The newbies of nuclease-based gene editing

The role of base and prime editing in therapeutics

The new genetic engineering approaches of base editing and prime editing promise the capacity for precise gene modifications, so could have a substantial impact on the development of therapeutics to treat rare disease and the next generation of cell-based therapies. As newbies in the fast-paced world of nuclease-based gene editing, base and prime editing are being investigated intensely for their advantages and limitations when it comes to clinical application.

Since the discovery of DNA restriction enzymes in the late 1960s enabled the manipulation of DNA, the idea of altering DNA for therapeutic gain has been with us. The past 10 years has seen an unprecedented pace of change in the rapidity and precision with which we can edit DNA. Such advances have been driven by the use of nucleases, such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats-CRISPR associated protein (CRISPR-Cas), but the precision of these can be less than desired when contemplating genetic changes *in vivo* that would be required to correct monogenic diseases of childhood, such as Duchenne muscular dystrophy, or making many simultaneous genetic alterations to a cell *ex vivo* before placing it back into the patient from whence it came.

The challenge of DNA double-strand breaks

The CRISPR-Cas9 system has been widely adopted as the go to method for gene editing, indeed this approach has progressed impressively from its commercial debut in 2012 to its clinical trial debut in 2016. While ZFNs, TALENs and CRISPR-Cas are being used to drive gene and cell therapy pipelines, they all share one substantial challenge in that their mode of action generates DNA double-strand breaks. These breaks are more often than not repaired in an error prone fashion by the cell's repair machinery and as such can cause unintended genetic changes within the wider genome of engineered cells. Where these nuclease-based gene editors are targeting just one single gene, there are methods that can be used to make them error free.

However, it is becoming clear that for some cell therapies to be successful, such as the use of chimeric antigen receptor (CAR) T cells in the treatment of solid tumours, multiple gene engineering events will be required to achieve efficacious therapies. There is a real risk that if one employs standard gene-editing nucleases to make multiple edits, it could lead to genome-altering insertions, deletions and/or chromosomal translocations. The impact of this on a patient could be that the gene or cell therapy is effective but the off-target genetic changes lead to deleterious side-effects, impacting patient recovery and potentially survival.

While base editing and prime editing make use of the CRISPR-Cas gene editing system, they use modified forms of these components that limit the introduction of DNA double-strand breaks. This means that the off-target effects of these technologies in terms of insertions, deletions or translocations should be substantially reduced. However, the true nature and extent of off-target effects arising from these new gene editing approaches has yet to be fully investigated.

Mechanics of base editing and prime editing

Base editing uses a deaminase enzyme to make a specific base-pair change in the DNA. The base pair alteration is a transition (Figure 1), and can be A to G or C to T depending on which deaminase is used. Prime editing uses a different approach that involves a reverse transcriptase that enables both transitions and transversions (Figure 1), as well as multiple base-pair swaps, small insertions and small deletions.

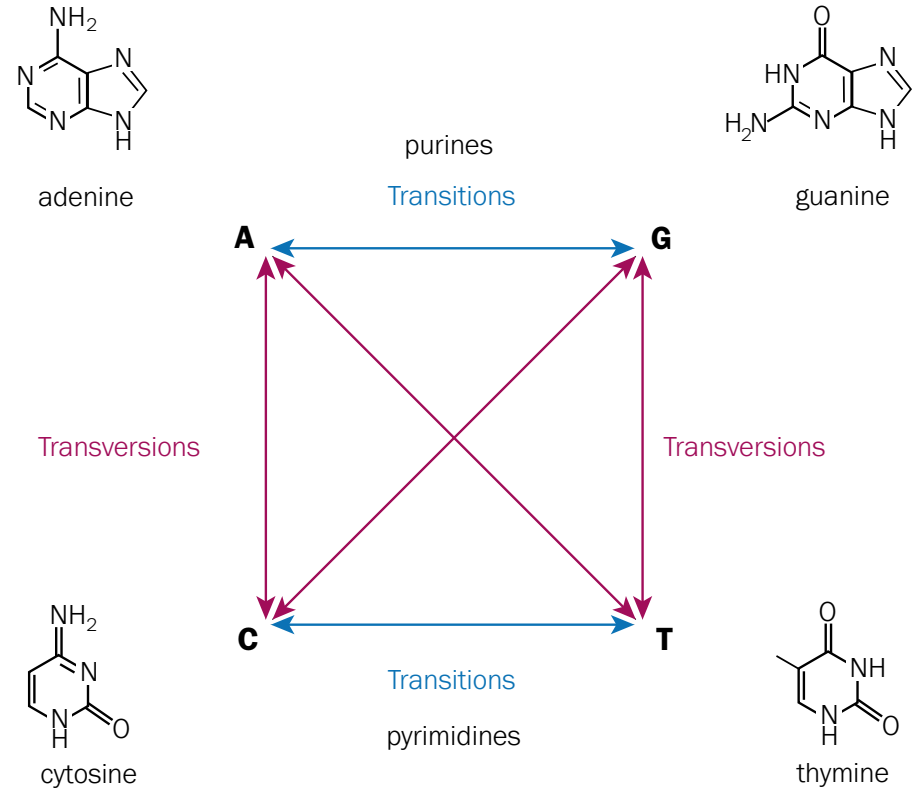
Aside from its potential use to correct transitions in the DNA that cause human disease, base editing is well suited for introducing stop codon(s) into genes to knock out gene transcription from one or more genes. This is potentially ideal for allogeneic CAR T cell therapy in patients with solid tumours where it is expected that multiple genes need to be silenced to provide a cloak of invisibility to these cells such that they are not 'seen' as foreign by the treated patient's immune system, as well as prolonging the survival of the cells in the patient and enabling them to function in an immune suppressive tumour microenvironment. As base editors do not cut both strands of the DNA in order to introduce a base-pair transition, and have a relatively high gene engineering efficiency, it suggests that base editing will have a starring role in the next generation of cell-based therapies where multiple genetic changes are required.

Prime editing has the flexibility to correct nearly any single nucleotide polymorphism within a target genome, and add in multiple base pairs using a template that is part of the prime editing machinery. Prime editing can introduce insertions or deletions. These functionalities together are ideal for gene therapies where corrections of small stretches of DNA are required for establishing normal gene function and a positive outcome for the patient. However, as the newest of the newbies on the block, the efficacy and efficiency of prime editing is still under investigation. Prime editing can introduce unintended insertions and deletions into the genome compared with base editing, but still at levels below those evident when using CRISPR-Cas gene engineering.

One additional advantage of base editing and prime editing is that they can modify the genome with precision and efficacy in non-dividing cells. In the past, introducing either single base changes or modifying small stretches of DNA required a gene knock-in. Knocking stretches of DNA into cells requires a process known as homologous recombination, which is most efficient in dividing cells, and is limited in primary cells, especially those that do not divide. In a therapeutic setting where non-dividing cells are often the target for gene therapy, established

methods for gene knock-ins are too inefficient to have utility. As base editing and prime editing are not reliant on these established methods for introducing one or more base pair changes, they might prove to be the systems of choice for altering DNA in primary, non-proliferative cells. Early data suggest that base editing has a higher level of gene editing efficiency compared with prime editing, which could offer base editing a clear advantage in modifying primary cells for therapeutic use[1].

Figure 1. Mechanics of base editing and prime editing



What don't we know yet?

The concern with the established methods of CRISPR, ZFNS and TALENs for making multiple genetic changes in a cell is their off-target effects. The off-target effects of base editing and prime editing are still under investigation. While their reduced capacity for introducing changes in the DNA likely to bring about substantial genetic effect such as translocations and large deletions is evident, the off-target effects of both systems are still largely unknown. Similarly, factors influencing the editing efficiency of either technology require more investigation.

Similar to CRISPR-Cas gene editing, the design of the guide RNA will be crucial to the efficacy and precision of base editing and prime editing. Fortunately, optimisation of guide RNA design has been necessary for the CRISPR-Cas gene engineering revolution and key learnings from the past decade should be applicable to both base editing and prime editing.

The off-target activities of the reverse transcriptase and deaminase for prime editing and base editing, respectively, also need investigation. It is well known among virologists that reverse transcriptases are error prone and the level of error that might occur with prime editing is currently unclear. It is possible that prime editing might be able to accurately change one or two bases per edit but beyond that the error rate increases. Similarly, for base editing the off-target deamination activity of the deaminase is being investigated and will need to be carefully controlled to ensure that the deaminase does not randomly deaminate base pairs from the target site bound by the guide RNA.

In summary

With any new technology, success is always dependent on the details and 'real world' data. If over the next year peer-reviewed data show that base editing has a high gene engineering efficiency with limited off-target effects compared with the established methods of CRISPR, ZFNs and TALENs, then it will potentially be the gene editor of choice for cell-based therapies where multiple knockouts are required. Similarly, if prime editing proves to be precise and efficacious at transition, transversion and introducing short stretches of DNA to correct a gene of interest, then this might become the method of choice for gene therapy. In the fast-moving field of research that is gene editing today, we should expect much greater understanding of the capabilities and limitations of base editing and prime editing and their applications to gene and cell therapy within the next 12 months.

References:

1. Anzalone AV, Randolph PB, Davis JR, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*. 2019;576(7785):149-157. doi:10.1038/s41586-019-1711-4

Figure 1: https://commons.wikimedia.org/w/index.php?curid=19884125

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