Main Submission

Next Steps in Cancer Therapy: Integration of Gene Editing in Inducible Pluripotent Stem Cells

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When Paul Berg, the father of genome engineering, received the Noble Prize in 1980 for inserting the lambda phage gene into monkey Simian 40 oncogenic virus, a revolutionary era began. Since then, several tools have been created to optimize the manipulation of DNA for targeted gene editing, such as adenoviral transduction vectors, zinc finger nucleases, transcription activator-like effector nucleases (TALENs), and the new and simple clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9). Of these, TALENs and CRISPR/Cas9 are the predominant tools for clinical cancer therapy used with injectable differentiated cells. We foresee future intersections between genome editing and stem cell-based techniques that will pave the way for the introduction of universal donor stem cells (UDSCs) in the treatment of cancer.

Today, many cancer researchers are continuing Berg's path of gene editing while exploiting cancer hallmarks. Cancer manifests itself through sustaining proliferative signaling, inhibiting the activity of growth suppressors, impairing DNA repair and apoptotic mechanisms, deregulating normal epigenetic patterns, inducing chemo-resistance, and enhancing excessive angiogenesis, leading to uncontrollable invasion and metastasis (1). Many have used CRISPR/Cas9 to manipulate cancer-related genes in human-derived cells and human cell lines to understand lymphoma, lung cancer, and various other types of cancers. Findings from these cell-based genome-editing studies warrant the application of genetically engineered cells in a clinical setting (2,3).

These cell-based discoveries led to the introduction of genetically engineered cell therapy in clinical trials for the treatment of cancer. The first application of this method used genetically engineered immune cells to target and attack cancerous cells. One success story is the trial for the treatment of two leukemic infants. This group injected donor-derived TALEN engineered T cells into the cancer patients, while concurrently using immunosuppressive chemotherapy to avoid immune rejection of the injected cells designed to attack the cancer cells (4). Furthermore, an ongoing lung cancer clinical trial using CRISPR, headed by Dr. Lu You, utilizes a similar strategy of immune cell enhancement to provide targeting of cancer cells (5). If this trial succeeds, it will further emphasize the significance of gene-edited injectable cell therapy in developing future clinical cancer treatments. However, the concurrent immunosuppressant usage in the conducted studies points to the persistent risk of immune rejection of the injected cells.

Attempts to minimize the risk of immune rejection have used inducible pluripotent stem cells (iPSCs) in integration with gene editing for cancer therapy. In this method, the patient's somatic cells are collected, de-differentiated into self-renewing iPSCs that can be genetically modified, and then re-differentiated into any cell type of interest for injection into the same patient. This approach avoids the risk of immune rejection of the immunotherapy. However, the generation of iPSCs and the reprogramming into fully differentiated immune cells faces its own challenges. It can be costly, time-consuming, and highly variable in epigenetic status, genomic stability, and the pluripotency potential of differentiating into various cell types. Moreover, autologous immune cell rejection may still occur due to unpredicted alterations in the surface antigens of the iPSC-derived immune cells (6).

To further bypass the challenges of immune rejection when using personalized iPSCs, groups are currently employing the CRISPR/Cas9 system to generate UDSCs that are devoid of antigens typically targeted by the immune system (6). Future success in such studies will lead to the generation of UDSC line banks to readily provide countless possibilities of differentiated cells containing the desired genetic modification, specifically designed for each patient. Moreover, UDSC lines can be generated using various human stem cell types, such as iPSCs and bone marrow-derived multipotent progenitor cells (6,7).

Interestingly, while several groups are comparing TALEN and CRISPR independently, others are combining them to develop inducible-CRISPR methods, which can promote genetic modification at multiple loci following iPSC stimulation. The application of these technologies is crucial for mechanistic interrogation of complex and pleiotropic genetic mutations that are often observed in cancer genetics (8,9). Additionally, efforts have been undertaken to improve CRISPR/Cas9 technology for use in the clinical field to address the off-target concerns and to investigate the integration of the entire Cas9 plasmid construct into the targeted genomic loci (10). In the future, these improvements can be applied to UDSCs, leading to more precise and patient-specific genetic modifications proceeding the injection.

The era in which gene editing applications link the basic science field to the clinical world is approaching faster than anticipated. TALEN has already been successfully used in donor T cells for the treatment of leukemia. Simultaneously, CRISPR/Cas9 is now recognized as one of the simplest, easiest, and most efficient gene editing technologies. Today, the CRISPR/Cas9 system is being tested to treat lung cancer for the first time in humans. We envision the integration of TALEN and CRISPR/Cas9 with personalized iPSCs, to thereby generate UDSCs, as the next promising step towards enhancing cancer therapy. ■

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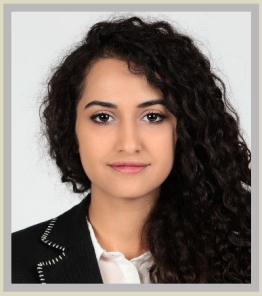

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Farigol received her honours BSc with high distinction from the Department of Physiology at the University of Toronto. With an extensive family history of cardiovascular diseases and diabetes, she was inspired to contribute to these areas of research. 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.