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Editorial

Bringing base editing to the clinic: The next generation of genome editors

CRISPR-Cas technologies have emerged as a promising platform for engineering multiplex-edited cells and potentially curing genetic diseases. The first generation of CRISPR-Cas utilizes RNA-guided nucleases to produce DNA double-strand breaks (DSBs). This locus-specific DNA cleavage enables targeted gene knockouts, as well as transgene insertions. While these systems offer remarkable programmability and precision, the induction of DSBs poses a risk of oncogenicity and might compromise cellular viability.[1] Efforts to expand the CRISPR-Cas toolbox have led to modified Cas proteins that perform gene editing without the need for DSBs. A notable example is base editing that combines the programmability of the Cas system with the ability of deaminases to precisely induce point mutations.[2] These point mutations can be used to facilitate highly efficient, DSB-free gene knockouts or correct certain genetic diseases. To date, most base editors have been delivered using linear mRNA. To enhance stability, this requires the addition of a 5′ cap and a 3′ polyadenylation, processes that can be resource intensive.

In a study published in *Molecular Therapy – Methods & Clinical Development*, Woodruff and colleagues showcased the benefits of using circular RNA (circRNA) for encoding base editors.[3] Unlike conventional linear RNAs, circRNAs are a type of endogenous RNA that forms a covalently closed loop without 5′ caps or 3′ tails. This structure provides resistance to exonucleases and makes them economical to produce at scale. To achieve this, the authors utilized previously described permuted intron-exon self-splicing (PIE) RNAs[4] to circularize the mRNA encoding the BE4max cytosine base editor. Through a simple incubation step, these PIE RNAs undergo so-called "back-splicing", resulting in a circRNA encoding the desired gene. In their experiments, circRNA displayed a superior translational capacity in primary human T cells compared with linear RNA. This advantage was evident in a clinical-scale CAR T cell production, with circRNA-transfected T cells showing increased BE4max protein expression over their linear RNA counterparts. The authors then demonstrated that BE4max circRNA can be used to effectively disrupt T cell inhibitory receptors when

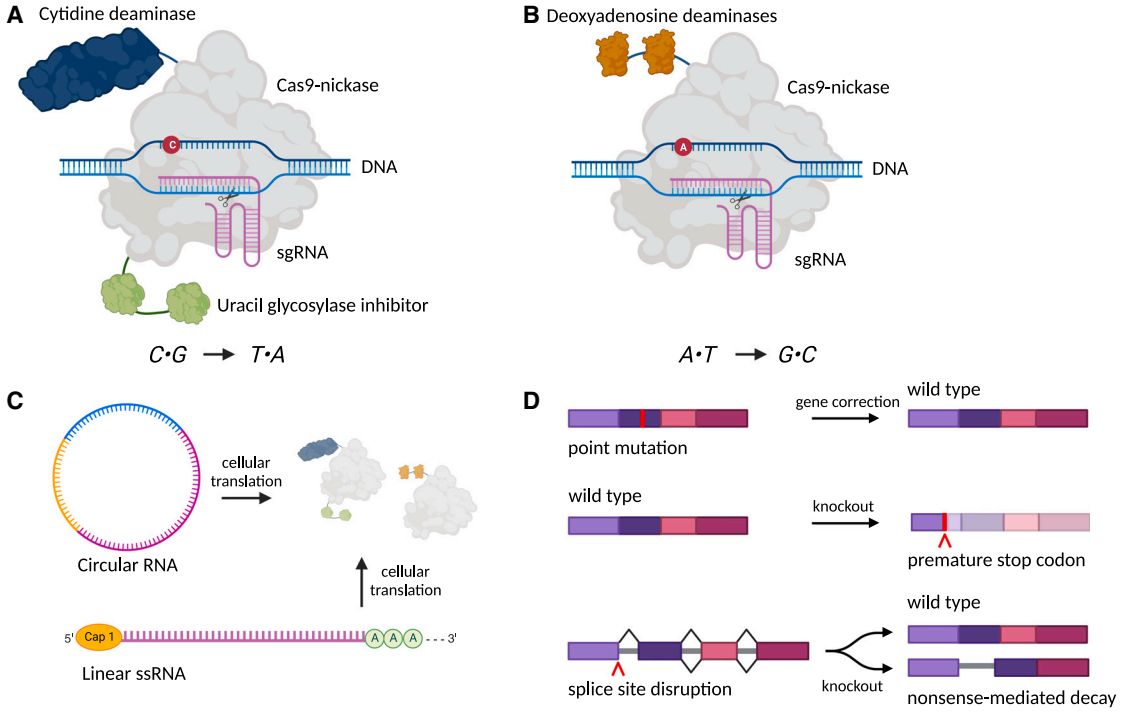

Figure 1. Therapeutic base editors
(A and B) Cytidine and adenine base editors.[7]
(C) Base editor-encoding RNAs.
(D) Mechanisms of therapeutic base editing. Created with Biorender.com.

Table 1. Examples of base editing in cellular therapies

Modality	Mechanism	Example applications
Gene correction	*Reverting Point Mutations*	*Hutchinson-Gilford progeria syndrome: correction of RNA mis-splicing by reverting C→T mutation (malign Progerin → benign Lamin A).*[8]
	Generating Benign Isoform	*Sickle cell disease: mutation of pathogenic GTG codon (Val; Sickle cell disease) to GCG (Ala; benign Hb-Makassar isoform).*[10]
Gene disruption	*Splice Site Disruption*	*Familial hypercholesterolemia: in vivo hepatic knockout of PCSK9 via splice site disruption.*[11]
	Premature Stop Codon	*Multiplex-edited CAR T cells: Premature stop codon in TRBC (prevent graft-vs-host disease) and CD7 (prevent fratricide), splice site disruption of CD52 (alemtuzumab resistance).*[12]
Gene modification	*Inducing Point Mutations*	*Epitope editing: mutating binding motifs on healthy tissues to prevent on-target, off-tumor recognition by chimeric antigen receptors/monoclonal antibodies (e.g., CD135, CD117, IL-3RA on healthy transplants).*[6]
Gene activation	*Disrupting Suppressors*	*β-hemoglobinopathies: Reactivating fetal hemoglobin expression by disrupting γ-globin repressor gene BCL11A.*[14]

co-delivered with single guide RNAs targeting TIM3 and PD1. Notably, when targeting PD1, even a concentration as low as 0.25 μg circRNA/1 × 10^6 cells successfully disrupted gene expression in T cells, highlighting circRNA's potential as an effective method for gene therapy applications.

Base editors offer the advantage of efficient, multiplex gene editing with minimal chromosomal aberration risks[1] (Figure 1), positioning them as strong candidates for *in vivo* editing and cellular therapies requiring complex engineering. For instance, CAR T cell therapies for solid tumors present a formidable engineering challenge, given the lack of tumor-specific target antigens and the persistent risk of on-target, off-tumor toxicity (OTOT).[5] Recently, several groups used base editing to modify surface epitopes on engrafted hematopoietic stem and progenitor cells,[6] thus eliminating binding sites for CAR T cells. This strategy ensures that healthy grafts are safeguarded from OTOT, while malignant cells are left vulnerable to CAR T cell-mediated killing.

Moreover, as some genetic disorders are better suited for *in vivo* editing than *ex vivo* engineering, *in vivo* base editing has shown promise in tackling previously incurable diseases (Table 1). One significant accomplishment was the correction of the Hutchinson-Gilford progeria point mutation in mice, resulting in substantial increases in lifespan and vitality.[8] The efficient packaging and delivery of base editors are paramount for such *in vivo* applications. Currently, lipid nanoparticles and adeno-associated viruses lead the charge as delivery platforms. While the potential of circRNAs in this domain has been touched upon, it is clear that more research is needed to fully elucidate their role and efficacy.[9] Base editing is also being investigated in conditions such as reactivating fetal hemoglobin in β-thalassemia and sickle cell disease,[10] as well as *in vivo* silencing of PCSK9 in heterozygous familial hypercholesterolaemia.[11] In addition, they enhance cellular immunotherapies by enabling targeted gene knockouts.[12] As they inch closer to the clinic, the demand for GMP-grade base editors intensifies. Meeting this demand necessitates streamlining manufacturing processes to curb complexities and costs while ensuring high editing efficacies.[13]

AUTHOR CONTRIBUTIONS

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