Main Submission

Query into the Future of Gene Editing: Possibilities and Apprehensions

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Since the discovery of 22,300 protein-coding genes by the Human Genome Project, geneticists have generated tools to manipulate DNA using engineered nucleases such as clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) (1-3). Concurrent advancements in stem cell research have allowed for de-differentiation of somatic cells back to a pluripotent state, known as induced pluripotent stem cells (iPSCs), which can then be re-differentiated into any cell type (4). This review is focused on the safety and ethical concerns of the intersection between gene editing and iPSCs to create genetically corrected injectable cells for therapeutic purposes. To emphasize the importance of genetically engineered injectable cells, we will discuss the application of this approach for treatment of chronic disorders, prevalent in the western world.

Although the use of genetic engineering allows for site-specific genetic alterations (3), future consequences of this approach remain in juvenile stages. Older gene editing techniques, such as transcription activator-like effector nuclease (TALEN) and adeno-associated virus (AAV), present with limitations of non-specific site targeting, cytotoxicity, and low vector transfer efficiency. In one case of heart disease, AAV vectors caused fever and muscle spasm in patients, emphasizing the importance of performing prior safety trials (5). Fortunately, the new CRISPR/Cas9 has been revolutionizing the field by its simplicity, low toxicity, and high efficiency. The ability to simultaneously deliver multiple single-guided RNAs using CRISPR/Cas9 has allowed for editing genes in polygenetic forms of diabetes and heart disease (6). The first clinical trial is currently being conducted using CRISPR/Cas9 in a small human lung cancer population, in which the safety of these methods will be monitored.

The employment of iPSCs in gene editing allows for the introduction of desired cell-specific genetic alterations (4). However, even less is known regarding future consequences of this approach. Risks associated with using stem cells for gene editing include the type of cells used, the procurement, culturing, the level of manipulation, and site of injection. These risk factors may lead to tumourigenesis, immune activation, and bio-transmission of pathogens. Limitations in safety databases, such as low numbers of treated patients and limited long-term follow-ups, leads to a lack of scientific understanding of the long-term consequences (7). Currently, scientists are investigating ways to manage these risks. To better address the immune rejection issues of iPSCs, scientists are investigating the use of CRISPR/Cas9 to form universal donor stem cells (UDSCs), which lack antigens that are usually targeted by the immune system (8).

With these limitations in perspective, scientists are monitoring the application of gene-edited iPSC-derived injectable cells in cultures and small clinical trials. This novel approach has been applied to treatment of monogenetic cardiovascular disease and type I and II diabetes (T1D and T2D, respectively). One recent study corrected phospholamban-dependent cardiomyopathy and generated human PSC-derived cardiomyocytes (9). Genetically engineered iPSCs have also been applied to T1D, a disease resulting from the autoimmune destruction of pancreatic β-cells. It has been proposed that by differentiating β-cells from UDSCs, the immune attack can be bypassed (8). Concordantly, genome editing has been used in T2D, a disease that results in peripheral tissue insulin resistance and pancreatic β-cell exhaustion. Furthermore, genome-edited iPSCs have been used to show that the haploinsufficiency of key insulin-related genes is sufficient for early exhaustion-induced β-cell death, identifying targets for gene correction (10). Moreover, we propose that these methodologies may be applied to re-inject iPSC-derived hematopoietic stem cells, genetically engineered to be less pro-inflammatory, to dampen the inflammation and insulin resistance in peripheral tissues.

The findings of these studies, which reaffirm the importance of this field, need to be cautiously assessed at every stage. The obscurity in future consequences

of genome editing also challenges the Hippocratic oath of non-maleficence. If genome editing is the future direction of medicine, there needs to be a consensus on the extent of its clinical application. There is the likelihood of this technique being first available to the wealthiest, and used for purposes of self-improvement instead of the treatment of life-threatening illnesses. Critics argue that the fiscal load of genome editing will not allow its advancement into medical practice. However, the cumulative lifetime financial and medical burden of chronic disorders begs for an alternative approach that may not only manage these diseases but treat them. Therefore, before genome editing reaches this capacity, there needs to be an open dialogue for establishing governmental policy framework, patents, and regulations that are systematically monitored.

Today, the application of genetic engineering on iPSCs is an attractive approach that removes the drawbacks of donor genetics. While there remain several safety and ethical issues with this method, the discoveries achieved for cardiomyopathies and diabetes from this scientific intersect cannot be disregarded. Therefore, it is important to further navigate the ethical and safety concerns through immediate strategic actions by scientists and governing bodies, and to accelerate this treatment for these highly prevalent and chronic diseases. ■

References

1. Pertea M, Salzberg SL. Between a chicken and a grape: estimating the number of human genes. *Genome Biol*. 2010;11(5):206.
2. Marraffini LA. CRISPR-Cas immunity in prokaryotes. *Nature*. 2015;526(7571):55-61.
3. Brookhouser N, Raman S, Potts C, Brafman DA. May I cut in? Gene editing approaches in human induced pluripotent stem cells. *Cells*. 2017;6(1).
4. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126(4):663-676.
5. Pacak CA, Byrne BJ. AAV vectors for cardiac gene transfer: experimental tools and clinical opportunities. *Mol Ther*. 2011;19(9):1582-1590.
6. Kelly M, Semsarian C. Multiple mutations in genetic cardiovascular disease: a marker of disease severity? *Circ Cardiovasc Genet*. 2009;2(2):182-190.
7. Herberts CA, Kwa MS, Hermsen HP. Risk factors in the development of stem cell therapy. *J Transl Med*. 2011;9:29.
8. CA C. *Universal donor stem cells: Removing the immune barrier to transplantation using gene editing*. Presented at: American Diabetes Association 76th Scientific Sessions. 2016 June 10-14.
9. Karakikes I, Stillitano F, Nonnenmacher M, Tzimas C, Sanoudou D, Termglinchan V, et al. Correction of human phospholamban R14del mutation associated with cardiomyopathy using targeted nucleases and combination therapy. *Nat Commun*. 2015;6:6955.
10. Zhu Z, Li QV, Lee K, Rosen BP, Gonzalez F, Soh CL, et al. Genome editing of lineage determinants in human pluripotent stem cells reveals mechanisms of pancreatic development and diabetes. *Cell Stem Cell*. 2016;18(6):755-768.

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Parastoo is a PhD candidate in the Department of Biochemistry at the University of Toronto. She completed her Bachelor of Science studying biochemistry, human biology and physiology at the University of Toronto in 2015. As her undergraduate research, she investigated the effect of Korean white ginseng on cardiovascular disease and type II diabetes. Her current research is at the intersect of immunology and metabolic disease and investigates the contribution of diet induced obesity on the developing immune cells. She has been awarded the Banting and Best Diabetes-Novo Nordisk Studentship for 2017-2018.

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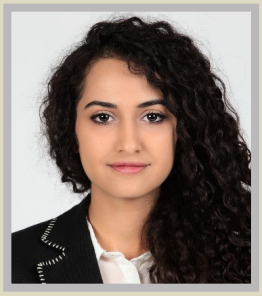
Neshat graduated with high distinction from a Bachelor of Science at the University of Toronto. During her undergraduate training, she was involved in several research projects focusing on food intake regulation, and dietary control of peptide hormones. She is currently finishing her Master of Science in nutrigenomics, also at the University of Toronto, where she investigates the effects of genetic variation in Fructokinase and Aldolase B on biomarkers of the metabolic syndrome.



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Farigol received her honours BSc with high distinction from the Department of Physiology at the University of Toronto. With an extensive family history of cardiovascular diseases and diabetes, she was inspired to contribute to these areas of research. She began her research on the myogenic response in the microvasculature of stroke animal models. She then worked on generating organoids (intestinal buds) from ileal stem cells to study glucagon-like peptide-1 and its effects on insulin secretion. She is currently pursuing her MSc at the University of Toronto. She is the recipient of a Queen Elizabeth II Graduate Scholarship for 2016-2017.