RNA base editing: programmable protein machine

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RNA (ribonucleic acid) is a unique molecule which plays important and diverse roles in biology. In cells, RNA is ephemeral; it acts as a mediator, carrying genetic information from the nucleus to the protein-making machinery in the cytoplasm. A point mutation in RNA transcript could make the protein much shorter or larger than normal[1]. Aberrant expression of RNAs effects the regulation of many cellular functions and gene networks. Most human genetic disorders such as sickle cell anaemia are due to just a single base-pair substitution, or by errors in RNA splicing, or its regulation[2]. Precise editing of aberrant RNA provides a platform to correct the errors, without altering the genetic make-up of template DNA.

The CRISPR (clustered regularly interspaced short palindromic repeat)-mediated gene-editing technology is one of the greatest advances in the field of genome engineering and functional genomics[3,4]. CRISPR/Cas13a (previously known as C2c2, an effector of type-VI CRISPR/Cas system) was initially identified in 2015, capable of targeting and cleaving single-stranded RNA (ssRNA)[5]. Cas13a is structurally and functionally different from CRISPR/Cas9 and Cpf1. Unlike CRISPR/Cas9 and Cpf1, Cas13a cleaves the RNA transcript rather than DNA substrate, and also does not require any protospacer adjacent motif (PAM) sequence for targeting specific RNA recognition by guide RNA (gRNA). Cas13a contains two HEPN (higher eukaryotes and prokaryotes nucleotide-binding) domains having dual RNAase activities – one for CRISPR RNA (crRNA) processing and the other for target RNA cleavage[6,7]. However, like dCas9/Cpf1, the catalytically inactivated Cas13a (dCas13a) retains its ability to bind to a target-specific RNA site, and so can function as an RNA-guided RNA-binding protein. Abudayyeh *et al.*[8] had reported that Cas13a is most active for targeted knockdown of RNA in *Eschericihia coli* after screening 15 Cas13a orthologues. They also demonstrated that Cas13a robustly cleaves the target RNA in mammalian and plant cells with ~28 long nucleotide spacer, without requiring any specific protospacer flanking site (PFS) sequence. They also confirmed that deactivated Cas13a can be used as a programmable RNA-binding protein[8]. The target gene knock down by CRISPR/Cas13a shows higher specificity than RNAi technology, making it potentially well-suited for therapeutic applications.

Recently, Cox *et al.*[9] have shed light on a novel RNA editing system based on CRISPR technology using ADAR (adenosine deaminase acting on RNA). RNA adenosine deaminase has the ability to change adenine (A) into an inosine (I) by hydrolytic deamination at C^6 position (Figure 1 *a*). The translation machinery reads an inosine as a guanosine; so it base-paired with cytosine. In this manner, it changes amino acids in translated exons that generate functional protein diversity. A-to-I editing is the most common type of post-transcriptional nucleotide modification in humans. It occurs in a wide range of coding and non-coding RNAs[10].

To make a robust RNA base editor, Cox *et al.*[9] tested a squillion of mammalian codon-optimized version of Cas13 enzymes (21 Cas13a, 15 Cas13b and 7 Cas13c orthologoues) for RNA knockdown activity in mammalian cells. After several iterations, they found Cas13b from *Prevotella* sp. P5-125 (PspCas13b) to have the highest level (62.9%) of RNA interference activity compared to other Cas13a orthologoues. PspCas13b is highly sensitive to mismatch in target RNA and does not require a PFS. They engineered an RNA editing system which is composed of two components: a catalytically inactivated CRISPR/Cas13b (mutated HEPN domain) and deaminase domain ADAR ($ADAR2_{DD}$). The resulting base editor (dCas13b–$ADAR2_{DD}$) directly converts A into I on the target base pair without cutting the transcript or relying on cellular repair mechanisms (Figure 1 *b*). Cox *et al.*[9] named this system RNA Editing for Programmable A to I Replacement (REPAIR).

To improve editing efficiency, they fused hyperactive $ADAR2_{DD}$ with dCas13b and used this fusion construct to target RNA by many gRNAs having different spacer lengths (30, 50, 70 and 84 nucleotide) with an A–C mismatch at the target A. This modification improves the editing efficiency up to 28% with 50 nt long spacer. Cox *et al.*[9] named this optimized version REPAIRv1, which effectively supplants the targeted A into I on endogenous mRNAs. To demonstrate the therapeutic potential of REPAIRv1, the researchers targeted two disease mutations in mammalian cells: 878G->A (*AVPR2* W293X) in X-linked diabetes insipidus and 1517G->A (*FANCC* W506X) in Fanconi anaemia. They were able to correct these mutations at the RNA level by 35% of *AVPR2* and 23%

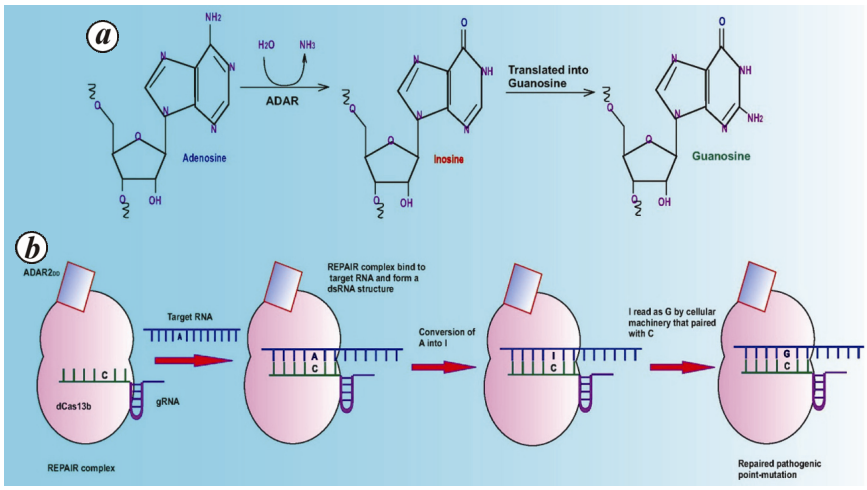

Figure 1. RNA base editor. ***a***, Deamination of adenosine to inosine by adenosine deaminase acting on RNA. ***b***, Precise targeted base editing by REPAIR complex.

of *FANCC*. Cox *et al.*[9] also tested the robustness of REPAIRv1 by targeting 34 different disease-relevant G>A mutations from the ClinVar database. They successfully edited 33 out of 34 sites with 28% editing efficiency in HEK293T cells. This can be used to edit full-length mutated transcripts because there are no sequence constraints.

The efficient and safe delivery of REPAIRv1 construct into specific tissues is a major limitation because it is too large to be packed into adeno-associated virus (AAV) vectors, which allows less than 4.2 kb foreign DNA. AAV vectors are most commonly used in gene therapy to transport foreign genetic material into human cells. They circumvent this packaging problem by reducing the size of $ADAR2_{DD}$ (from ADAR gene to make it conveniently packaged into AAV) without compromising on editing efficiency. So the size of REPAIRv1 decreases from 4473 bp to 4152 bp, which could be easily packaged into the 4.7 kb AAV with a gRNA expression cassette. RNA knockdown by REPAIRv1 editing system was highly specific, but showed subtle off-target activity[9]. Thus, this study provides new therapeutic platform for diseases caused by point mutations.

Cox *et al.*[9] further modified the REPAIRv1 editing system to improve specificity and suppressed off-target edits using mutant $ADAR2_{DD}$ (E488Q/T375G). The resulting variant system is called the REPAIRv2 editing system. The authors tested the specificity of REPAIRv2 to target endogenous transcript *KRAS* or *PPIB* and found that it had no detectable off-target effects and managed to edit specific RNA letters within efficiency ranging from 20% to 40%. A whole transcriptome comparison showed that REPAIRv2 highly reduced the off-target edits from 18,385 observed with REPAIRv1, to only 20.

In contrast to DNA base editors[11,12], the RNA editing system does not require any PAM sequence at the target locus or endogenous DNA damage repair mechanisms, such as non-homologous end joining (NHEJ) and homologous directed repair (HDR). In addition, it would alleviate the ethical concern associated with DNA editing because the edited RNA will degrade over a short period[13]. REPAIR allows transient and non-permanent modifications which are temporary in nature, so we can treat many disorders caused by temporary changes in the cell state. RNA base editor has the potential to modulate the biological activity of RNAs without the risk of permanently modifying the native genetic information. REPAIR will act as a powerful RNA editing tool for studying non-coding RNA and essential genes at transcript level, genome imaging in live cells as well as potential therapeutic in a more targeted and personalized manner in the future.

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