CRISPR-Cas9: The Unintended Consequences of Molecular Makeover

Christina My and Hannah Baughman*

ABSTRACT/BACKGROUND

CRISPR-Cas9 is the one of the sharpest and most precise gene editing tools discovered. This tool enables a wide variety of DNA modifications such as base substitution, insertion, deletion. Its applications are rapidly expanding, including disease modeling, therapeutic development, and agricultural improvement, showing an increase in a widespread range of applications. However, the CRISPR-Cas9 system can lead to unintended consequences, and a deeper understanding of these consequences is necessary as the usage of CRISPR tools increases. In this review, I discussed the possible unintended consequences of CRISPR-Cas9 followed by the consideration of the tools and how it can be responsibly used. This review focuses on three potential unintended consequences of CRISPR-Cas9: off-target effects, immune response, and chromosome aberrations. To compile this review, I drew from published, peer-reviewed articles and focused on the most recent and relevant studies that provided valuable insight into the unintended effects of CRISPR-Cas9. By critically analyzing these sources, I aim to provide a balanced overview of the current understanding of the potential risk of CRISPR-Cas9. Off-target effects involve unintended modifications at sites other than the target, which can lead to mutations and genomic instability. In addition, the immune response can be triggered by the introduction of foreign protein, potentially leading to immune-related complications. Lastly, induction of chromosome loss involves a large-scale genomic alteration, which can have severe consequences. An understanding of these unintended consequences is crucial for the future development and application of CRISPR-Cas9, ensuring that it is used safely and effectively.

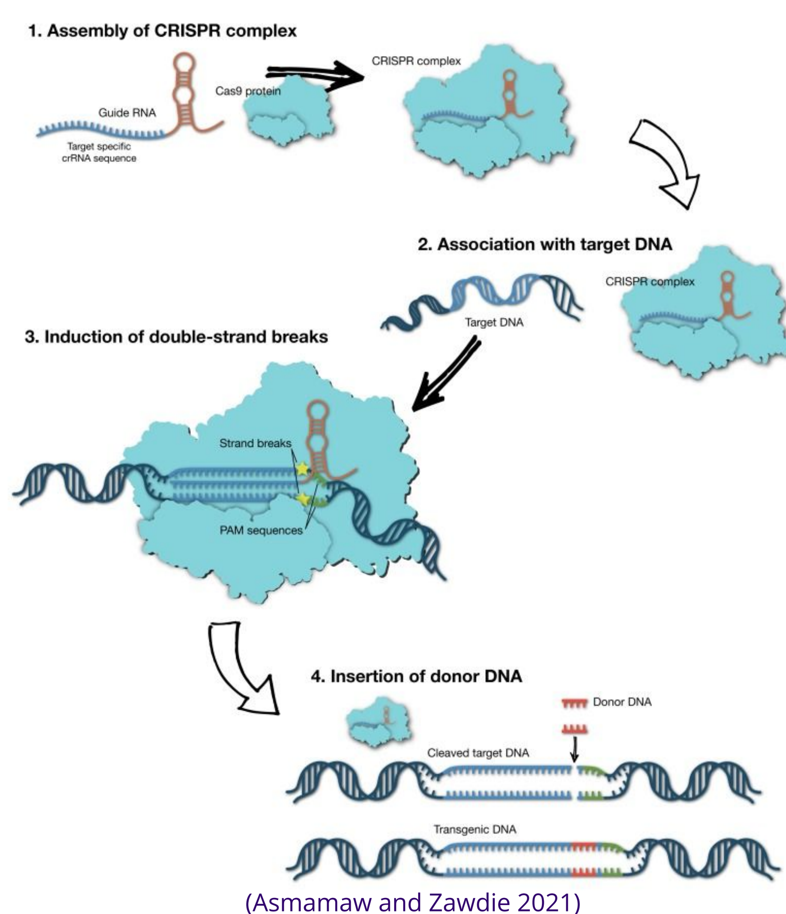

Figure 1: This figure illustrates the CRISPR-Cas9 gene editing process. Initially, a guide RNA (gRNA) is designed to match the target DNA sequence. The gRNA is then combined with the Cas9 protein to form the CRISPR-Cas9 complex, which is introduced into the cell. The gRNA directs the complex to the specific DNA location, where the Cas9 enzyme makes a precise double-strand cut. Following the cut, the cell's DNA repair mechanisms, either non-homologous end joining (NHEJ) or homology-directed repair (HDR), attempt to repair the break, resulting in targeted genetic modifications.

METHODS

To evaluate the unintended consequences of CRISPR-Cas9, a literature review of 20 primary scientific articles was conducted. Studies were collected from various reputable medical and scientific journals.

UNINTENDED CONSEQUENCES

Off-Target Effects:

One of the primary concerns with CRISPR-Cas9 editing is the potential for off-target effects, where the Cas9 enzyme may inadvertently cleave DNA at sites similar but not identical to the target sequence. This could lead to unintended mutations and genetic alterations elsewhere in the genome.

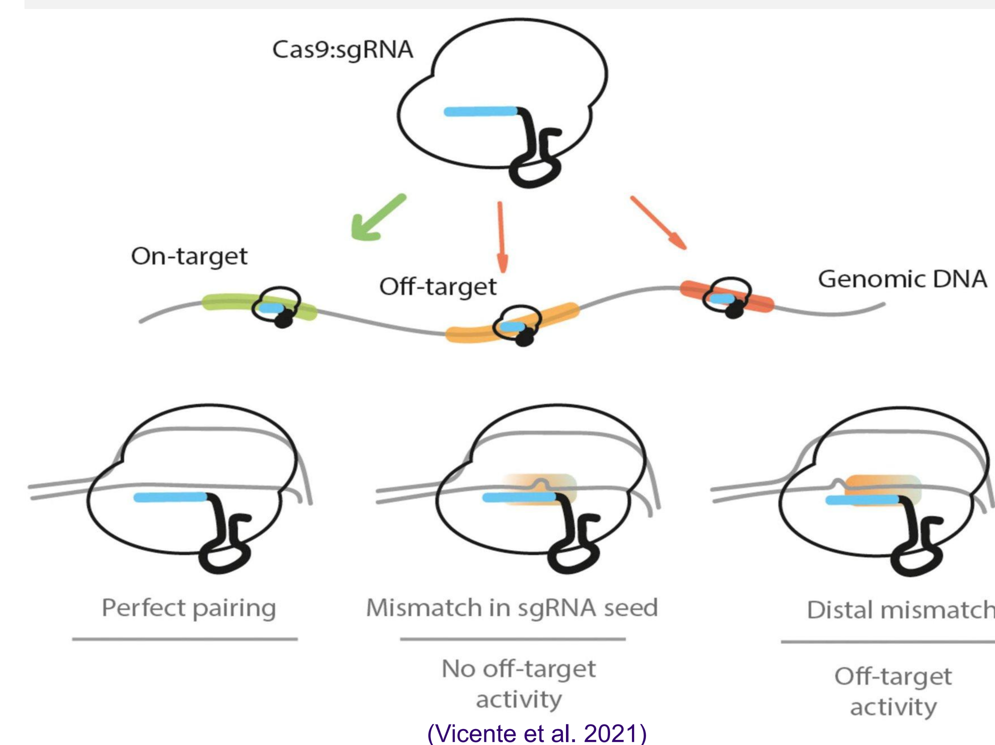

Figure 2: The Cas9 sgRNA ribonucleoprotein complex targets genomic DNA within the cell nucleus. It specifically targets PAM-adjacent sequences, potentially encountering multiple recognition sites. When the target DNA and the sgRNA exhibit perfect base pairing, Cas9 induces a double-strand break (DSB). However, if mismatches occur within the first 7-12 base pairs (proximal to the PAM), base pairing is insufficient to induce a DSB. Additionally, mismatches in the PAM-distal (5′ end of the sgRNA) nucleotides may still allow Cas9 to induce a DSB, leading to off-target activity.

Possible Immune Response Leading to Cytotoxicity

CRISPR guide RNAs (gRNAs) with a 5′-triphosphate group can trigger RNA-sensing innate immune responses in human and murine cells, leading to significant cytotoxicity. Transfection of the primary human T cells with Cas9 RNP and 5′-ppp gRNAs targeting the CCR5 gene significantly reduced cell viability and inhibited cell division. While, the 5′-OH sgRNAs did not induce cytotoxicity and allowed for successful cell expansion.

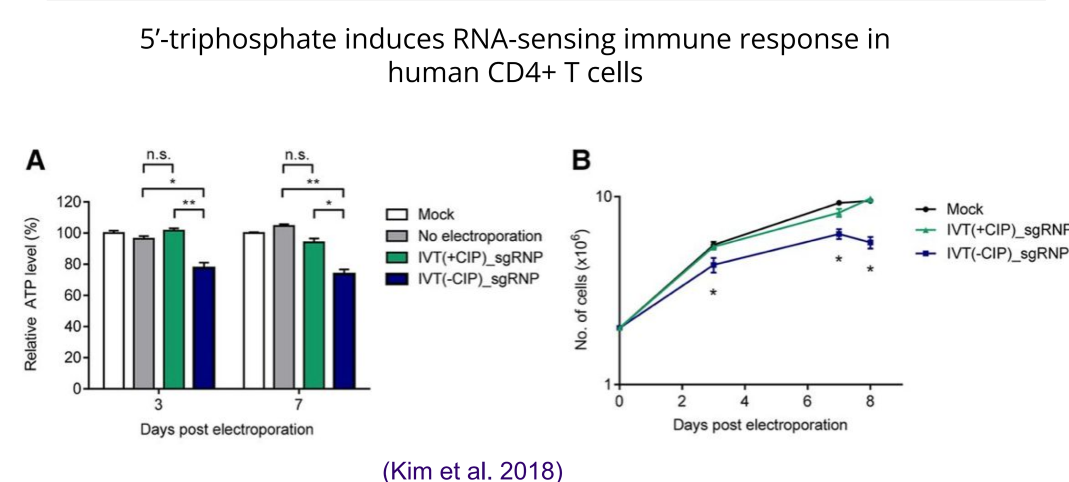

Figure 3: The treatment with 5′-ppp gRNA resulted in a significant reduction in cell viability (*P < 0.05, **P < 0.01), whereas 5′-OH-sgRNA did not induce cytotoxicity (Fig. A). Additionally, 5′-ppp gRNA-treated cells exhibited a lack of division after 7 days post-electroporation, contrasting with the successful expansion of cells transfected with CIP-treated 5′-OH sgRNA in complex with the Cas9 protein (Fig. B)

Chromosome Aberrations Induced By dsDNA Breaks

The results from Varga and Aplan 2005 revealed that among the clones studied, five exhibited significant insertions matching gene segments from distant chromosomal regions, while two others displayed complex rearrangements involving DNA insertions from adjacent chromosome 7 regions. One clone, 5-22, showed a Southern blot pattern consistent with chromosomal translocation, with EF1α promoter sequences joined to a sequence from chromosome 15 α satellite centromeric repeat sequences. Overall, the characterized clones showed a variety of chromosomal alterations, with 56% having interstitial deletions, 24% having deletions accompanied by small (<30 bp) insertions, and 20% having deletions accompanied by large (>30 bp) insertions.

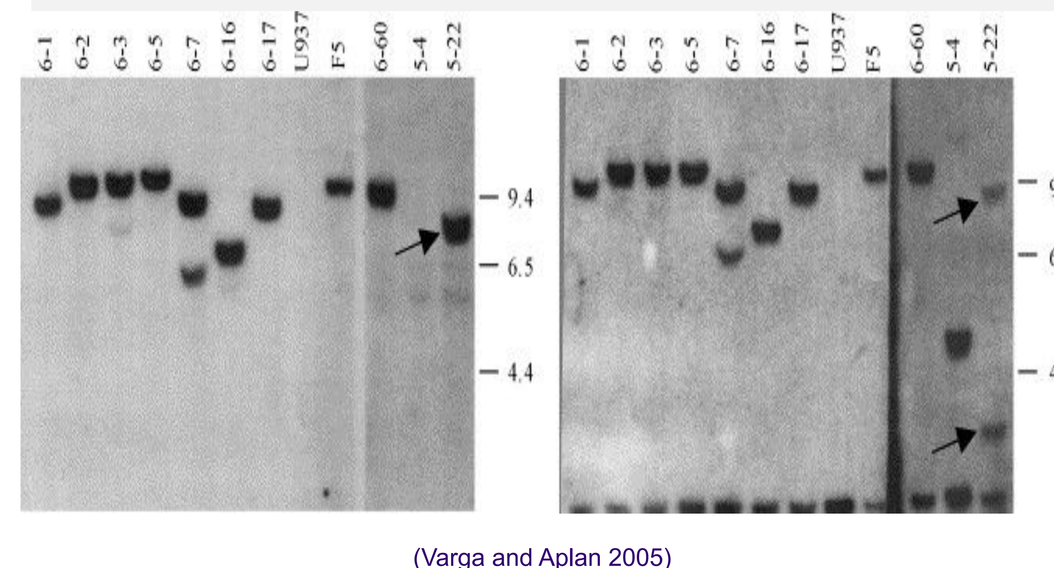

Figure 4: HindIII-digested genomic DNA from individual clones was hybridized to a neo gene specific probe (left panel) or a chromosome 7 (TV 7) specific probe (right panel). The two hybridization signals are of the same size, indicating repair of the double-strand break (DSB) accompanied by an interstitial deletion. In clone 5-4, the deletion extended beyond the neor gene, as verified by inverse PCR sequence analysis. In clone 5-22, the chromosome 7 specific probe shows two signals of even intensity, neither corresponding to the size of the neo specific signal (arrows). The endogenous chromosome 7 signal is seen at 2.3 kb in all lanes. Size standards are indicated in kb.

CONCLUSION

- Off-target effects can lead to unintended genetic modifications, disrupting normal gene function and activating harmful pathways.
- CRISPR-Cas9 machinery can trigger immune responses, potentially causing inflammation and rejection of modified cells and/or tissues.
- Transfection of primary human T cells with Cas9 ribonucleoprotein (RNP) and 5′-ppp gRNAs targeting the CCR5 gene led to decreased cell viability and inhibited cell division, whereas 5′-OH sgRNAs did not induce cytotoxicity and enabled successful cell expansion.
- The study by Varga and Aplan in 2005 revealed significant chromosomal alterations among the clones studied, including insertions matching gene segments from distant chromosomal regions and complex rearrangements involving DNA insertions from adjacent chromosome 7 regions.
  - One clone (5-22) exhibited a Southern blot pattern consistent with chromosomal translocation, suggesting a potential risk of genomic instability associated with CRISPR-Cas9 editing.
  - characterized clones demonstrated a variety of chromosomal alterations, with a majority (56%) showing interstitial deletions and a significant proportion (24% and 20%) exhibiting deletions accompanied by small (<30 bp) or large (>30 bp) insertions, respectively.

CONSIDERATION AND FUTURE DIRECTION

- Need to define ethical boundaries for CRISPR-Cas9 use.
- Importance of precision editing to minimize unintended genetic changes.
- Long-term effects studies crucial for assessing impact on health.
- Ethical discussions and regulatory frameworks needed for responsible use.
- Enhance the specificity and efficiency of CRISPR-Cas9.

ACKNOWLEDGMENT

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REFERENCES