COMMENTARY

CRISPR-Cas9 in Gene Therapy: Much Control On Breaking, Little Control On Repairing

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Abstract

Recent advances in CRISPR-Cas9 genome editing tool have made great promises to basic and biomedical research as well as gene therapy. Efforts to make the CRISPR-Cas9 system applicable in gene therapy are largely focused on two aspects: 1) increasing the specificity of this system by eliminating off-target effects, and 2) optimizing *in vivo* delivery of the CRISPR-Cas9 DNA constructs to target cells and limiting the expression of Cas9 and gRNA to prevent toxicity immune responses. However, there is an unnoted but crucial consideration about the mode of DNA repair at the lesion caused by CRISPR-Cas9. In this commentary, I briefly highlight recent publications on *in vivo* use of the CRISPR-Cas9 system in gene therapy. I then discuss the undesired on-target DNA repair events that can occur as a result of the activity of CRISPR-Cas9. Overall, this commentary underscores the need for more study on controlled DNA repair in systems targeted with CRISPR-Cas9 genome editing tools.

Keywords: CRISPR-Cas9; Gene Therapy; DNA Repair

Commentary

Advances in CRISPR-Cas9 genome editing technology have made many great promises to basic and biomedical research, as well as human therapeutics [1]. Recent reports show successful *in vivo* interrogation of genes by CRISPR-Cas9 [2, 3]. It is now accepted that site-specific manipulation of genome is no longer a limitation to experiments. However, for *in vivo* gene therapy, precise genome editing can still be a bottleneck, as targeting a specific site on genome should be coupled with a controlled DNA repair. Otherwise, unwanted outcomes of genome editing can cause further on-target damage.

Since its development as a genome-editing tool, the CRISPR-Cas9 system has been widely used for making changes in the sequence of DNA. There are now numerous reports on the successful *in vitro* use of this system in different cell types. This includes insertion of new elements into specific sites of DNA, deletion of target DNA sequence and making mutations in the sequence of DNA, with or without a template. The wide spectrum of capabilities in targeted DNA modification has created an excitement about the use of CRISPR-Cas9 system in gene therapy. There are well-founded concerns about the use of CRISPR-Cas9 system in gene therapy. This includes the tolerance of cells towards expression of an exogenous protein such as CAS9, and specificity of the CRISPR-Cas9 system and its potential off-target site [4, 5]. Indeed, a great deal of research in the past two years has been geared towards increasing the specificity of CRISPR-Cas9 activity [6]. However, precise

genome editing is not easily achieved for the purpose of in vivo gene therapy in which it is imperative that targeting of a specific site on the genome be accompanied by a controlled form of DNA repair.

The CRISPR-cas9 system induces site-specific double-strand breaks (DSB) DNA. Repair of the DSBs depends on one of the two repair systems: homologous recombination (HR) or non-homologous end joining (NHEJ). When repairing a mutated gene back to wild-type is desired, gene therapy often relies on HR with a provided DNA template that carries the desired sequence modification [7]. Besides the efficient delivery of vectors to the specific target cells and controlled expression of Cas9, there are considerations about the repair of the DSBs that should be taken into account while using CRISPR-Cas9 system in gene therapy. Availability of the exogenous DNA template at the time of repair, and making the HR mode of repair more favorable over NHEJ, which is naturally a cell-cycle dependent choice [8], are two limiting factors in successful in vivo genome editing for gene therapy. New studies report on successful modulation of DNA repair in vitro, along with genome editing by CRISPR-Cas9. In the first study, chemical inhibitors of cell cycle are used to synchronize the cells before genome editing, therefore increasing certain modes of DNA repair [9]. Another study reports on certain small molecules that can tip the preference of DNA repair system towards the desired mode [10]. In addition, two recent studies have focused on small molecules that inhibit NHEJ to promote HR [11, 12] . Although being in its early days, adaptation of such control over DNA repair system can make the CRISPR-Cas9 a more promising tool for human gene therapy.

As an example, consider a gene therapy scenario in which fixing a point mutation in an exonic region of a gene is desired (Figure 1). A highly target-specific guide-RNA (gRNA) is designed and delivered to the target cells together with Cas9 nuclease. In addition, a homology template DNA is delivered to the target cells along with CRISPR-Cas9 constructs. If: 1) the homology DNA template is not available at the time of repair, or 2) in the specific cell cycle condition the NHEJ is more favorable over HR, the DSB will be repaired by NHEJ. The error prone nature of NHEJ repairs can cause insertions/deletions at the site of DSB that can: 1) make further detrimental changes in the function of the targeted gene and 2) lend the locus untargetable in the future.

In laboratories, *in vivo* studies in model organisms and in human cell lines do not have these limits as screening and selection helps researchers to find the desired DNA modification. The current continuing research on genome editing by CRISPR-Cas9 should be accompanied by more studies on control of DNA repair system in targeted cells.

Competing interests
The authors declare that they have no competing interests.

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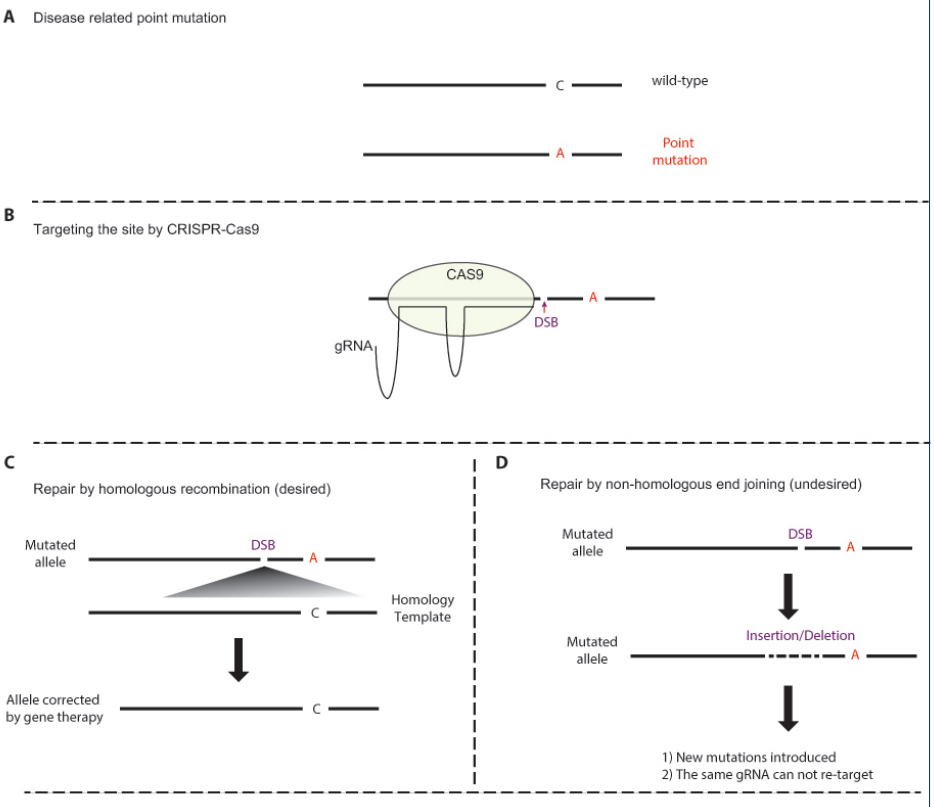

Figure 1 An example gene therapy scenario by CRISPR-Cas9 in which correction of a point mutation is desired (A). The locus is targeted by a gRNA and Cas9 to make a DSB (B). The repair of the DSB can be performed by either of the repair modes: (c) Homologous recombination, which is the desired mode and (D) Non-homologous end joining that can further cause insertion and deletions. This is undesired as it can introduce new mutations and also make re-targeting with the same gRNA impossible.