Base editing and stem-cell based therapies

Twenty-five years ago James Thomson's research group at the University of Wisconsin-Madison reported the successful extraction and in vitro culturing of human embryonic stem cells (ESCs) — pluripotent stem cells derived from fertilized human embryos — which had the ability to differentiate into any type of cell present in the human body.[1] From this discovery, the potential of ESCs was immediately apparent.

While this landmark discovery generated considerable excitement in the scientific and medical communities, it was tempered by concerns related to the source and supply of ESCs as well as significant ethical considerations. In the United States, a 1996 law prohibited federal funding for research involving the creation or destruction of human embryos, and in 2001 President George W. Bush banned federal funding for research on any newly created human embryonic cell lines.[2] While President Barack Obama revoked his predecessor's ban in 2009, the 1996 law has continued to hamper US-based researchers.[3]

The birth of induced pluripotent stem cells (iPSCs)

Despite rapid progress being made in countries that supported stem cell research, the ethical concerns of destroying human embryos for the purpose of harvesting ESCs remained a global commonality. These concerns were largely negated in 2006 when Kazutoshi Takahashi and Shinya Yamanaka reported they had successfully converted murine somatic cells into pluripotent stem cells, calling them "induced pluripotent stem cells" (iPSCs).[4] The following year, Takahashi, Yamanaka, and co-workers reported the successful conversion of adult human dermal fibroblasts into iPSCs.[5] A month after Yamanaka's report, the Thomson Group published their own findings wherein they converted human somatic cells into iPSCs.[6]

Because iPSCs are derived from fully differentiated somatic cells, they avoid the ethical quandaries and governmental red tape associated with their embryo-harvested counterparts. Furthermore, as the precursor somatic cells are taken from an individual patient, iPSCs can be used for autologous (self-donor) therapy. This removes the potential for immune rejection of any patient-matched iPSC-generated transplant/infusion and, in theory, enables any individual to have their own line of iPSCs to use as-needed. The fact that iPSCs can be generated from a multitude of mature human cells including blood cells, skin cells, and even renal cells found in urine, means the supply constraints of ESCs are not a concern.[7,8]

iPSCs stimulate intense research efforts

Since their discovery, iPSCs have been the subject of intense research efforts. Their role as precursors for the generation of tissue, organ, and disease models has been

especially useful for the screening and rational repurposing of drugs, and crucial for furthering our understanding of developmental biology and disease pathophysiology. Meanwhile, their ability to differentiate into any human cell or structure has naturally led to a particular focus upon regenerative medicine. A Japanese patient with macular degeneration was the first to undergo iPSC-based therapy; while not curative, the lack of negative events and the positive patient-reported outcomes spurred further clinical trials in both Japan and the United States.[9–12] Researchers have also shown that β-like pancreatic cells with glucose-responsive insulin production can be generated from iPSCs, representing a potential treatment for diabetes.[13] iPSC-induced regeneration of cardiac tissue and function following acute cardiac injury has been demonstrated in a variety of animal models, offering hope for future therapeutic applications.[14]

Other research has focused upon a variety of neurological conditions. Yuan and co-workers showed that iPSC-derived neural stem cells were able to survive transplantation into rat models of ischemic stroke, that they spontaneously differentiated into neurons and astrocytes, migrated to ischemic areas, and improved neurologic function compared with the control groups.[15] The use of iPSC-based regenerative therapies for treating spinal cord injuries has shown promise in many rodent-based studies, and the first trial in humans has been approved but delayed by the COVID-19 pandemic.[16,17]

Gene editing opens new doors for iPSCs

Recent advances in gene editing technologies have led to rapid progression of adoptive cell immunotherapies which leverage the body's immune system against diseases such as cancer. Antitumor activity of immune effector cells has been enhanced in a directed manner through insertion of chimeric antigen receptors (CARs) which target a protein on the surface of malignant cells. This approach has typically been investigated using T cells, and while there have been demonstrated successes against CD19- expressing hematologic cancers, solid tumors have proven to be recalcitrant. Additionally, the process of generating these CAR-T cells is logistically complex, expensive, and requires healthy T cells. Natural killer (NK) cells derived from iPSCs have a number of beneficial qualities including the ability to generate large numbers, improved safety profiles, and cytotoxic activities that do not require human leukocyte antigen (HLA) matching; this allows for the simpler development of NK-based allogeneic immunotherapies.[18] By engineering iPSC-derived NK cells to express novel CARs, Li and co-workers were able to target ovarian cancer cells in a murine xenograft model and improve survival.[18] Genetic engineering has also been utilized to improve the *in vivo* proliferation and persistence of NK cells, and encouraging preclinical results have laid the groundwork for the initiation of multiple clinical studies targeting cancers such as leukemia, pancreatic cancer, and prostate cancer.[19]

CRISPR/Cas9 has revolutionized the field of adoptive cell immunotherapy, as the gene editing technology is comparatively simple relative to older gene editing strategies and highly scalable. By introducing or knocking out target genes in iPSCs, researchers can create unlimited quantities of cells bearing specific mutations for use in model systems of disease. CRISPR/Cas9 editing has also been used extensively in the research and development of CAR-T cell therapies, which has both highlighted its utility as well as revealed inherent vulnerabilities.[20] In particular, the mechanism of action of CRISPR/Cas9 involves the introduction of DNA double-strand breaks (DSBs) which can activate p53-dependent DNA damage response pathways and lead to mutagenesis events such as translocations and chromosomal rearrangements. As more genes are identified as being either beneficial or detrimental to immune effector efficacy and persistence, it becomes necessary to introduce greater numbers of genetic modifications. However, as multiple edits are simultaneously carried out using CRISPR/Cas9, the free DNA ends resulting from DNA DSBs can anneal and repair in undesired and unpredictable ways, potentially leading to activation of oncogenes or inactivation of tumor suppressor genes. These off-target and potentially dangerous events represent a significant drawback of CRISPR/Cas9-based production of immunotherapies.

Unlocking the full potential of iPSCs with base editing

To address this shortcoming, the research groups of David Liu (Harvard University), Akihiko Kondo (Kobe University), and Shengkan Jin (Rutgers University) have developed an alternative strategy called base editing.[20] This approach alters single nucleotides by exploiting DNA mismatch and base excision repair pathways, and does not rely upon DSBs Base editors are able to chemically alter single nucleotides, leading to conversion of cytosine

(C) to thymine (T) and adenine (A) to guanine (G). They are composed of a catalytically impaired Cas nuclease for targeted DNA binding and an effector protein which catalyzes the chemical conversion of one nucleotide to another through deamination. Base editors can be used to inactivate target genes by introducing premature stop codons or splice site disruptions through single nucleotide conversions, and because no DSBs are introduced, the risks of translocations and chromosomal rearrangements are abrogated.[21] Base editing technologies have become incredibly precise and efficient, with multiplex base editing becoming the norm for making multiple simultaneous edits without detectable translocations.

The goal of many researchers is to advance the therapeutic technology to the point where allogeneic, "off the shelf" therapies are available, meaning an immunotherapeutic could be mass produced and administered to anyone. iPSCs and their ability to generate unlimited numbers of cells offer an alternative approach to harvesting T cells from healthy donors for this purpose. Differentiation into NK or T cells and subsequent genetic engineering with base editing could be the combination which leads to this goal being achieved. As iPSCs continue to be used for research and generating immune effector cells for immunotherapies, it is of paramount importance to ensure their safety profiles are robust. By using base editing for genetic modifications rather than CRISPR/Cas9, researchers are able to introduce multiple edits to improve *in vivo* persistence, proliferation, and efficacy with high efficiency and low risk of off-target effects. The benefits extend to tissue and organ regeneration as well, as researchers are able to precisely manipulate iPSCs with base editing to direct and improve outcomes. The two technologies, both of which are rapidly expanding, complement one another and should propel the field forward. Important concerns regarding safety profiles and *in vivo* persistence remain and are currently being evaluated, and standardization methods are being developed to ensure predictable results. Regardless, the impacts of base editing and iPSCs upon the fields of basic science, regenerative medicine, and immunotherapy are already significant despite their recent discoveries. It is not too optimistic to think that the combination of iPSCs and base editing could produce multiple breakthroughs in multiple fields and diseases in the coming years.

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