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 immune responses. Moreover, 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 concerns about the off-target activity and immune responses triggered by the use CRISPR-Cas9 in gene therapy. Following this, I focus on the undesired on-target DNA repair events that can occur as a result of the activity of CRISPR-Cas9. This concise commentary sets itself apart from previous perspectives by focusing on the modes of DNA repair employed following a CRISPR-Cas9 induced genomic insult, and by carefully weighing the benefits of the outcomes. In particular, the present manuscript underscores the need for more study on controlled DNA repair in systems targeted with CRISPR-Cas9 genome editing tools.

Keywords: Gene Therapy, CRISPR-Cas9, DNA Repair

COMMENTARY

In their recent review in Science, Doudna and Charpentier highlight the advances in CRISPR-Cas9 genome editing technology and discuss its many great promises for basic and biomedical research, as well as human therapeutics (1). As they emphasize, 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.

Some important concerns have been raised about the use of CRISPR-Cas9 system in gene therapy. These include 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 (2)(3). In the past two years, a great deal of research has been carried out to increase the specificity of CRISPR-Cas9 activity, which results in a site-specific double-strand break (DSB) in the 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 (4).

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 (5), are two limiting factors in successful in-vivo genome editing for gene therapy. Consider this gene therapy scenario: a gRNA and CRISPR-Cas9 effectively target a gene and make a DSB in a specific locus. If the homology DNA template is not available at the time of repair, or in that specific condition the NHEJ is more favorable over HR, the DSB will be repaired by NHEJ. Because of the inaccurate nature of NHEJ repair system, this 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) make the locus not targetable with the same gRNA.

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 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.

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