Base Editing and Its Application to Cell and Gene Therapy

CRISPR-Cas gene editing has been a force to be reckoned with in gene engineering for years, and it doesn't seem likely this is going to change anytime soon

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Propelled by the promise of faster, cheaper, and more accurate tools to selectively alter particular sequences in the genome, the past decade has seen the field of gene engineering dominated by CRISPR-Cas gene editing. For all of their ease and rapidity, conventional CRISPR-based approaches have a substantial flaw when it comes to gene editing for therapeutic use: unmodified Cas9 proteins result in DNA double strand breaks (DSB), which, although tolerable for a research tool, pose an additional safety concern for cell or gene therapy. The generation of unintended DNA strand breaks can be mitigated by various approaches, but the more recent invention of base editing offers another solution. Base editing uses an 'attenuated' nickase version of Cas9 linked to a deaminase enzyme and can achieve gene knockout or gene correction through the alteration of single bases without the formation of DNA DSBs. Base editing is a relatively nascent field and one that is still finding its niche within the wider gene editing and gene therapy arena. To better understand the current and future uses of base editors, it is better to focus on the development of these editors and on the emerging data that indicate how base editors might be used to treat genetic-based diseases with unmet clinical need.

The Basics of Base Editors

CRISPR-Cas-based gene editing relies on a short guide RNA sequence to direct the Cas enzyme to a specific DNA sequence where it introduces a DNA DSB. The cells intrinsic DNA repair pathways detect and repair this break, but for cells in culture, most DSBs are repaired using an imprecise mechanism that commonly leads to random insertions and deletions (indels) of DNA base pairs. It is the random nature of these repairs that can be used to efficiently disrupt or knockout gene transcription (1). However, the rate of single base changes as a result of the DNA repair is low (0.1-5%), making this approach inefficient for modifying single base

pairs. Base editors are a derivation of the CRISPR-Cas system combining a modified version of the Cas enzyme only capable of generating a nick on one strand of the DNA, directly linked to a deaminase enzyme. They still make use of short guide RNA sequences to direct the complex to the target loci on the DNA, where the Cas enzyme nicks the non-target strand to promote the deamination of single base pairs of DNA.

Base editors have been configured with both cytidine (CBEs) and adenine deaminase enzymes in order to orchestrate C:G to T:A and A:T to G:C conversions, respectively (2-3). Thus, base editors are able to edit the majority of pathogenic mutations known to contribute to human disease. The first base editors made use of a catalytically dead Cas9 enzyme capable of binding DNA in an RNA-guided way but without introducing a DSB, but subsequent iterations using a nickase version of Cas9 were shown to be more efficient at biasing the DNA repair machinery towards the desired edited outcome. In the case of CBEs, the deaminase APOBEC1 targets cytidines within the editing window and deaminates them to give uracil. As uracil is not a standard base pair in DNA, this triggers a set of repair pathways within the cell. Most often, the offending U is converted back to C by a DNA glycosylase. To increase the conversion of the C to a T, uracil DNA glycosylase inhibitors have been added to the base editing complex to bias the conversion of the U to T. Once this change is made, the opposing guanidine is converted to A through another DNA repair pathway, giving a T:A base pair instead of the initial C:G pair (3).

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Since their initial configuration, the development of base editors has focused on enhancing desirable characteristics, such as editing a precise C or A; improving target specificity (minimising off-target effects); and reducing the already low levels of indel formation even further. Although initial modifications were tested using small-scale directed alterations, recent publications have developed screening approaches that examine vast numbers of base editing variants. APOBEC1, the deaminase commonly used for CBEs, has been shown to deaminates Cs that are preceded by a G (GC sequences) poorly, and the position of the target C within the base editing window also impacts editing efficiency. Using phage-assisted continuous evolution deaminase variants, such as evoAPOBEC1-BE4max, with improved on-target editing, reduced sequence context requirements have been identified (4).

The Issue of Off-Targets

Off-target effects are not limited to chemical and biologic drugs and are a considerable issue for gene editing. The ability to detect any unwanted edits is an area of intense research, which is needed to facilitate the use of CRISPR-based editors in the generation of human therapeutics. For base editors, off-targets can manifest in several forms. Bystander edits – base changes occurring outside of the target residue but within the editing window – are a difficult area to address and vary with the target gene and sequence within the editing window. These are of particular concern when the aim is to precisely repair a point mutation that causes a monogenic disease, but less so when trying to introduce changes such as a stop codon to prevent gene transcription.

Base editors, like other CRISPR-based editing platforms, bind specific sequences in the DNA through the guide RNA. Guide RNAs can bind to stretches of DNA that have high sequence homology to the target sequence, and this can result in Cas9-dependent off-target editing. The deaminase itself also has intrinsic DNA binding properties and it can bind to and deaminate DNA in a guide RNA and Cas9-independent fashion. This can lead to off-target deamination at loci that cannot be predicted or screened for in the same way that Cas9-dependent off-targets can be. Deaminases are also able to bind RNA, leading to unwanted base changes in RNA as well as DNA (5).

Off-targets and how to reduce them are a subject of much debate and discussion. New variants of Cas enzymes and deaminases are being assessed to identify versions with more favourable off-target profiles. In a recent publication, a novel dual-selection method was described where on-target base editing was coupled to the detection of Cas9-independent/deaminase-dependent off-target editing and was able to identify and validate deaminase variants with up to 100-fold lower off-target editing (6). Such deaminase variants would be an attractive option when considering base editors for cell and gene therapy.

A Tool for Genetic Medicine

The potential use of base editors for the correction of pathogenic single nucleotide polymorphisms has attracted much interest and emerging data highlight the transformative potential of this technology. Although efficient, conventional CRISPR does not always result in functional knockouts because they are achieved by indel-induced frameshift mutations. Some indels will consist of three base pair changes or multiples thereof, so frame shifts do not occur. In contrast, base editing can induce gene knockouts through targeted C:T base changes that lead to the introduction of a stop codon, and this more predictable approach could be considered favourable when manufacturing a therapeutic.

Base editing for therapeutic use has focused on diseases where a single base change is curative and where the affected cell type is likely to be amenable to genetic engineering outside of the body. In the case of haemoglobinopathies such as sickle cell anaemia and ß-thalassemia, base editing has been used to engineer single base changes in gamma globin genes HBG1 and HBG2 to either directly edit the disease-causing point mutation or to disrupt repressor binding and reactive foetal haemoglobin expression. Promising data in haematopoietic stem and progenitor cells are an exciting step towards a permanent solution for treating blood disorders (7).

Aside from the correction of certain monogenic diseases, base editing is also of interest to the chimeric antigen receptor (CAR) T cell field. CAR T cells are T cells that have been engineered to recognise and destroy tumour cells. They can be derived from either T cells belonging to a patient (autologous) or from a healthy donor (allogenic), and each comes with a unique set of requirements and challenges. Autologous CAR T cells mitigate the risk of immune rejection when infused back into the patient from whom they were isolated, but require a complex manufacturing process that is not easy to scale for a large number of patients. In contrast, allogenic CAR T cells could provide an 'off-the-shelf' product that overcomes some of these manufacturing difficulties. Both strategies require T cells to be edited, introducing the CAR into one of the genes encoding the T cell receptor and knocking out

key genes that enable longevity of the CAR T cells *in vivo*. Base editing has been used to knockout genes encoding cell surface targets on primary T cells such as the T cell receptor alpha chain, B2M, and PD-1, with high efficiency as both single and multiple targets (8). Here, the fundamental difference between base editing and conventional CRISPR-Cas editing comes into play. Base editors can be used to knockout several genes in combination with minimal risk of chromosomal translocations or rearrangements compared with CRISPR-Cas, and such alterations pose a significant safety question for any therapeutic strategy.

A significant hurdle in applying base editing to cell and gene therapy has been finding a suitable delivery mechanism. Current base editors are too large to be packaged efficiently using the preferred method of adeno-associated virus (AAV). Innovative approaches, such as splitting the base editors into a dual AAV system, which reconstitutes the base editing complex in transduced cells, can achieve therapeutically relevant levels of gene editing (9). Delivering editors in the form of messenger RNA or protein/RNA complexes has been hugely beneficial, both by increasing the cell types amenable to base editing and also to minimise any off-target editing because of their transient nature. Promising data using delivery modalities such as lipid nanoparticles and virus-like particles offer alterative, non-viral solutions (10).

The Future

Cell and gene therapy has emerged as one of the most exciting areas of biotechnology, and base editing looks set to play its part in providing treatment options for a wide range of genetic diseases. So far, there has been support from regulatory bodies such as the FDA to see CRISPR-based therapeutics enter clinical trials, and with this comes a better understanding of what is required both in terms of efficiencies, but, perhaps more importantly, safety. Base editors are anticipated to stand up well to both of these benchmarks.

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