise for tissue regeneration, but these cells are also highly prone to acquiring pathogenic mutations that may result in malignancy during expansion in their target tissue. Today there is greater scrutiny around characterizing unintended consequences of cell therapies. For example, in the United States, the Food and Drug Administration (FDA) has created guidelines concerning the assessment of safety when developing cell therapy products.[7]

While best practices are being developed by regulatory agencies, what features should investigators require in their assays and analysis plans?

Single-cell resolution. Not all cells will successfully undergo reprogramming to a pluripotent state and some cells will harbor harmful mutations. Only through single-cell analysis can investigators successfully identify which iPSCs have acquired the correct features without deleterious mutations.

Even allelic balance. Mutations in iPSCs may only occur in one copy of the gene. Only through assays demonstrating good allelic balance can investigators be confident that both alleles are without pathogenic mutations.

High sensitivity and precision in SNV calling. Unintended mutations in iPSCs may be as small as a single base pair. Leveraging an assay with high precision and sensitivity enables researchers to confidently determine whether such mutations have been acquired.

Uniform whole genome coverage. Unintended AAV-vector insertions can occur throughout the genome and mutations in non-coding regions can result in mutagenesis. An assay with uniform genome coverage enables better characterization of unintended mutations.

Bioinformatics analysis support. Single-cell whole genome sequencing has unique bioinformatics considerations. Identifying a collaborator or service provider with expertise in single-cell genomics is a critical aspect in streamlining data analysis.

With these considerations in mind, investigators require a comprehensive solution that enables them to analyze the entire genomes of single cells.

ResolveDNA from Bioskryb Genomics leverages primary template-directed amplification (PTA)

to enable precise and accurate single-cell whole genome sequencing. PTA chemistry, available only through BioSkryb Genomics in ResolveDNA and ResolveOME kits, overcomes biases, allelic dropout, low and variable genome coverage, poor reproducibility, and artifacts associated with existing whole genome amplification approaches.[8, 9] This patented chemistry has been used to characterize the variability of on-target edits and catalog the number of off-target indels, copy number variations, and translocations that occur in individual cells following gene editing with CRISPR/Cas9.[8, 10]

For investigators who want to move beyond inference of gene expression and define the mechanisms of gene expression on a single-cell level, BioSkryb Genomics offers ResolveOME.[11] ResolveOME provides single-cell genome and transcriptome amplification in a unified workflow. This approach combines PTA-mediated whole genome amplification with full-transcript reverse transcription, allowing novel links to be drawn between genomic and transcriptomic changes. For example, in acute myeloid leukemia cells with acquired resistance that demonstrated increased *CEBPA* expression without genomic copy number increases, ResolveOME enabled the identification of a candidate distal promoter/enhancer SNP approximately 20kb 5' of the *CEBPA* transcriptional start site.[11] Deploying ResolveOME in iPSC-based therapies could lead to similar discoveries, notably which mutations lead to gene and cell type expression changes.

These complex analyses require bioinformatics support. Supporting the analysis and interpretation of data generated with ResolveDNA and ResolveOME is BaseJumper®. BaseJumper is a bioinformatics platform built for biologists that enables multiomic data analysis. With this suite of products, along with custom service offerings through ResolveServices℠, BioSkryb Genomics provides solutions for more thorough characterization of iPSC-based therapies.

To talk to a BioSkryb Genomics scientist about how ResolveDNA, ResolveOME, ResolveServices, and BaseJumper can empower research with iPSC-based therapies, email a member of our team at:

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2810 Meridian Pkwy, Suite 110, Durham, NC 27713

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