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REVIEW ARTICLE

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Acceleration of cancer science with genome editing and related technologies

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Genome editing includes various edits of the genome, such as short insertions and deletions, substitutions, and chromosomal rearrangements including inversions, duplications, and translocations. These variations are based on single or multiple DNA double-strand break (DSB)-triggered in cellulo repair machineries. In addition to these "conventional" genome editing strategies, tools enabling customized, site-specific recognition of particular nucleic acid sequences have been coming into wider use; for example, single base editing without DSB introduction, epigenome editing with recruitment of epigenetic modifiers, transcriptome engineering using RNA editing systems, and in vitro detection of specific DNA and RNA sequences. In this review, we provide a quick overview of the current state of genome editing and related technologies that multilaterally contribute to cancer science.

KEYWORDS
base editing, cancer science, CRISPR-Cas9, genome editing, transcriptome editing

1 | INTRODUCTION

The definition of a "gene" is a unit of genetic information, which is a rather conceptualistic term. In living cells, there are coding genes and non-coding genes, which function as protein and RNA, respectively. Expression of a gene is regulated by *cis* and *trans* elements, along with various epigenetic modifications of DNA and histones. Genes can be used, manipulated, and analyzed in vitro for various purposes including creation of cell-free transcription and translation, and diagnostics of genetic diseases, cancers, and infectious diseases.

Genome editing tools can engineer genes with customizable DNA-binding and cleaving properties that are applicable to living cells and organisms. They are unambiguously useful for sequence alteration of genomic DNA, resulting in simple gene knock-out and knock-in, and more complex edits such as multiplex mutagenesis and chromosome engineering (Figure 1). However, considering the many features of genes described above, the editing technology of genes has become diverse. In fact, over the past few years, diverse methods and technologies have rapidly been developed, improved, and applied in various ways.

In this review, we summarize the up-to-date information on genome editing technology that has dramatically innovated and accelerated cancer science.

2 | GENOME EDITING RELOADED: ATTRACTIVE DERIVATIVES COME OF AGE

As a result of rapid technological developments in the field of genome editing, it is quite difficult to remain up to date in this field of study. So far, we have published many reviews regarding genome editing containing the newest information available at the time of each publication. For example, a general outline of this technology including historical background was reviewed in 2014,[1] a more focused review on CRISPR-Cas9 was published in 2015,[2] and a comprehensive overview of transcription activator-like effector (TALE) nuclease (TALEN) systems,[3] updates on CRISPR tools,[4] and recent advances on gene knock-in systems[5] were summarized in 2017. Within these reviews, we

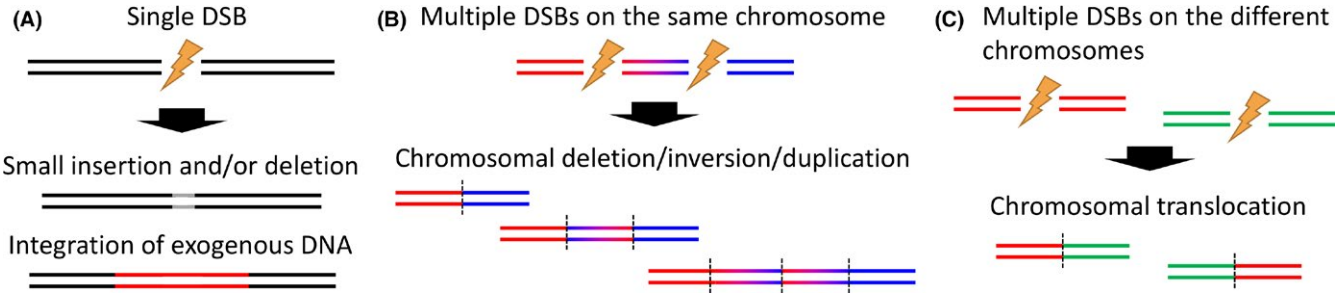

FIGURE 1 Concept of double-strand break (DSB)-mediated genome editing. Simple gene knock-out and knock-in are achieved by introducing single DSB (A), and various chromosomal engineering is achieved by introducing multiple DSB (B,C)

have introduced various achievements in technological development including our highly active variant of TALE/TALEN, named Platinum TALE/TALEN (Figure 2A).[6]

After these publications, in the context of basic tool development and classical genome editing methodology, there were a number of important innovations recently reported, such as xCas9 with broader PAM specificities,[7] HypaCas9[8] and bridged nucleic acid-incorporated CRISPR RNA[9] for hyper-accurate DNA recognition, and Cas9-HE for highly efficient homology-dependent repair.[10] In addition, we recently developed the local accumulation of DSB repair molecules (LoAD) system, which enables repair pathway-biased genome editing (Figure 2B).[11] However, along with these mainstream innovations, we strongly feel the need to discuss the variety of constructive, derivative, applied, or repurposed technologies of genome editing in order to broaden the horizons of the users of this technology.

3 | A CLOSER LOOK AT FRONT-LINE TECHNOLOGIES

To enable a quick overview of the topics covered in this article, we created a simplified timeline of the development of each technology (Figure 3). DNA-binding tools, mainly CRISPR-Cas systems, and sometimes zinc finger (ZF)- or TALE-based chimeric proteins, have recently been used in multiple ways. For the purpose of sequence change with an alternative route, chemical replacement of DNA bases was achieved by fusing deaminase with catalytically inactive Cas9 (dCas9). High-capacity and highly variable genome editing enabled genome-wide screening and DNA barcoding/recording technologies. Transcriptional engineering has been achieved by transcriptional control and epigenome editing technologies. More recently, unique and interesting approaches such as RNA targeting and editing, CRISPR-based diagnosis, and proximity labeling of particular genomic locus have also appeared.

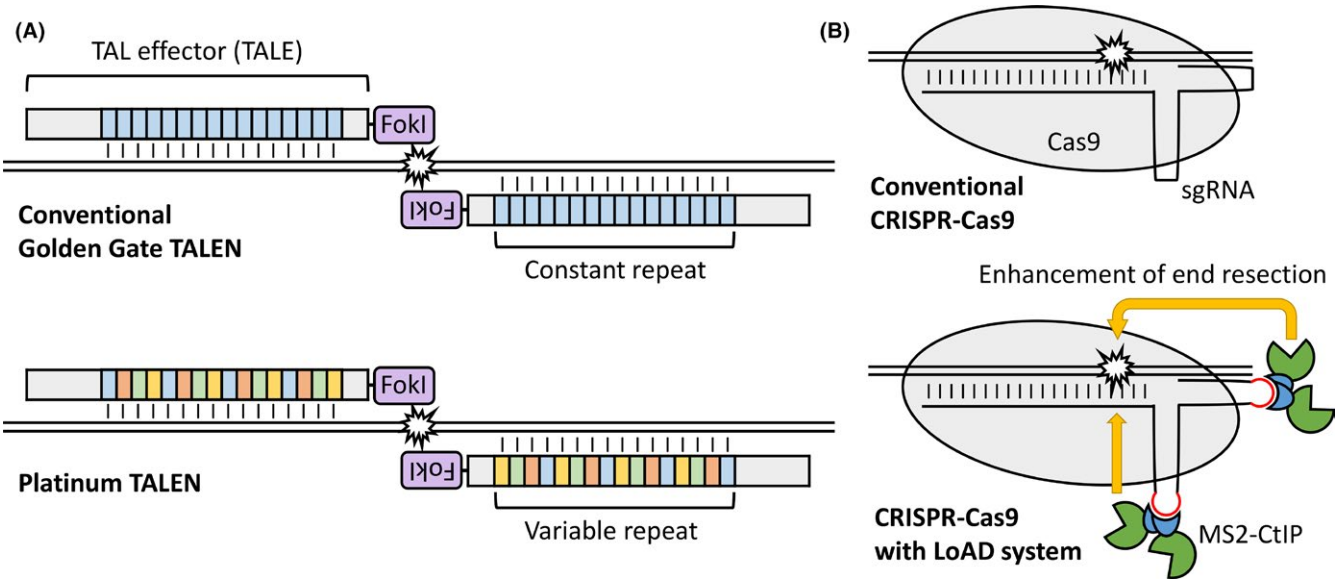

FIGURE 2 Schematics of current genome editing tools. Regarding transcription activator-like effector nuclease (TALEN) systems, conventional Golden Gate TALEN contains constant repeat without non-repeat-variable di-residue (non-RVD) variations (A, top), whereas our improved variant of TALEN, Platinum TALEN, contains variable repeat with non-RVD variations, resulting in higher nuclease activity (A, bottom). Regarding CRISPR-Cas9, conventional system introduces DSB at the target site only (B, top), whereas our local accumulation of DSB repair molecules (LoAD) system coupled with CRISPR-Cas9 allows enhancement of end resection as well as DSB introduction, leading to biased genome editing (B, bottom)

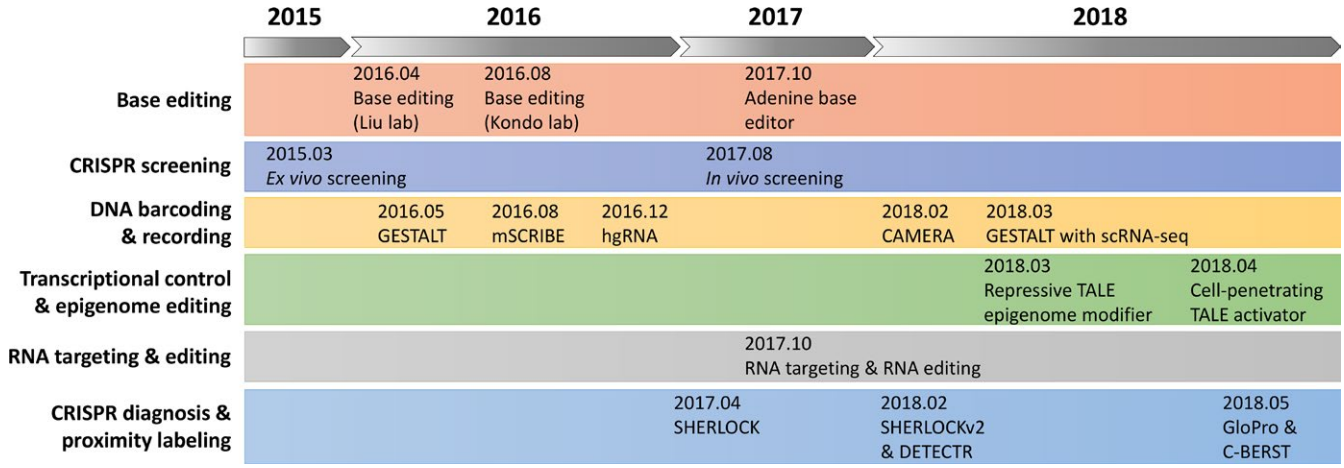

FIGURE 3 Timeline of selected publications covered in the present review. The time point of each publication shows the timing of first online appearance

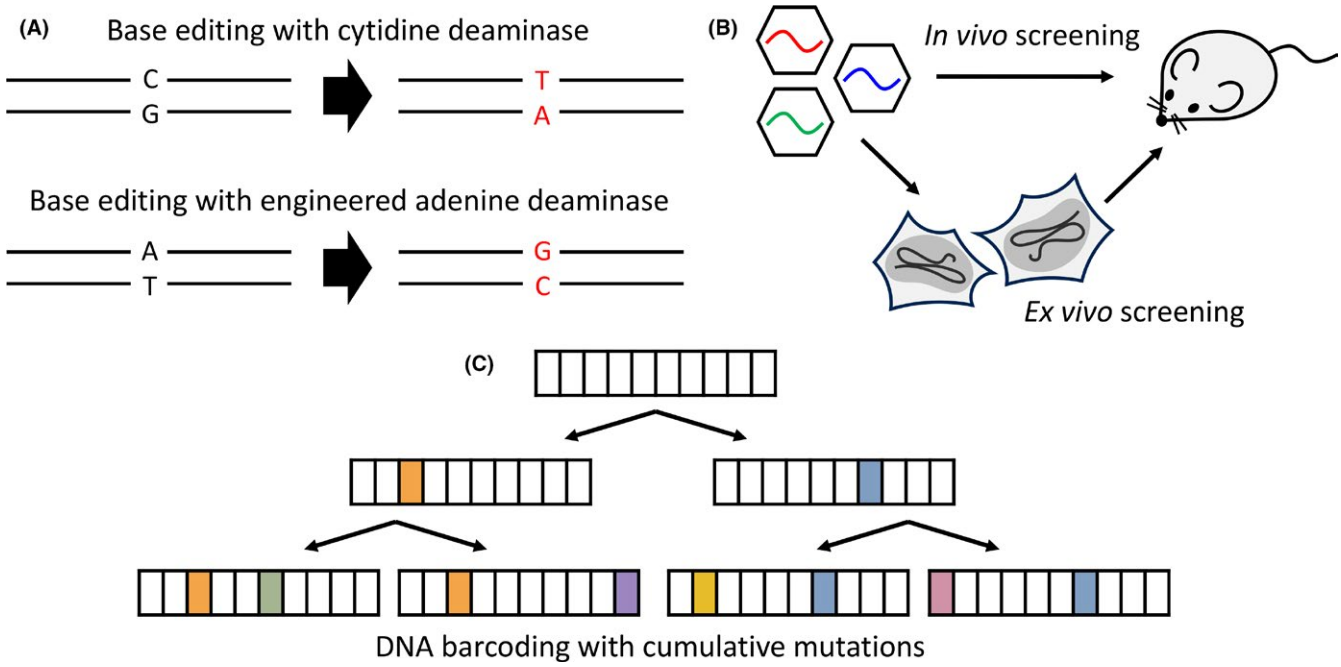

FIGURE 4 Selective schematics of genome editing-related technologies altering DNA sequence. The concepts of base editing (A), ex vivo and in vivo CRISPR screening (B), and DNA barcoding with the GESTALT method (C) are illustrated

3.1 | Base editing

Single base-pair editing is a subtle but quite important modification methodology of the genomic sequence for disease modeling and correction. Various sophisticated strategies depending on conventional DSB-mediated genome editing have been reported (reviewed by Ochiai),[12] and there is ongoing development in this area. One recent example is microhomology-mediated end-joining (MMEJ)-dependent strategy, named MhAX,[13] implementing a MMEJ-dependent gene editing strategy called the PITCh system[14,15] in cassette excision.

DSB-free base editing system was first reported by the laboratory of Liu et al,[16] followed by the laboratory Kondo et al.[17] The basic concept of base editing is cytidine deaminase-mediated conversion of cytosine into uracil, followed by thymine (Figure 4A, top). Cytidine deaminase such as APOBEC1 was linked to dCas9 or Cas9n (Cas9 nickase) to target a specific genomic locus. Liu and colleagues have continued updating their system to broaden its targeting range, increase its specificity, and heighten its efficiency.[18–20] Note that deaminase catalyzes single-strand DNA; therefore, deaminase fused with ZF or TALE was likely to show less activity compared to that fused with dCas9 or Cas9n.[21] The applicability of base editing systems has been initially proven in yeasts and mammalian cells,[16,17] followed by various organisms including plants, mice, sea urchins, and bacteria.[22–25] The high specificity of the system was confirmed by genome-wide assessment.[26] Notably, another type of base

editor, referred to as adenine base editor (ABE), catalyzes adenine into guanine, and was recently produced by Liu's group (Figure 4A, bottom),[27] and was quickly applied to the proof-of-concept study of mouse gene editing and a remedy model of hereditary disease.[28]

Specific applications of base editors were also reported, including the creation of stop codon (iSTOP and CRISPR-STOP)[29,30] and saturation mutagenesis (TAM and CRISPR-X).[31,32] Saturation mutagenesis was previously achieved by DSB-mediated strategies,[33,34] but base editing-dependent strategies seem much easier to carry out and are highly capable of specifically inducing base substitutions. In the CRISPR-X article, the authors hyper-mutated the target gene of the cancer therapeutic drug bortezomib, *PSMB5*, and identified known and novel mutations that confer bortezomib resistance.[32]

3.2 | Genome-wide screening

Genome editing nucleases have been typically used in reverse genetics, but three milestone papers published in *Science* and in *Nature Biotechnology* opened up a new era of CRISPR-mediated forward genetics.[35–37] In brief, the lentiviral RNAi screening system was replaced with CRISPR-Cas9. Genome-wide lentiviral single-guide RNA (sgRNA) library was pooled and infected with cultured cells, the intended anticancer drug resistance was carried out, and the enriched sgRNA analyzed by next-generation sequencing. The principle, variations, and current status of genome-wide screening were reviewed in other publications in more detail.[38,39]

In the context of cancer science, we would like to emphasize several key studies using genome-wide screening. The first study is ex vivo screening of genes involved in tumor growth and metastasis (Figure 4B, bottom arrows).[40] The screening component was introduced in non-metastatic mouse cancer cells and, then, they were transplanted into adult mice and tumor growth- and metastasis-related mutations were screened. After this publication, various related studies depending on the transplantation-based approach have been conducted.[41–43] In addition, adeno-associated virus (AAV) vector-mediated direct delivery of pooled sgRNA library enabled bona fide in vivo screening, although the capacity of the library size was tightly restricted in this strategy (Figure 4B, top arrow).[44,45] Such ex vivo and in vivo screening studies were nicely summarized recently by Chow and Chen.[46]

The second study is a series of studies reporting long non-coding RNA (lncRNA) screening. A recent study uncovered the diversity of lncRNAs and speculated that thousands of lncRNAs were related to human diseases.[47] However, conventional knockout screening is difficult to apply to lncRNAs, because frameshift mutations cannot occur in non-coding RNAs. Therefore, alternative approaches were conducted in lncRNA screening, including double-cut excision with paired sgRNA library,[48] CRISPR interference (CRISPRi) with dCas9-repressor,[49] and gain-of-function screening with dCas9-activator.[50] Furthermore, integrated dual screening of coding and non-coding genes showed the networks of coding genes and lncRNAs in drug resistance.[51] The lncRNA screening systems will play a major role in deepening our understanding of the relationship between lncRNA and cancer.

3.3 | DNA barcoding and recording

As introduced already, genome editing with reverse and forward genetics significantly contributed to the characterization of cancer; however, from the viewpoint of clinical oncology, the vast amount of mutations derived from cancer evolution make things extremely complicated.[52] If the family tree of cancer evolution could be shown by complete lineage tracing, we would obtain a strong weapon to cope with this enormous challenge. Possible solutions were provided by three independent groups, which could roughly be classified in two strategies; DNA barcoding and recording.

The GESTALT method, incorporating the barcode into the genome that was capable of introducing numerous mutation patterns, enabled whole-organism lineage tracing in zebrafish (Figure 4C).[53] Independently introduced and evolved mutation patterns in the barcode were analyzed by next-generation sequencing, by which lineage tracing was achieved. Later, the combination of improved GESTALT (or a related method) and single-cell RNA-sequencing (scRNA-seq) technologies were applied in combinatorial profiling of lineages and cell types.[54,55] This technology might be appropriate for cancer lineage and stage profiling.

The mSCRIBE[56] and homing-guide RNA (hgRNA)[57] methods were DNA recording approaches using self-targeting sgRNA. In these systems, sgRNA-coding DNA sequence was modified to be targeted by self-transcribed sgRNA. After the introduction of mutations, mutated sgRNAs retarget their own template DNA. These systems were also able to track the cell lineage. Moreover, a base editing-dependent recording system named CAMERA 2 was established to write cellular events such as external stimuli-induced signaling.[58] Another interesting CRISPR-based memory device enabled encoding a digital movie in a living cell population.[59] These DNA tape recorder systems enable a new age concept in handling and managing genomic DNA in living cells.

3.4 | Transcriptional control and epigenome editing

Although each methodology is variable, all the technologies introduced above are accompanied by DNA sequence alterations. In contrast, controlled gene expression or manipulation of epigenetic modifications can also modulate the phenotype without changing the sequence of DNA. As partly described in the paragraph "Genome-wide screening", transcriptional control systems such as dCas9-activator and dCas9-repressor have been established. In addition, direct conversion of epigenetic status has been achieved by epigenetic modification enzymes fused with dCas9 or other related systems. We recently summarized the technical background of such transcriptional control and epigenome editing technologies and their application in cancer science;[60] therefore, the several newest investigations in this context that were not published at the time of

publication of the previous review are only introduced in the present article.

First, we reported very recently that integrated, multiplex adenoviral CRISPRi vector simultaneously carrying dCas9 fused with transcriptional repressor domain, KRAB, and multiple sgRNAs efficiently showed an antitumor effect in cultured cells and in vivo.[61] We previously established a plasmid-based all-in-one CRISPR-Cas9 vector system,[62] and expanded it for the paired nickase and dimer-type FokI-dCas9 nuclease systems.[63] In this study, we further repurposed the system for dCas9-KRAB and transferred it to the adenoviral vectors. Although the applicability of this system was shown only for the treatment of lung and esophageal squamous cell carcinoma in this article, it should be used to attack various cancers in the future.

Second, TALE-based transcription factor and epigenetic modifier are also promising tools in this field (Figure 5A). Our recent examination showed that reprogramming into induced pluripotent stem cells could be achieved by adding only one recombinant platinum TALE protein fused with a transcriptional activation domain and cell-penetrating peptide.[64] Similarly, another group showed that only one TALE with KRAB and DNA methyltransferase domains sufficiently suppressed the target gene in cultured cells.[65] As the unintended global effect was observed in the dCas9-epigenetic modifier,[66] these techniques might be attractive alternatives, although comprehensive off-target analysis must be carried out in TALE-based systems as well as in dCas9-based ones.

3.5 | Other emerging technologies: RNA targeting and editing, CRISPR-based diagnosis, and proximity labeling

Finally, we take a brief look at other characteristic technologies developed recently. RNA-guided RNA targeting enzyme named Cas13 is a noteworthy system in terms of transcriptome editing and in vitro diagnosis. Cas13 could reportedly be used in gene knockdown (Figure 5B) and targeted RNA binding for live cell tracking of transcripts.[67] Similar applications were shown by using other Cas proteins such as CasRx.[68] Deaminase-mediated RNA base editing system, referred to as REPAIR, was also established.[69]

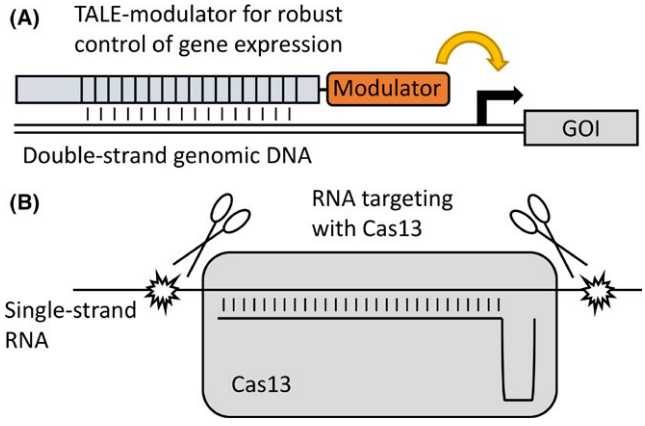

FIGURE 5 Selective schematics of genome editing-related technologies without altering DNA sequence. The concepts of transcription activator-like effector (TALE)-based transcriptional modulation (A) and RNA targeting with Cas13 (B) are illustrated. GOI, gene of interest

Cas13 was further repurposed for diagnosis of viral nucleic acids and cell-free DNA for cancer detection. The primary system was called SHERLOCK, which enabled low-cost, high-sensitivity, and robust detection from quite a limited amount of target DNA and RNA.[70] The system depends on non-specific RNase activity triggered by a specific target RNA sequence. Similar activity was also later discovered in Cas12a, where non-specific single-strand DNase activity was triggered by a specific DNA target sequence, and the application of diagnosis, named DETECTR, was demonstrated.[71] At the same time, multiplexed SHERLOCK (SHERLOCKv2)[72] and HUDSON method-coupled SHERLOCK affording various clinical samples[73] were also reported.

GloPro and C-BERST, both enabling dCas9-APEX-mediated proximity labeling of targeted genomic DNA region, were established for site-specific proteomics.[74,75] A previous system called engineered chromatin immunoprecipitation (enChIP) was based on tagged dCas9-mediated precipitation;[76] however, these new systems could easily biotinylate the proximate proteins by expressing only dCas9-APEX with the sgRNA in the presence of biotin-phenol and H_2O_2. These technologies should also contribute to cancer science in terms of proteomics analysis of the promoter/enhancer regions of cancer-related genes.

4 | CONCLUSION

We believe that DSB-mediated standard genome editing is a world-changing technology, no doubt drastically altering life science studies including cancer science. However, in addition, many new technologies are currently waiting for an opportunity to create a paradigm shift (or perhaps multiple paradigm shifts). To make the most of these fledgling technologies, we must keep an observant eye on them.

On another front, recent investigations cautioned the risk of tumorigenesis,[77,78] unexpected heterogeneity,[79] and variable on-target editing outcomes[80] when applying CRISPR-Cas9. Safety concern has become much more important in proportion to the drastic increase in genome editing applications.

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CONFLICTS OF INTEREST

Authors declare no conflicts of interest for this article.

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