EDITING THE ENGINEERED: AN AMALGAMATION OF IPSC AND BASE EDITING TECHNOLOGY

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Abstract

Induced pluripotent stem cells (iPSCs) are stem cells with the ability to differentiate into different cell lineages and are derived from adult somatic cells. iPSCs allow for the *in vitro* study of various disorders, thus providing an opportunity for therapeutic intervention when combined with gene editing. Gene editing is defined as the precise modification of a gene and its most common application is to modify a genotype which is responsible for the disease phenotype making it the basis of treating monogenic disorders. Well-established protocols for the generation of genetically corrected patient-derived iPSCs involve first the reprogramming of somatic cells to iPSCs, which are then subjected to gene editing. This however leads to longer culture time with higher risk for *in vitro* alterations. Simultaneous base editing and reprogramming of somatic cells thereby pave the way for a straightforward approach to circumvent this, providing high efficiency of reprogramming and editing while simultaneously reducing culture time and *in vitro* changes drastically.

iPSCs: a renaissance in stem cell biology

The advent of induced pluripotent stem cell technology [1] has brought with it immense potential applications and has reshaped our approach to experiments in human genetics, stem cell biology, and biomedical research [2,3]. Ectopic expression of a set of reprogramming transcription factors in a somatic cell can direct the cell into a pluripotent state, transforming it into what is called the induced pluripotent stem cell (iPSC). The applications of iPSCs are wide-ranged from disease modelling and drug screening to personalised cell-based gene therapy[2–4]. This broad spectrum of uses can be attributed to the uncomplicated and systematic reprogramming process in the presence of a cocktail of transcription factors, also known as the Yamanaka factors (*Klf4*, *Sox2*, *Oct4,* and *cMyc*)[5]. In addition, iPSCs maintain their state of pluripotency even after they have undergone extensive rounds of culturing, retaining their ability to be reprogrammed to any cell of the body. Gene editing in iPSCs is a specific application that is aimed towards the better understanding of the pathophysiological mechanisms that underly genetic diseases [6]. This is specifically the case in monogenic disorders, which involve a mutation or dysfunction in a single gene that eventually causes a disease phenotype. Editing the gene implicated in a disorder to induce the disease mutation can be used to model the disease or to generate genetically corrected patient-derived iPSCs. This thereby presents an opportunity for therapy using a combined approach of iPSC technology with gene editing techniques [7].

Base editing: the molecular 'search and replace' tool

Base editing is a CRISPR/Cas9-based genome editing technique that can be used to induce a point mutation in iPSC control lines or to correct a pathogenic mutation in patient-derived iPSCs[8]. The characteristic feature that makes this technique desirable is its ability to edit without the introduction of double strand breaks (DSBs) in the genome. Unrepaired DSBs, being lethal to cells, have diverse mechanisms of repair, which can result in several on and off-target DNA indels (insertions and deletions), chromosomal aberrations and eventually apoptosis [9]. The base editor's ability to surpass this gives it an edge over the conventional CRISPR/Cas9 nuclease system. Base editors are primarily composed of two parts, namely, the catalytically dead Cas9 (dCas9) and an enzyme with DNA modifying ability. dCas9 is catalytically inactive due to mutations present in its structure: Asp10Ala and His840Ala, which render its nuclease function inactive while retaining the ability to bind DNA in a single guide RNA (sgRNA) programmed manner[10]. The conjugation of this dCas9 with the DNA modifying enzyme directs the conversion of the bases in a direct and irreversible manner without the induction of DSBs [10,11]. The dCas9 domain binding, once directed by the sgRNA at the target site, further causes the single stranded DNA (ssDNA) to be edited to form an R loop. This ssDNA R-loop is the substrate for the DNA modifying enzyme where base editing occurs. There exists a window of editing, a narrow region of bases in the DNA which are exposed by the dCas9 binding leading to the action of the DNA modifying enzyme and consequent gene editing.

Deamination of the cytosine base to adenine occurs about 100-500 times in a day per cell in humans. This same base change is associated with half of the known pathogenic single nucleotide polymorphisms (SNPs) [12,13]. The conversion of the A.T base pair to a G.C pair at the targeted loci is the most straightforward way to correct the disease-causing mutation in the gene[14,15]. Adenine base editors (ABE) are intended for this particular base change: an ABE-dCas9 fusion binds to the DNA sequence in a sgRNA-programmed way where the DNA modifying enzyme i.e., the deoxyadenosine deaminase, catalyses the adenine to inosine base change. Inosine is recognized as a guanine during DNA replication and as a result the A.T pair is subsequently replaced by a G.C pair. A combined use of the base editors through an efficient RNA-based delivery system with simultaneous vector reprogramming of somatic cells leads to the single straightforward editing of cells while generating a monoclonal human iPSC line. This methodology is one with unprecedented efficiency where the simultaneous approach also drastically reduces the culture time and thus the risk for any alterations that occur *in vitro.*

Treatment of monogenic disorders: are iPSCs the yin to the gene editing's yang?

The foundation of combining gene editing with reprogramming somatic cells was laid by countless experiments which eventually led to the optimised protocol with a high efficiency in obtaining the desired results. The lengthy procedure of first reprogramming the cells into a pluripotent state, later being subjected to gene targeting, editing and subsequent selection of the desired genotype was found to be cumbersome and error-prone. It was also reported that with an increase in the duration of culture time, there is an increased chance of undesirable changes in genomic integrity; namely oncogenic gene activation, duplication, and induced karyotypic abnormalities [16,17].

One of the first attempts made towards combining the two processes involved the correction and reprogramming of fibroblasts obtained from patients which reported a targeting efficiency of about 8% in the iPSC population generated [18]. The targeting of the *DNMT3B* locus in the reprogrammed iPSCs was done from a fibroblast cell line of a patient suffering from retinitis pigmentosa which resulted in correction of the mutation. Another cell line was also used which was aimed at correcting the *ADA* and *PRPF8* gene loci of the patient derived cells which cause SCID. *DNMT3B* is a DNA base cytosine methylator and thus an epigenetic factor behind retinitis pigmentosa, an inherited retinal disease [19]. This protocol, however, depended on the function of a single-stranded oligonucleotide as it reduces the chances of Cas9 protein cutting after homologous recombination occurred while acting as a selectable marker for successful clones. The success of these experiments then paved the way for optimisation of the one-step protocol to increase the efficiency of correction.

The next attempt made at editing with simultaneous reprogramming aimed for the elimination of any steps involving the employment of a selectable marker [20]. This approach resulted in a substantial increase in the reprogramming efficiency solely due to the use of Cas9-Gem which made sure there were minimal secondary mutations. Cas9-Gem is formed by the fusion of Cas9 nuclease with the human geminin protein to ensure it gets degraded in the G1 phase of the cell cycle when non homologous end joining (NHEJ) DNA repair predominates. This fusion protein caused a two-to-three-fold decrease in the frequency of NHEJ, resulting in a bias towards the homology directed repair (HDR) pathway [20].

The most recent work in this field has resulted in further optimisation of this one-step procedure [21] wherein the use of a seventh generation ABE [22] called ABEmax coupled with reprogramming using episomal vectors (extrachromosomal closed circular DNA that replicates autonomously in the host cell which can be of bacterial or viral origin) was employed. High and transient expression of the ABEmax was employed with the use of an *in vitro* transcribed RNA construct that mimicked the human mRNA structure. Electroporation of patient-derived fibroblasts with this construct and the sgRNA led to the generation of hundreds of colonies that were monoclonal in nature. This process established about 96% efficiency while being free of DSBs, thereby reducing the risk of DNA damage. This protocol also reduced cell toxicity and off-target effects as the ABEmax components were all RNA-based. The use of episomal reprogramming vectors ensures pluripotency is maintained over passages while culturing [21].

Future prospects

With advances in stem cell technology and gene editing techniques, there is great potential for the one-step procedure of gene editing

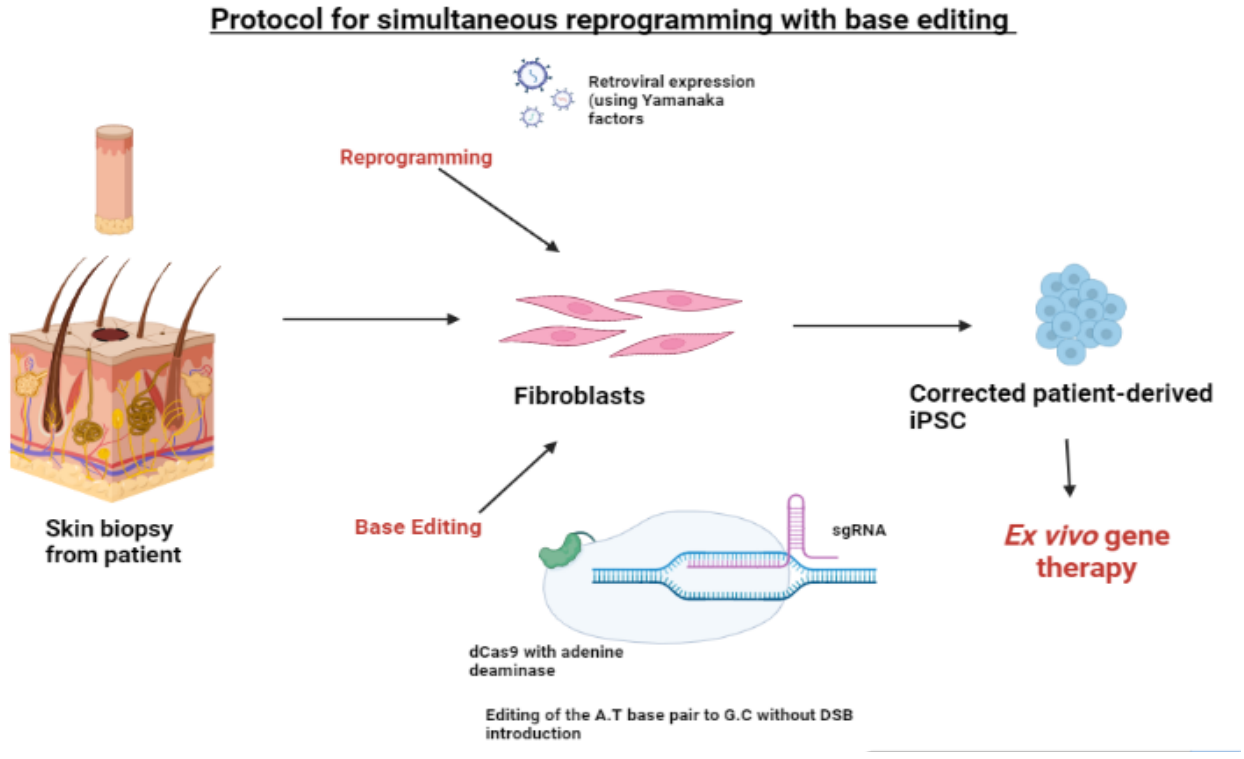

Figure 1: *One-step protocol consisting of simultaneous fibroblast reprogramming with base editing to generate genetically corrected patient-derived iPSCs (created using BioRender).*

and reprogramming to be employed for biomedical research, eventually providing therapy formonogenic diseases in a cleaner and more efficient way. With rapid advances in gene therapy towards clinical application [23], its use can be envisioned in the future where pathogenic SNPs with any of the four base changes can be edited in an accurate manner without DSBs and while avoiding any off-target edits, limitations due to the protospacer adjacent motif sequence, etc. Further work on the delivery of these constructs via nanoparticles shows immense potential for therapy development from bench to bedside [24–26].

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