Review Article

The Development and Application of a Base Editor in Biomedicine

Fang Wang,1,2 Yuqiang Zeng,1,2 Yi Wang,1,2 and Yuyu Niu1,2

1Yunnan Key Laboratory of Primate Biomedical Research, Institute of Primate Translational Medicine, Kunming University of Science and Technology, Kunming, Yunnan 650500, China
2Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming, Yunnan 650500, China

Correspondence should be addressed to Yuyu Niu; niuyy@lpbr.cn

Received 6 May 2020; Accepted 15 July 2020; Published 14 August 2020

Academic Editor: Kexuan Tang

Copyright © 2020 Fang Wang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Using a base editor to generate monogenic disease models and correct pathogenic point mutations is a breakthrough technology for exploration and treatment of human diseases. As a burgeoning approach for genomic modification, the fused CRISPR/Cas9 with various deaminase separately has significantly increased the efficiency of producing a precise point mutation with minimal insertions or deletions (indels). Along with the flexibility and efficiency, a base editor has been widely used in many fields. This review discusses the recent development of a base editor, including evolution and advance, and highlights the applications and challenges in the field of gene therapy. Depending on rapid improvement and optimization of gene editing technology, the prospect of base editor is immeasurable.

1. Introduction

Benefit from the progress of gene therapy, we are entering an era in which genome editing tools could be used to manipulate gene sequences flexibly and precisely. Gene editing greatly drives the innovation of the treatment from symptoms to genetic basis of human genetic diseases. The first explosive event about gene editing came from Scherer and Davis in 1979, who develop a method that could be used to introduce foreign sequences into the chromosomes of yeast [1]. Then, researchers continuously finished precise gene targeting by homologous recombination in Drosophila [2], mouse [3], and human [4]. Afterwards, some engineered DNA-cleaving enzymes were discovered, including zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). Although both of them demonstrated the potentiality of therapeutic genome editing, they required a lot of time and labor. Subsequently, the development of clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR/Cas9) offers a simpler technology which has been adopted widely, owing to its easier DNA-binding and modifying capabilities [5, 6]. CRISPR/Cas9 protein-RNA complexes were recruited to target DNA sequence via base pairing with a specified single guide RNA (sgRNA) and natively create a double strand breaks (DSBs), triggering cellular DNA repair by nonhomologous end joining (NHEJ) or homology-directed repair (HDR) to achieve genome editing eventually [7, 8]. Based on this property of CRISPR/Cas9, scientists have developed a variety of derivatives according to different gene editing requirements. For example, transcriptional repressors or activators were fused into catalytically inactivated Cas9 (dCas9) to achieve gene repression or activation [9–13]. In addition, in order to overcome the defect that conventional CRISPR/Cas9 induced abundant and unpredictable insertions or deletions (indels) and exhibited low efficiency in correcting point mutations, researchers developed a base editor—a new elegant Cas9 derivative which could efficiently generate precise point mutations with minimal indels. In this review, we will elaborate the development and application of a base editor in gene therapy.

1.1. The Evolution of Base Editor

1.1.1. The Birth of Base Editor. Before 2016, researchers delivered CRISPR/Cas9 with a donor DNA template to achieve gene correction. At present, the point mutations using HDR still remain inefficient (typically 0.1–5%), especially in
The deoxyadenosine deaminase catalyses conversion of A to I, following DNA repair or replication by nicking in the nonedited strand. The enzyme that is tethered to the nCas9 converts the single-strand target C to U. Then, the BE3 nicks the nonedited strand containing the G, triggering DNA repair to induce G:U convert A:U. Eventually, A:U is converted to A:T during DNA replication or repair.

The ABE contains a modified deoxyadenosine deaminase (TadA*), a wild type TadA, and an nCas9. Initially, ABEs bind the target DNA guided by sgRNA. Then, the deoxyadenosine deaminase domain catalyzes the conversion of adenine (A) to inosine (I). Within the constraints of a polymerase active site, the inosine would be read or replicated as G. Eventually, the T: A base pair can convert to C: G base pair permanently [19] (Figure 1(b)).

To further increase the editing efficiency of the base editor (CBE and ABE), Koblan et al. optimized the nuclear localization signals (NLS) and codon usage, as well as reconstructed the ancestral deaminase component [20]. Up to now, the newest versions of base editors are BE4max, AncBE4max, and ABEmax.

With the emergence of CBEs, other two teams reported new base editor-targeted AID-mediated mutagenesis (TAM). They fused activation-induced cytidine deaminase (AID) or AID ortholog PmCDA1 with nuclease-inactive CRISPR/Cas9 for efficient genetic modifications, which enabled to perform highly efficient site-directed mutagenesis and high-throughput screening of functional variants [21–23].

1.1.2. The Advance of Base Editor. Although base editor can help us to convert bases easily, there are still some problems needed to be addressed. The requirement of editing window and protospacer adjacent motif (PAM) greatly limits the scope of base editor.

When there are multiple editable Cs or As within or nearby the “editing window” (positions 4-8 for CBE or 4-7 for ABE, counting the PAM as positions 21-23), base editor could introduce the conversion of bases edit in addition to the target base. To solve this problem, researchers further optimized the cytidine deaminase domains via inducing specific mutations, which eventually narrowed the width of the editing window from ~5 nucleotides to as little as 1-2 nucleotides [24]. For instance, YE1-BE3, YE2-BE3, EE-BE3, and YEE-BE3 are modified versions of BE3 with narrower active windows, but still show stable activity of base editing compared to regular BE3. Besides, the team of Tan obtained two high-precision base editors that BE3-PAPAPAP mainly edits within an activity window from −14 to −16, and base editors with CDA1 truncations mainly edit at position −18 [25, 26]. Conversely, in some cases, the editing windows need to be expanded to achieve targeted base editing. The groups

Figure 1: Schematic of CBEs and ABEs work in target DNA. (a) The working mechanism of BE3. Cytidine deamination by APOBEC1 enzyme that is tethered to the nCas9 converts the single-strand target C to U. Then, the BE3 nicks the nonedited strand containing the G, triggering DNA repair to induce G:U convert A:U. Eventually, A:U is converted to A:T during DNA replication or repair. (b) The working mechanism of ABE. ABEs is composed of the fusion of TadA (wild type) and TadA* (TadA variant after protein evolution) and nCas9. The deoxyadenosine deaminase catalyses conversion of A to I, following DNA repair or replication by nicking in the nonedited strand. Eventually, the original T:A is replaced with C:G in the target site.
of Jiang and coworkers developed base editor (BE-PLUS) with expanded C to U (T) programming scope [27]. Either narrower or broader strategy both enlarged the genome-targeting scope.

Except editing window, the PAM requirement also limits the number of editable sites. To broaden the targetable genome sequences of base editor, scientists have exploited numbers of Cas9 variants or homologue. Kim and coworkers, respectively, replaced the regular SpCas9 with four Cas9 variants to generate VQR-BE3 (NGAN), EQR-BE3 (NGAG), VRER-BE3 (NGCG), and SaKKKB-E3 (NNNRRT) [24]. Moreover, they used phage-assisted continuous evolution method to evolve a new SpCas9 variant (xCas9) with an expanded PAM including NG, GAA, and GAT [28]. Meanwhile, to break the G/C-rich protospacer-adjacent motif (PAM) restriction, the team of Jia Chen developed a CRISPR-Cas12a-based BE. They fused the rat cytosine deaminase APOBEC1 with a catalytically inactive version of Lachnospiraceae bacterium Cas12a (also named Cpf1) to achieve C to T conversion in human cells with a T-rich PAM [29]. Moreover, two team demonstrated new CBE variant (eA3A-BE3) which replaced the regular cytidine deaminases—rAPOBEC1 with human APOBEC3A—that have narrower editing windows that can reduce bystander mutations and mediate efficient C to T conversion in regions with high methylation levels [30, 31]. Recently, Richter and coworkers developed a new ABE variant—ABE8e—which activity has been increased 590-fold than ABE7.10’s. ABE8e offers substantially improved editing efficiencies when paired with a variety of Cas9 or Cas12 homologs [32]. Up to now, there are several base editors’ variants have been developed. These variants not only expand the editable range but also improve the editing efficiency of target sites (Table 1). All the variants hold great potential for both basic research and clinical application in biomedicine.

Moreover, in order to treat genetic disorders which were caused by multiletter mutations, such as Tay-Sachs disease caused by an insertion of four DNA letters into the HEXA gene [33], Anzalone et al. developed the prime editing (PE), a “search-and-replace” genome editing technology that mediates targeted insertions, deletions, and all 12 possible base-to-base conversions without requiring DSBs or donor DNA templates [34]. The new editor, PE, consists of an nCas9, a reverse transcriptase (RT), and a prime editing guide RNA (pegRNA). It can directly copy genetic information from the pegRNA into the target genomic locus (Figure 2). Because nicking the nonedited strand favors repair of that strand, resulting in preferential generation of desire replacement in cells, they developed PE3 which uses the Cas9 H840A nickase to nick the nonedited strand to further increase editing efficiency [34]. However, when a single target nucleotide is present within the base editing window, or when bystander edits are acceptable, primer editor is little efficient and generate more indels than current base editor.

Except in DNA level, base editing in RNA can also provide powerful capabilities for life sciences. To date, researchers had developed several base editors which can deaminate A to I, depending on the characteristic of ADAR family. The ADAR can mediate endogenous conversion of adenosine to inosine via hydrolytic deamination. The inosine is functionally equivalent to guanosine in the process of translation and splicing of the cell’s protein building [35, 36]. In the early days, researchers developed an RNA editor that linked the catalytic domain of an ADAR enzyme to a guiding antisense RNA oligonucleotide [37–42]. Therefore, the ADAR deaminase domain (ADARDD) can be recruited into the target RNA, which relies on the Watson-Crick base pairing between the antisense RNA and the target transcript.

In 2017, Shmakov’s team developed a precise and flexible technology, Programmable adenosine to inosine Replacement (REPAIR), in RNA level by using the type VI CRISPR-associated RNA-guided RNase Cas13 [43–45]. REPAIR includes a catalytically dead RNA-guided Cas13b enzyme (dPspCas13b), an ADAR, and a sgRNA. CrRNA is targeted to the specific site by hybridization to create a dsRNA structure and recruit dCas13b-ADARDD. And a mismatched cytidine in the crRNA opposite the target adenosine could enhance the editing reaction [46] (Figure 3). Except REPAIR, RNA base editing tools are also included, (RNA Editing for Specific C to U Exchange) RESCUE [47] and (Leveraging Endogenous ADAR for Programmable Editing of RNA) LEAPER [48], and they all mediated by ADAR enzymes in mammalian cells. The RNA editing allows a temporary correction of a disease-causing mutation without permanent alteration to the genome and could be a potentially safer option when it comes to gene-fixing therapeutics. At the same time, RNA editing can also help us interrogate genes and noncoding RNA as well as control cellular processes at the transcript level.

1.2. The Application of Base Editor in Biomedicine

1.2.1. Disease Modeling. Base editor can induce specific base changes without DSBs and donor templates, which make it a convenient, high-efficiency approach for engineering nucleotide substitutions at target sites. There have been numerous reports showing that single-base editing systems can be successfully applied to bacteria [59, 60], plants [49, 61–64], zebrafish [65, 66], mammals, and even human [50, 67–74]. These studies all demonstrated the power of base editor in drug target research, crop improvement, animal disease modeling, gene function screening, disease treatment, and so on. This review will focus on the modeling and treatment of different disease to describe the prospect of base editor in biomedicine.

In 2017, Kim’s team firstly showed that CBE could be an efficient method to generate mice models with targeted point mutation [68]. Secondly, they proved that ABEs can also be used to generate disease mice models that obtain Tyr mutant with albino phenotype [70]. Furthermore, Li’s team demonstrated that CBE or ABE system can be applied to generate rabbit models with the high mutation efficiency of 44–100%. They used CBEs to induce C to T conversion to generate a premature stop codon in Mstn and Tyr gene, respectively, and obtained two models that were double-muscled and albinism diseases. They also used CBEs to induced C to T conversion in LMNA gene leading in creating a cryptic splice donor site that produces a mutant lamin A
| First author                  | Publication years | Species                     | Category of base editor | Cas protein | Deaminase | Editing windows | PAM               |
|------------------------------|-------------------|-----------------------------|-------------------------|-------------|-----------|----------------|-------------------|
| Yuan Zong [49]               | 2017              | Rice, wheat, and maize      | PBE                     | nSpCas9     | rAPOBEC1  | 3-9            | NGG               |
| Puping Liang [50]            | 2017              | Human                       | YEE-BE3 SaBE3 SaKKH-BE3 | nSpCas9     | nSaCas9   | 5-6            | 3-12 for SaBE3    |
|                              |                   |                             |                         | rAPOBEC1-YEE|           |                | NNGRT for SaBE3   |
| Y Bill Kim [24]              | 2017              | Human                       | VQR-BE3 EQR-BE3 RER-BE3 | nSpCas9-VQR | rAPOBEC1  | 4-11 for VQR-BE3 and EQR-BE3 | 3-10 for VQR-BE3 and EQR-BE3 |
| Pranam Chatterjee [51]       | 2018              | Human                       | ScCas9-ABE7.10 ScCas9   | ScCas9(n)   | TadA-TadA* | 4-9            | NNNG              |
| Noah Jakimo [52]             | 2018              | Human                       | Spy-mac nCas9-BE3       | nSp-macCas9 | rAPOBEC1  | 4-7            | NAAN              |
| Hiroshi Nishmasu [53]        | 2018              | Human                       | Target-AID-NG           | SpCas9(n)   | PmCDA1    | 2-8            | NG                |
| Xiao Wang [31]               | 2018              | Human                       | hA3A-BE3 dCpf1-BE       | dCpf1-BE    | rAPOBEC1  | 8-13 for dCpf1-BE and dCpf1-eBE | 10-12 for dCpf1-BE-YE and dCpf1-eBE-YE |
| Xiaosa Li [29]               | 2018              | Human                       | dCpf1-1BE-YE dCpf1-eBE  | dLbCpf1     | rAPOBEC1  | 4-8            | NG, GAA and GAT   |
| Johnny H. Hu [28]            | 2018              | Human                       | xCas9(3.7)-ABE CjABE    | nxCas9      | APOBEC1   | 4-8            | NGG               |
| Xinjian Li [54]              | 2020              | Human                       | CjABE CBE-NRHR CBE-NRCH | nCjCas9     | TadA-TadA* | 9              | GGGGACC           |
|                              |                   |                             | CBE-NRTH                 | nSpCas9-NRHR | APOBEC1 for CBE | 4-8 for CBE |
|                              |                   |                             | ABE-NRHR                 | nSpCas9-NRCH | TadA-TadA* for ABE | 4-7 for ABE |
|                              |                   |                             | ABE-NRCH                 | nSpCas9-NRTH |                   |                 |
|                              |                   |                             | ABE-NRTH                 |              |                   |                 |
| Shannon M. Miller [55]       | 2020              | Human                       | CjABE                   |              |           |                 | NRRH             |
|                              |                   |                             | SpABE8e                 | nSpCas9     |           |                 | 4-8 for SpABE8e   |
|                              |                   |                             | SaABE8e                 | nSaCas9     |           |                 | NNGG for SaABE8e  |
| Michelle F. Richter [32]     | 2020              | Human                       | LbABE8e                 | dLbCas12a   | TadA-8e   | 3-14 for SaABE8e | NNGGRT for SaABE8e|
|                              |                   |                             | LbABE8e                 | dLbCas12a   |           |                 | TTTV for LbABE8e  |
|                              |                   |                             | LbABE8e                 | dLbCas12a   |           |                 | and enAsABE8e     |
|                              |                   |                             | dLbCas12a               | dLbCas12a   |           |                 | enAsABE8e         |
| Xiang Lin [56]               | 2020              | Human                       | SaCas9n-KKH-miniABEmax  | nSaCas9-KKH | TadA*     | N.R.           | NNNNRT            |
|                            |                   |                             |                         |              |           |                 |                   |
| First author          | Publication years | Species | Category of base editor | Cas protein | Deaminase | Editing windows | PAM               |
|----------------------|-------------------|---------|-------------------------|-------------|-----------|----------------|------------------|
| Ziyang Hu [57]       | 2020              | Human   | SauriBE4max             | nSauriCas9  | APOBEC1 for CBE | 4-13 for CBE    | NNGG             |
|                      |                   |         | SauriABEmax             |             | TadA-TadA+ for ABE | 6-14 for ABE    |                  |
|                      |                   |         | SpG-BE4max              | nSpCas9     | rAPOBEC1 for CBE | 3-9 for CBE     |                  |
| Russell T. Walton [58]| 2020              | Human   | SpG-ABEmax              |             | TadA-TadA+ for ABE | 5-7 for ABE     | NRN for SpRY     |
|                      |                   |         | SpRY-CBE                |             |                        |                  | (R is A or G)    |
|                      |                   |         | SpRY-ABE                |             |                        |                  |                  |
protein, “progerin,” obtaining the third model which was Hutchinson-Gilford progeria syndrome (HGPS). Then, they used ABE7.10 to generate A to G conversion in Dmd (T279A) and obtained X-linked dilated cardiomyopathy (XLCM) model [69]. They got four kinds of disease models collectively, and the mutant rabbits showed the typical phenotypes observed in patients. Intriguingly, Liu and coworkers created mouse model harboring multiple mutations by using a combination of ABE and SaBE3. The mouse models recapitulated respective clinical defects and proved the specificity of ABE [75]. The study of Xie’s group also showed that CBEs could induce C to T conversions at multiple sites in pig embryos simultaneously, and the mutation efficiency approximated 40~50% [67]. These studies mentioned all prove that base editor can be applied to generate mammal’s models, which could mimic the mutations associated with human disease and could be used to guide the treatment of disease to some extent.

1.2.2. Disease Treatment. To explore the feasibility and safety of base editor in gene therapy, researchers first studied in mammalian genetic disease models. Ryu’s team demonstrated that delivering ABEs via transsplicing adeno-associated viral vectors to muscle cells in a mouse model of Duchenne muscular dystrophy enables the correction of the pathogenic mutation in the Dmd gene [70]. Two studies in nature medicine demonstrated that the base editor could be used to treat genetic disease in mice model of human autosomal recessive liver disease phenylketonuria or hereditary tyrosinemia type 1 [76, 77]. Recently, Thomas Gaj and
coworkers established an intein-mediated transsplicing system that could deliver CBEs in vivo. They injected dual AAV particles encoding a split-intein CBE, introducing a nonsense-conding substitution into a mutant SOD1<sub>G93A</sub>, and achieving significantly slowed progression of ALS disease in mouse model [78].

Further, verifying the safety of base editor in clinical gene therapy, researchers are now focusing on human embryos and cells. In 2017, Huang’s team reported the efficient correction of HBB (28 A>G) mutation in human primary cells and human embryos by BE3 or BE3’s variants with corresponding sgRNA [50]. The HBB gene (28 A>G) mutation caused a common genetic disease, β-thalassemia, which is a major problem of global health. Researchers found that the mutation in HBB gene will lead to the reduction of hemoglobin β chain (β-globin) and erythrocytes, finally inducing oxygen shortage, bone deformity, organ dysfunction, and even organ failure in many parts of the human body [79]. At present, although the β-thalassemia patients could get treatment with blood transfusion and iron chelation, they still got numerous complication such as arrhythmia and hypothyroidism. Even the only curative therapy, bone marrow transplantation, is also limited by the antigen compatibility of human leukocyte. So, the disease is extremely lethiferous in humans currently. Huang’s studies proved that using base editor in anemia could not only cure the disease but also prevent the disease from being passed onto future generations. Geurts and coworkers applied SpCas9-ABE and xCas9-ABE on four cystic fibrosis (CF) organoid sample. Their studies showed that both genetic mutations and functional disorders were repaired in all four cases, indicating that 20% of 664 patients in CF intestinal organoid biobank can be repaired by ABE [80].

At present, there have many prominent cases of base editor used in gene therapy for genetic disease (Table 2). Li and partners have successfully applied base editor in a cancer treatment for primary glioblastomas (GBM). The 124C>T in TERT gene would increase telomerase promoter activity and lead to the overexpression of TERT and preservation of telomere, enabling tumor cells to proliferate and evade senescence eventually. And it had been confirmed that there are 83% of existing TERT (124C>T) mutation lesions in GBM [81]. They developed a base editor variant which is composed of an nCas9 of <i>Campylobacter jejuni</i> and an adenine base editor (CjABE). They utilized CjABE to correct the 124C>T TERT promoter mutation. The local injection of adenovirus expressing TERT-specific sgRNA and CjABE could reduce the TERT transcription and protein expression by blocking the binding of members of the E2F transcription factor family to the TERT promoter, eventually facilitating the senescence and proliferative arrest of cancer cells [54].

All the studies demonstrate that the base editor can correct pathogenic gene mutations and have great prospect in gene therapy.

1.3. The Challenges of Base Editor. As with conventional CRISPR/Cas9 technology, there are two major bottlenecks that are off-target and delivery methods when applying base editor in practical applications. Although base editor cannot produce as many deletions and complex genomic rearrangements as that CRISPR/Cas9 does [88, 89], there are some shortcoming. Due to the property of deaminases which can modify RNA and single-stranded DNA at sites other than the intended target, the base editor can alter the DNA. Last year, two papers in science both reported the high levels of genome-wide off-target effects by CBES [90, 91]. Yang and coworkers developed the Genome-wide Off-target analysis by Two-cell embryos Injection (GOTI) to detect off-target mutations. They injected CRISPR/Cas9 or base editor (CBE or ABE) into two-cell stage and compared the WGS results of edited and nonedited blastomeres at E14.5. Their study showed that the off-target single-nucleotide variants (SNVs) were rare in embryos of either CRISPR/Cas9 or ABEs, and the frequency close to the natural mutation rate. Surprisingly, the number of SNVs in embryos edited by CBES was over 20-fold higher than that in others. Jin’s team demonstrated that CBES but not ABE induced substantial genome-wide off-target mutations which were mostly the C to T conversion by comparing the WGS results from rice plants edited by CBES (BE3 and HF-BE3) or the ABE, with unedited population as control [90]. Moreover, the study of McGrath and coworker also revealed there were lots of unintended point mutations in human stem cells edited by CBES [92]. Significantly, the three teams all demonstrated that the absence of sgRNA did not change the levels of nonspecific off-target edited by CBES. The teams of Yang and Gao both showed an enrichment of SNVs located in highly transcribed genes. So, the result indicated that the APOBEC1 or UGI elements maybe responsible for the substantial off-target, because, in the natural state, APOBEC1 can bind single-stranded DNA (ssDNA) [93], and UGI can increase the spontaneous mutation rate [94, 95]. The random encounters between the deaminase domain of base editor and transient ssDNA may induce random nondirected off-target base editing [96]. Hence, decreasing the ability of APOBEC1 binding to ssDNA or the high levels of UGI may be good choices to reduce SNV’s [97]. Recently, the team of Doman focused on the deaminase domain of APOBEC1 and engineered YF1 variants to narrow the on-target base editing window by screening of deaminase mutant. The new variants retain the substrate-targeting scope of high-activity CBES as well as maintain minimal numbers of Cas9-independent off-targets [98]. In addition, the increase sensitivity of Cas9_R63A/q768A variant to mismatches within the target DNA maybe another good way [99]. Surprisingly, Kim and cooperators showed that except converting adenine to guanine, ABES can also convert cytosine to guanine or thymine in narrow editing window and in a confined TC<N sequence context [100]. Moreover, two papers in nature verified that base editor could induce off-target in RNA. Grunewald’s team found that both CBES and ABES can cause extensive transcriptome-wide RNA edits in human cells and that CBES-induced RNA editing occurs in both protein-coding and non-protein-coding sequences [101]. Zhou and coworkers also demonstrated that BE3 and ABET.10 produced thousands of off-target in RNA level [102]. All of the studies warm us to seriously consider the problem of off-target before clinical therapy. Until now, there are several approaches that could be used to predict off-target sites
Table 2: Summary of application of base editor in gene therapy.

| First author | Publication years | Species (tissues)   | Category of base editor | Delivery | Disease              | Gene   | Mutation efficiency | Off-target                                      |
|--------------|-------------------|---------------------|-------------------------|----------|----------------------|--------|---------------------|-------------------------------------------------|
| Alexis C. Komor [15] | 2016            | Mouse astrocytes  | BE3                     | Plasmids | Alzheimer’s disease  | APOE4  | 58-75%              | 4.6–6.1% indels at the targeted locus         |
| Alexis C. Komor [15] | 2016            | Human cell         | BE3                     | Plasmids | Breast cancer        | P53    | 3.3-7.6%            | ≤0.7% indel formation                        |
| Puping Liang [50]    | 2017            | Human embryos     | YEE-BE3                 | Injecting mRNA and gRNA | β-Thalassemia     | HBB    | 22.9%               | No off-target in top 10 predicted sites       |
| Alexandra C. Chadwick [82] | 2017          | Adult mice        | BE3                     | Adenoviral vectors | High blood cholesterol | PCSK9  | Average 24%         | ≤1% indel formation                         |
| Seuk-Min Ryu [70]    | 2018            | Adult mice        | ABE                     | Adeno-associated virus | Duchenne muscular dystrophy | DMD    | 3.3 ± 0.9%          | No off-target mutations at homologous sites with up to three mismatches |
| Luke W Koblan [20]   | 2018            | Human fibroblasts | BE4, BE4max–P2A–GFP, AncBE4max–P2A–GFP nSaKKH-BE3 | Plasmids | Glycosylation type If | MPDU1  | 32.77%              | N.R.                                           |
| Lukas Villiger [76]  | 2018            | Adult mice        | dLbRR-BE                | Adeno-associated virus | Autosomal recessive liver disease phenylketonuria | PAH    | Average 25.1%       | No C→T or G to T conversions or indel formations in ten potential off-target loci |
| Alexandra C. Chadwick [83] | 2018      | Adult mice        | BE3                     | Adenoviral vectors | Hyperlipidemic | ANGPTL3 | 35%                | No off-target in top 10 predicted sites       |
| Yanting Zeng [84]    | 2018            | Human embryos     | BE3                     | Injecting mRNA and gRNA | Marfan syndrome | FBNA   | 89%                 | No off-target and indels were detected in 32 potential off-target sites |
| Maria Paz Zafra [85] | 2018            | Mouse intestinal organoids | FNLS-BE3                             | Plasmids | Colorectal cancers19 | Apc    | >97%               | Less than 1% indels                          |
| Xinjian Li [54]      | 2020            | Human U87 and U251 cells | CjABE                           | Adeno-associated virus | Glioblastoma | TERT   | 70-80%             | N.R.                                           |
| Xiang Lin [56]       | 2020            | Human SMA motor neurons | SaCas9n-KKH-ABE                  | Injecting mRNA and gRNA | Spinal muscular atrophy | SMN2   | 5-40%               | No detectable off-target effects in DNA level |
| Colin K.W.Lim [78]   | 2020            | Adult mice        | BE3                     | Adeno-associated virus | Amyotrophic lateral sclerosis | SOD1   | N.R.               | N.R.                                           |
| Chun Qing Song [86]  | 2020            | Adult mice        | ABE6.3                  | Plasmids | Tyrosinemia          | FAH    | 9.5 ± 4.0%         | One off-target site                          |
| Jonathan M. Levy [87] | 2020           | Adult mice        | BE3                     | Adeno-associated virus | Niemann-Pick disease | Npc1   | 10-80%             | One off-target site                          |
[103–105]. Nevertheless, the predictions are usually far different from the WGS—a cumbersome and expensive approach. So, we need to develop reliable predictive software.

The other area that needs to be optimized is delivery strategy. Four general methods for delivery are electroporation, lipofection, viral vectors, and nanoparticles. Electroporation and lipofection are the primary methods used in vitro. Electroporation involves pulsing cells with high-voltage currents that create transient nanometre-size pores in the cell membrane to facilitate the delivery of base editor to cells. However, due to the particularity of operation, electroporation is limited to cell transfection in vitro. Lipofection reagent wraps plasmid vector DNA, forming DNA-lipid complex which could be absorbed via endocytosis of cell membrane, but the toxicity can cause massive cell death. Nanoparticle is another alternative way to deliver base editor via endocytosis and micropinocytosis. And nanoparticle is inexpensive and relatively easy to produce rather than the cumbersome and expensive approach. Predictably, if the base editor stays in the cell too long, it will cause more off-target mutations. Therefore, using the preassembled CRISPR/Cas9 RNPs with sgRNA can reduce possible off-target mutations due to the short half-life [16, 106, 107].

2. Conclusions

Gene editing is fascinating the medicine of the future and opening a window to actual personalized precision medicine. Depending on the character that CRISPR system could be anchored to target DNA or RNA sequences with relevant gRNA, base editor shows precise and highly predictable nucleotide substitution at target sites without DSBs and donor templates following little indels. The base editor guarantees the stability of the genome to some extent, when they are applied for gene editing. With the expansion of the application range of base editor, its accuracy and security need to be further ameliorated. As described in this review, two strategies could significantly reduce the off-target effect by reducing the intimacy between the base editor and nontarget site and optimizing the delivery method. There is no doubt that the base editor provides a powerful strategy for exploring the mechanisms and treating monogenetic disease, which have the potential to broadly impact the biomedicine.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Authors’ Contributions

Fang Wang and Yuqiang Zeng contributed equally to this work.

Acknowledgments

We thank Dr. Qiaoyan Yang from the Leon H Charney Division of Cardiology, New York University School of Medicine, for helpful suggestions and modifications to this manuscript. This work was supported by the National Key Research and Development Program (2016YFA0101401).

References

[1] S. Scherer and R. W. Davis, “Replacement of chromosome segments with altered DNA sequences constructed in vitro,” Proceedings of the National Academy of Sciences of the United States of America, vol. 76, no. 10, pp. 4951–4955, 1979.
[2] Y. S. Rong and K. G. Golic, “Gene targeting by homologous recombination in Drosophila,” Science, vol. 288, no. 5473, pp. 2013–2018, 2000.
[3] S. L. Mansour, K. R. Thomas, and M. R. Capecechi, “Disruption of the proto-oncogene int-2 in mouse embryo-derived stem cells: a general strategy for targeting mutations to non-selectable genes,” Nature, vol. 336, no. 6197, pp. 348–352, 1988.
[4] O. Smithies, R. G. Gregg, S. S. Boggs, M. A. Koralewski, and R. S. Kucherlapati, “Insertion of DNA sequences into the human chromosomal beta-globin locus by homologous recombination,” Nature, vol. 317, no. 6034, pp. 230–234, 1985.
[5] M. Jinek, K. Chylinski, I. Fonfara, M. Hauer, J. A. Doudna, and E. Charpentier, “A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity,” Science, vol. 337, no. 6096, pp. 816–821, 2012.
[6] L. Cong, F. A. Ran, D. Cox et al., “Multiplex genome engineering using CRISPR/Cas systems,” Science, vol. 339, no. 6211, pp. 819–823, 2013.
[7] P. Mali, L. Yang, K. M. Esvelt et al., “RNA-guided human genome engineering via Cas9,” Science, vol. 339, no. 6211, pp. 823–826, 2013.
[8] M. Jinek, A. East, A. Cheng, S. Lin, E. Ma, and J. Doudna, “RNA-programmed genome editing in human cells,” eLife, vol. 2, article e00471, 2013.
[9] L. A. Gilbert, M. H. Larson, L. Morsut et al., “CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes,” Cell, vol. 154, no. 2, pp. 442–451, 2013.
[10] L. S. Qi, M. H. Larson, L. A. Gilbert et al., “Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression,” Cell, vol. 152, no. 5, pp. 1173–1183, 2013.
[11] D. J. Burgess, “Translational genetics: CRISPR therapies - making the grade not the cut,” Nature Reviews. Genetics, vol. 19, no. 2, p. 63, 2018.
[12] D. Bikard, W. Jiang, P. Samai, A. Hochschild, F. Zhang, and L. A. Marraffini, “Programmable repression and activation of bacterial gene expression using an engineered CRISPR-Cas system,” Nucleic Acids Research, vol. 41, no. 15, pp. 7429–7437, 2013.
[13] S. Konermann, M. D. Brigham, A. E. Trevino et al., "Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex," *Nature*, vol. 517, no. 7536, pp. 583–588, 2015.

[14] F. A. Ran, P. D. Hsu, J. Wright, V. Agarwala, D. A. Scott, and F. Zhang, "Genome engineering using the CRISPR-Cas9 system," *Nature Protocols*, vol. 8, no. 11, pp. 2281–2308, 2013.

[15] A. C. Komor, Y. B. Kim, M. S. Packer, J. A. Zuris, and D. R. Liu, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage," *Nature*, vol. 533, no. 7603, pp. 420–424, 2016.

[16] H. A. Rees, A. C. Komor, W. H. Yeh et al., "Improving the DNA specificity and applicability of base editing through protein engineering and protein delivery," *Nature Communications*, vol. 8, no. 1, article 15790, 2017.

[17] A. C. Komor, K. T. Zhao, M. S. Packer et al., "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C·G-to-T·A base editors with higher efficiency and product purity," *Science Advances*, vol. 3, no. 8, article eaao4774, 2017.

[18] F. d’Adda di Fagagna, G. R. Weller, A. J. Doherty, and S. P. Jackson, "The Gam protein of bacteriophage Mu is an orthologue of eukaryotic Ku," *EMBO Reports*, vol. 4, no. 1, pp. 47–52, 2003.

[19] N. M. Gaudelli, A. C. Komor, H. A. Rees et al., "Programmable base editing of A·T to G·C in genomic DNA without DNA cleavage," *Nature*, vol. 551, no. 7681, pp. 464–471, 2017.

[20] L. W. Koblan, J. L. Domcan, C. Wilson et al., "Improving cytidine and adenine base editors by expression optimization and ancestral reconstruction," *Nature Biotechnology*, vol. 36, no. 9, pp. 843–846, 2018.

[21] S. J. Bae, B. G. Park, B. G. Kim, and J. S. Hahn, "Multiplex gene disruption by targeted base editing of Yarrowia lipolytica genome using cytidine deaminase combined with the CRISPR/Cas9 system," *Biotechnology Journal*, vol. 15, no. 1, article e1900238, 2020.

[22] Y. Ma, J. Zhang, W. Yin, Z. Zhang, Y. Song, and X. Chang, "Targeted AID-mediated mutagenesis (TAM) enables efficient genomic diversification in mammalian cells," *Nature Methods*, vol. 13, no. 12, pp. 1029–1035, 2016.

[23] K. Nishida, T. Arazoe, N. Yachie et al., "Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems," *Science*, vol. 353, no. 6305, p. aa18729, 2016.

[24] Y. B. Kim, A. C. Komor, J. M. Levy, M. S. Packer, K. T. Zhao, and D. R. Liu, "Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions," *Nature Biotechnology*, vol. 35, no. 4, pp. 371–376, 2017.

[25] J. Tan, F. Zhang, D. Karcher, and R. Bock, "Engineering of high-precision base editors for site-specific single nucleotide replacement," *Nature Communications*, vol. 10, no. 1, p. 439, 2019.

[26] J. Tan, F. Zhang, D. Karcher, and R. Bock, "Expanding the genome-targeting scope and the site selectivity of high-precision base editors," *Nature Communications*, vol. 11, no. 1, p. 629, 2020.

[27] W. Jiang, S. Feng, S. Huang et al., "BE-PLUS: a new base editing tool with broadened editing window and enhanced fidelity," *Cell Research*, vol. 28, no. 8, pp. 855–861, 2018.
CRISPR-Cas systems,” *Molecular Cell*, vol. 60, no. 3, pp. 385–397, 2015.

[45] S. Shmakov, A. Smargon, D. Scott et al., “Diversity and evolution of class 2 CRISPR-Cas systems,” *Nature Reviews Microbiology*, vol. 15, no. 3, pp. 169–182, 2017.

[46] D. B. T. Cox, J. S. Gootenberg, O. O. Abudayeh et al., “RNA editing with CRISPR-Cas13,” *Science*, vol. 358, no. 6366, pp. 1019–1027, 2017.

[47] O. O. Abudayeh, J. S. Gootenberg, B. Franklin et al., “A cytosine deaminase for programmable single-base RNA editing,” *Science*, vol. 365, no. 6451, pp. 382–386, 2019.

[48] L. Qu, Z. Yi, S. Zhu et al., “Programmable RNA editing by recruiting endogenous ADAR using engineered RNAs,” *Nature Biotechnology*, vol. 37, no. 9, pp. 1059–1069, 2019.

[49] Y. Zong, Y. Wang, C. Li et al., “Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion,” *Nature Biotechnology*, vol. 35, no. 5, pp. 438–440, 2017.

[50] P. Liang, C. Ding, H. Sun et al., “Correction of β-thalassemia mutant by base editor in human embryos,” *Protein & Cell*, vol. 8, no. 11, pp. 811–822, 2017.

[51] P. Chatterjee, N. Jakimo, and J. M. Jacobson, “Minimal PAM specificity of a highly similar SpCas9 ortholog,” *Science Advances*, vol. 4, no. 10, article eaau0766, 2018.

[52] N. Jakimo, P. Chatterjee, L. Nip, and J. M. Jacobson, “A Cas9 with complete PAM recognition for adenine dinucleotides,” 2018.

[53] H. Nishimasu, X. Shi, S. Ishiguro et al., “Engineered CRISPR-Cas9 nuclease with expanded targeting space,” *Science*, vol. 361, no. 6409, pp. 1259–1262, 2018.

[54] X. Li, X. Qian, B. Wang et al., “Programmable base editing of mutated TERT promoter inhibits brain tumour growth,” *Nature Cell Biology*, vol. 22, no. 3, pp. 282–288, 2020.

[55] S. M. Miller, T. Wang, P. B. Randolph et al., “Continuous evolution of SpCas9 variants compatible with non-G PAMs,” *Nature Biotechnology*, vol. 38, no. 4, pp. 471–481, 2020.

[56] X. Lin, H. Chen, Y. Q. Lu et al., “Base editing-mediated splicing correction therapy for spinal muscular atrophy,” *Cell Research*, vol. 30, no. 6, pp. 548–550, 2020.

[57] Z. Hu, S. Wang, C. Zhang et al., “A compact Cas9 ortholog from Staphylococcus Auricularis (SauriCas9) expands the DNA targeting scope,” *PLoS Biology*, vol. 18, no. 3, article e3000686, 2020.

[58] R. T. Walton, K. A. Christie, M. N. Whittaker, and B. P. Kleinsteiver, “Unconstrained genome targeting with near-PAMless engineered CRISPR-Cas9 variants,” *Science*, vol. 368, no. 6488, pp. 290–296, 2020.

[59] W. Chen, Y. Zhang, Y. Zhang et al., “CRISPR/Cas9-based genome editing in Pseudomonas aeruginosa and cytidine deaminase-mediated base editing in Pseudomonas species,” *iScience*, vol. 6, pp. 222–231, 2018.

[60] Y. Wang, S. Wang, W. Chen et al., “CRISPR-Cas9 and CRISPR-assisted cytidine deaminase enable precise and efficient genome editing in Klebsiella pneumoniae,” *Applied and Environmental Microbiology*, vol. 84, no. 23, 2018.

[61] Y. Lu and J. K. Zhu, “Precise editing of a target base in the rice genome using a modified CRISPR/Cas9 system,” *Molecular Plant*, vol. 10, no. 3, pp. 523–525, 2017.

[62] B. C. Kang, J. Y. Yun, S. T. Kim et al., “Precision genome engineering through adenosine base editing in plants,” *Nature Plants*, vol. 4, no. 7, pp. 427–431, 2018.

[63] Z. Shimatani, S. Kashojiya, M. Takayama et al., “Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion,” *Nature Biotechnology*, vol. 35, no. 5, pp. 441–443, 2017.

[64] C. Li, Y. Zong, Y. Wang et al., “Expanded base editing in rice and wheat using a Cas9-adenosine deaminase fusion,” *Genome Biology*, vol. 19, no. 1, p. 59, 2018.

[65] Y. Zhang, W. Qin, X. Lu et al., “Programmable base editing of zebrafish genome using a modified CRISPR-Cas9 system,” *Nature Communications*, vol. 8, no. 1, p. 118, 2017.

[66] S. Tanaka, S. Yoshioka, K. Nishida, H. Hosokawa, A. Kakizuka, and S. Maegawa, “In vivo targeted single-nucleotide editing in zebrafish,” *Scientific Reports*, vol. 8, no. 1, article 11423, 2018.

[67] J. Xie, W. Ge, N. Li et al., “Efficient base editing for multiple genes and loci in pigs using base editors,” *Nature Communications*, vol. 10, no. 1, p. 2852, 2019.

[68] K. Kim, S. M. Ryu, S. T. Kim et al., “Highly efficient RNA-guided base editing in mouse embryos,” *Nature Biotechnology*, vol. 35, no. 5, pp. 435–437, 2017.

[69] Z. Liu, M. Chen, S. Chen et al., “Highly efficient RNA-guided base editing in rabbit,” *Nature Communications*, vol. 9, no. 1, pp. 2717, 2018.

[70] S.-M. Ryu, T. Koo, K. Kim et al., “Adenine base editing in mouse embryos and an adult mouse model of Duchenne muscular dystrophy,” *Nature Biotechnology*, vol. 36, no. 6, pp. 536–539, 2018.

[71] G. Li, Y. Liu, Y. Zeng et al., “Highly efficient and precise base editing in discarded human tripronuclear embryos,” *Protein & Cell*, vol. 8, no. 10, pp. 776–779, 2017.

[72] C. Zhou, M. Zhang, Y. Wei et al., “Highly efficient base editing in human tripronuclear zygotes,” *Protein & Cell*, vol. 8, no. 10, pp. 772–775, 2017.

[73] P. Billon, E. E. Bryant, S. A. Joseph et al., “CRISPR-mediated base editing enables efficient disruption of eukaryotic genes through induction of STOP codons,” *Molecular Cell*, vol. 67, no. 6, pp. 1068–1079.e4, 2017.

[74] C. Kuscu, M. Parlak, T. Tufan et al., “CRISPR-STOP: gene silencing through base-editing-induced nonsense mutations,” *Nature Methods*, vol. 14, no. 7, pp. 710–712, 2017.

[75] Z. Liu, Z. Lu, G. Yang et al., “Efficient generation of mouse models of human diseases via ABE- and BE-mediated base editing,” *Nature Communications*, vol. 9, no. 1, p. 2338, 2018.

[76] L. Villiger, H. M. Grisch-Chan, H. Lindsay et al., “Treatment of a metabolic liver disease by in vivo genome base editing in adult mice,” *Nature Medicine*, vol. 24, no. 10, pp. 1519–1525, 2018.

[77] A. C. Rossidis, J. D. Stratigos, A. C. Chadwick et al., “In utero CRISPR-mediated therapeutic editing of metabolic genes,” *Nature Medicine*, vol. 24, no. 10, pp. 1513–1518, 2018.

[78] C. K. W. Lim, M. Gapiske, A. K. Brooks et al., “Treatment of a mouse model of ALS by in vivo base editing,” *Molecular Therapy*, vol. 28, no. 4, pp. 1177–1189, 2020.

[79] A. Cao and R. Galanello, “Beta-thalassemia,” *Genetics in Medicine*, vol. 12, no. 2, pp. 61–76, 2010.

[80] M. H. Geurts, E. de Poel, G. D. Amatngalim et al., “CRISPR-based adenosine editors correct nonsense mutations in a cystic fibrosis organoid biobank,” *Cell Stem Cell*, vol. 26, no. 4, pp. 503–510.e7, 2020.

[81] P. J. Killela, Z. J. Reitman, Y. Jiao et al., “TERT promoter mutations occur frequently in gliomas and a subset of tumors
derived from cells with low rates of self-renewal,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 15, pp. 6021–6026, 2013.

[82] A. C. Chadwick, X. Wang, and K. Musunuru, “In vivo base editing of PCSK9 (proprotein convertase subtilisin/kexin type 9) as a therapeutic alternative to genome editing,” *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 37, no. 9, pp. 1741–1747, 2017.

[83] K. Musunuru and S. Kathiresan, “Cardiovascular endocrinology: is ANGPTL3 the next PCSK9?,” *Nature Reviews Endocrinology*, vol. 13, no. 9, pp. 503–504, 2017.

[84] Y. Zeng, J. Li, G. Li et al., “Correction of the Marfan syndrome pathogenic FBN1 mutation by base editing in human cells and heterozygous embryos,” *Molecular Therapy*, vol. 26, no. 11, pp. 2631–2637, 2018.

[85] M. P. Zafra, E. M. Schatoff, A. Katti et al., “Optimized base editors enable efficient editing in cells, organoids and mice,” *Nature Biotechnology*, vol. 36, no. 9, pp. 888–893, 2018.

[86] C. Q. Song, T. Jiang, M. Richter et al., “Adenine base editing in an adult mouse model of tyrosinaemia,” *Nature Biomedical Engineering*, vol. 4, no. 1, pp. 125–130, 2020.

[87] J. M. Levy, W. H. Yeh, N. Pendse et al., “Cytosine and adenine base editing of the brain, liver, retina, heart and skeletal muscle of mice via adeno-associated viruses,” *Nature Biomedical Engineering*, vol. 4, no. 1, pp. 97–110, 2020.

[88] M. Kosicki, K. Tomberg, and A. Bradley, “Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements,” *Nature Biotechnology*, vol. 36, no. 8, pp. 765–771, 2018.

[89] H. Y. Shin, C. Wang, H. K. Lee et al., “CRISPR/Cas9 targeting events cause complex deletions and insertions at 17 sites in the mouse genome,” *Nature Communications*, vol. 8, no. 1, article 15464, 2017.

[90] S. Jin, Y. Zong, Q. Gao et al., “Cytosine, but not adenine, base editors induce genome-wide off-target mutations in rice,” *Science*, vol. 364, no. 6437, pp. 292–295, 2019.

[91] E. Zuo, Y. Sun, W. Wei et al., “Cytosine base editor generates substantial off-target single-nucleotide variants in mouse embryos,” *Science*, vol. 364, no. 6437, pp. 289–292, 2019.

[92] E. McGrath, H. Shin, L. Zhang et al., “Targeting specificity of APOBEC-based cytosine base editor in human iPSCs determined by whole genome sequencing,” *Nature Communications*, vol. 10, no. 1, p. 5353, 2019.

[93] R. S. Harris, S. K. Petersen-Mahrt, and M. S. Neuberger, “RNA editing enzyme APOBEC1 and some of its homologs can act as DNA mutators,” *Molecular Cell*, vol. 10, no. 5, pp. 1247–1253, 2002.

[94] E. H. Radany, K. J. Dormfeld, R. J. Sanderson et al., “Increased spontaneous mutation frequency in human cells expressing the phage PBS2-encoded inhibitor of uracil-DNA glycosylase,” *Mutation Research*, vol. 461, no. 1, pp. 41–58, 2000.

[95] J. Di Noia and M. S. Neuberger, “Altering the pathway of immunoglobulin hypermutation by inhibiting uracil-DNA glycosylase,” *Nature*, vol. 419, no. 6902, pp. 43–48, 2002.

[96] H. A. Rees and D. R. Liu, “Base editing: precision chemistry on the genome and transcriptome of living cells,” *Nature Reviews Genetics*, vol. 19, no. 12, pp. 770–788, 2018.

[97] M. R. Willmann, “Base editors and off-targeting: the deaminase matters,” *The CRISPR Journal*, vol. 2, no. 2, pp. 71–73, 2019.

[98] J. L. Doman, A. Raguram, G. A. Newby, and D. R. Liu, “Evaluation and minimization of Cas9-independent off-target DNA editing by cytosine base editors,” *Nature Biotechnology*, vol. 38, no. 5, pp. 620–628, 2020.

[99] M. Bratovič, I. Fonfar, K. Chylinski et al., “Bridge helix arginines play a critical role in Cas9 sensitivity to mismatches,” *Nature Chemical Biology*, vol. 16, no. 5, pp. 587–595, 2020.

[100] H. S. Kim, Y. K. Jeong, J. K. Hur, J. S. Kim, and S. Bae, “Adenine base editors catalyze cytosine conversions in human cells,” *Nature Biotechnology*, vol. 37, no. 10, pp. 1145–1148, 2019.

[101] J. Grünewald, R. Zhou, S. P. Garcia et al., “Transcriptome-wide off-target RNA editing induced by CRISPR-guided DNA base editors,” *Nature*, vol. 569, no. 7756, pp. 433–437, 2019.

[102] C. Zhou, Y. Sun, R. Yan et al., “Off-target RNA mutation induced by DNA base editing and its elimination by mutagenesis,” *Nature*, vol. 571, no. 7764, pp. 275–278, 2019.

[103] S. Bae, J. Park, and J. S. Kim, “Cas-OFFinder: a fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases,” *Bioinformatics*, vol. 30, no. 10, pp. 1473–1475, 2014.

[104] D. Kim, S. Bae, J. Park et al., “Digenome-seq: genome-wide profiling of CRISPR-Cas9 off-target effects in human cells,” *Nature Methods*, vol. 12, no. 3, pp. 237–243, 2015.

[105] D. Kim, K. Lim, S. T. Kim et al., “Genome-wide target specificities of CRISPR RNA-guided programmable deaminases,” *Nature Biotechnology*, vol. 35, no. 5, pp. 475–480, 2017.

[106] D. Kim, D. E. Kim, G. Lee, S. I. Cho, and J. S. Kim, “Genome-wide target specificity of CRISPR RNA-guided adenine base editors,” *Nature Biotechnology*, vol. 37, no. 4, pp. 430–435, 2019.

[107] W. H. Yeh, H. Chiang, H. A. Rees, A. S. B. Edge, and D. R. Liu, “In vivo base editing of post-mitotic sensory cells,” *Nature Communications*, vol. 9, no. 1, p. 2184, 2018.