DNA base editing induces substantial off-target RNA mutations

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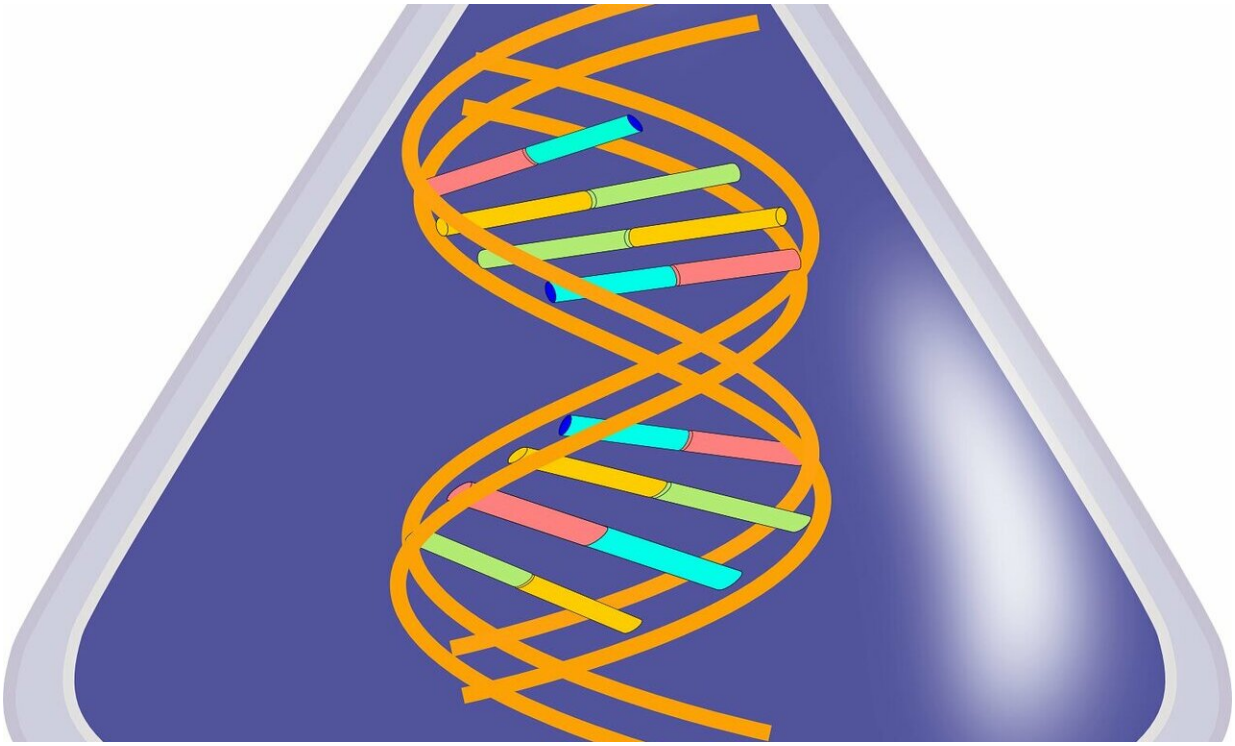

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In a study published in *Nature* on June 10, researchers from Dr. Yang Hui's Lab at the Institute of Neuroscience of the Chinese Academy of Sciences (CAS), and collaborators from the CAS-MPG Partner Institute for Computational Biology of CAS and Sichuan University demonstrated that DNA base editors generated tens of thousands of off-

target RNA single nucleotide variants (SNVs) and these off-target SNVs could be eliminated by introducing point mutations to the deaminases.

This study revealed a previously overlooked aspect of the risk of DNA base editors and further provided a solution to the problem by engineering deaminases.

DNA base-editing methods have enabled direct point mutation correction in genomic DNA without generating any double-strand breaks (DSBs), but the potential off-target effects have limited the application of these methods. Adeno-associated viruses (AAV) are the most common delivery system for DNA editing gene therapies. Since these viruses can sustain long-term gene expression in vivo, the extent of potential RNA off-target effects induced by DNA base editors is of great concern for their clinical application.

Several previous studies have evaluated off-target mutations in genomic DNA by DNA base editors. Meanwhile, the deaminases integral to commonly used DNA base editors often exhibit RNA binding activities. For example, the cytosine deaminase APOBEC1 used in cytosine base editors (CBEs) was found to target both DNA and RNA, and the adenine deaminase TadA used in adenine base editors (ABEs) was found to induce site-specific inosine formation on RNA. However, any potential RNA mutations caused by DNA base editors had not been evaluated.

In order to evaluate the off-target effect of DNA base editors at the level of RNA, the researchers counted the off-target RNA SNVs in each replicate of CBE- or ABE-treated cells, and then explored the possibility of eliminating the off-target RNA SNVs by engineering deaminases of DNA base editors.

They transfected one type of CBE, BE3 (APOBEC1-nCas9-UGI), or ABE, ABE7.10 (TadA-TadA*-nCas9), together with GFP and with or

without single-guide RNA (sgRNA), into HEK293T-cultured cells. After validating the high on-target efficiency of DNA editing by both BE3 and ABE7.10 in these HEK293T cells, they performed RNA-seq at an average depth of 125X on these samples and quantitively evaluated the RNA SNVs in each replicate.

The on-target editing efficiency was evaluated in each replicate of the CBE- or ABE-treated cells to guarantee efficient editing. Then the number of off-target RNA SNVs in CBE- and ABE-treated groups was compared with the GFP-only control group. They found strikingly higher numbers of RNA SNVs in DNA base editor-treated cells.

Furthermore, the researchers found that the mutation bias in BE3- or ABE7.10-treated cells was the same as that of APOBEC1 or TadA, respectively, indicating the off-target effects were caused by the overexpression of DNA base editors. They also identified CBE- and ABE-specific motifs and genetic regions of these off-target RNA SNVs.

To eliminate the RNA off-target activity of base editors, they examined the effect of introducing point mutations on APOBEC1 or TadA. Three high-fidelity variants, BE3W90Y+R126E, BE3 (hA3AR128A) and BE3 (hA3AY130F), reduced RNA off-target SNVs to the base level. Similarly, an ABE variant ABE7.10F148A also showed complete elimination of off-target effects.

This study obtained both high-fidelity variants for both CBEs and ABEs by introducing point mutations to the deaminases and provided a proposed method using rational engineering to increase the specificity of base editors.

More information: Off-target RNA mutation induced by DNA base editing and its elimination by mutagenesis, *Nature* (2019). DOI: 10.1038/s41586-019-1314-0 ,

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