Gene conversion following CRISPR/Cas9 DNA cleavage: an overlooked effect

Running title: CRISPR/Cas9 and gene conversion

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In the last few years the rapid pace in the development of gene editing technology has sparked hopes for curing rare diseases, leading to a potentially transformative impact in the field of gene therapy. CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/Cas9) gene editing technology consists of a bacterial endonuclease (Cas9) complexed with a small guide RNA matching a 20 nucleotide target DNA sequence in the genome, with this complex specifically recognizing the target site with a protospacer adjacent motif (PAM) sequence [1]. The cleavage of this target-genomic sequence generates a double stranded break of the DNA and initiation of the cellular repair machinery to use, or not use, a repair template. The DNA repair outcome is respectively a non-precise editing (indels) or precise editing via non-homologous end-joining (NHEJ) or homology directed repair (HDR) [2]. To date according to ClinicalTrial.gov database from the National Institute of Health (NIH), over 20 phase I clinical trials are currently underway and some have promising outcomes. Ensuring the safety of the CRISPR/Cas9 gene editing approach is therefore paramount to its implementation for *ex-vivo* or *in-vivo* editing in the clinics. This translates to ensuring high specificity and efficiency in targeting.

While the focus of therapeutic gene editing technology has been heavily focused on off-target effects and the control of Cas9 delivery to ensure safety and efficacy in targeting, an overlooked and important aspect of CRISPR editing is the occurrence of mitotic gene conversion at the target site. Gene conversion consists of, following homologous recombination, the transfer of DNA from one genomic location without double stranded break of the DNA, to another one location where the DNA has been cleaved. The importance of gene conversion and, in general, the use of an endogenous repair template for gene editing experiments came to light recently

following the highly publicized *Ma et al.* [3] observation on human embryos. This set of experiments consisted of repairing a dominant mutation in *MYBPC3* gene, responsible for a severe congenital hypertrophic cardiopathy. The authors repaired the mutation in the father's genome using HDR repair with delivery of Cas9 ribonucleoprotein (RNP) and a repair template to the fertilized embryos. They reported a high proportion of repaired alleles indicating the success of the approach. Surprisingly, the authors noted some edited embryos had the maternal allele present on the paternal target strand, suggesting the maternal allele was used as a HDR repair template. A hypothesis formulated by the authors is that the maternal allele was exchanged through inter-homology repair. While this interpretation is still highly disputed [4, 5], this leads to a series of intriguing research questions. How, or why, does the repair machinery choose to use an endogenous repair template following Cas9 cleavage and how prevalent is the use of an endogenous repair template foe gene conversion? If gene conversion is more prevalent than originally thought, would it be possible to use homologous regions in the genome as repair templates?

To elucidate these important questions, *Javidi-Parsijani et al.* [6] firstly examined the frequency of gene conversion for beta-hemoglobin (*HBB*) and delta-hemoglobin (*HBD*). Mutations in *HBB* are responsible for Sickle Cell Disease (SCD) or beta-thalassemia, two severe congenital disorders highly prevalent in the human population and of major therapeutic importance. Given the interest in therapeutically targeting beta-globin to cure severe congenital disorders and the evolutionary history of this locus, it is an ideal target to investigate the rate of gene conversion after gene editing. *HBB* and *HBD* are within the same locus and have a propensity for gene conversion and crossing over in an asymmetric fashion from *HBD* to *HBB [7]*. The overarching

hypothesis from the authors is following the double stranded break of the DNA, Cas9 increases the gene conversion rate and repair efficiency using an exogenous template.

The authors targeted *HBB* for homology directed repair using a donor template homologous to *HBB* but carrying 3 silent mutations including one in the PAM sequence to determine whether the repaired allele comes from the exogenous or the endogenous template. Using a GFP reporter assay in HEK293T cells, the authors assessed the *HBD* footprint in *HBB* and the exogenous repair rate on *HBB* locus. Importantly, they first evidenced that the rate of gene conversion observed was not caused by PCR mediated sequence shuffling or spontaneous gene conversion. Intriguingly, following sequencing of edited cells, and assessing the gene conversion rate from 4 variants uniquely present in *HBD*, the authors discovered that the gene conversion rate is correlated to the indel rate. By comparing various possible endogenous homologous regions on the beta-globin locus, they also noted that a minimum homology of 88 to 90% in sequence is required for gene conversion to occur. Finally, by editing *HBD* and *HBB* and observing *HBD* and *HBB* footprints, the authors evidenced that the observed gene conversion was unidirectional from *HBD* to *HBB* indicating that gene conversion was indeed caused by CRISPR/Cas9. In short the authors demonstrated that the *HBD* footprint in the *HBB* locus is caused by Cas9 cleavage.

The next pressing question is how efficient would it be to use gene conversion as a repair template? This would be an important contribution to the current debate in the field on whether an endogenous template could be used to improve HDR efficiency. To elucidate the effect of gene conversion on HDR repair, the authors assessed the competition between *HBD* gene conversion as an endogenous repair template versus an exogenous repair template delivered to

the cells. They found that exogenous donors were more favorably used as a template for HDR. Interestingly, the amount of gene conversion was proportional to the Cas9 cleavage efficiency in a linear fashion. Further quantification of the alleles by amplicon sequencing indicated that 1/10 of the alleles were from gene conversion versus exogenous template. Overall these set of experiments indicate that CRISPR/Cas9 induces a double stranded break, which favors the generation of indels but also favors exogenous repair for HDR.

Javidi-Parsijani et al. reported and demonstrated clearly that besides creation of insertions or deletions at the cleavage site, CRISPR/Cas9 can also mediate mitotic gene conversion between highly homologous sequences. This observation confirms previous reports and might partly explain the presence of endogenous repair following gene editing in model organisms, human and mouse embryos [3, 8, 9]. There are however many unknown and important research questions requiring further investigations on mitotic gene conversion following CRISPR editing. Important outstanding questions are: How the distance between the cleavage sites and the homologous sequences influences the recombination rate? Are sequence motifs specific in the target DNA to drive gene conversion rate? Are intrachromosomal recombination events preferred over interchromosomal events? Finally and importantly are the mechanisms of mitotic gene conversion distinct from homology-directed repair mechanisms? Additional assessments might confirm these observations and provide a better understanding on the detailed mechanisms of gene conversion. Finally, for gene therapy purposes, besides the assessment of off-target effects, quantification of gene conversion might be an important requirement to provide an accurate picture of on-target mutagenesis following CRISPR/Cas9 editing to ensure safety and efficacy of this approach.

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References:

1. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E: **A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity.** *Science* 2012, **337:**816-821.
2. Stark JM, Pierce AJ, Oh J, Pastink A, Jasin M: **Genetic steps of mammalian homologous repair with distinct mutagenic consequences.** *Mol Cell Biol* 2004, **24:**9305-9316.
3. Ma H, Marti-Gutierrez N, Park SW, Wu J, Lee Y, Suzuki K, Koski A, Ji D, Hayama T, Ahmed R, et al: **Correction of a pathogenic gene mutation in human embryos.** *Nature* 2017, **548:**413-419.
4. Adikusuma F, Piltz S, Corbett MA, Turvey M, McColl SR, Helbig KJ, Beard MR, Hughes J, Pomerantz RT, Thomas PQ: **Large deletions induced by Cas9 cleavage.** *Nature* 2018, **560:**E8-e9.
5. Egli D, Zuccaro MV, Kosicki M, Church GM, Bradley A, Jasin M: **Inter-homologue repair in fertilized human eggs?** *Nature* 2018, **560:**E5-e7.

6. Javidi-Parsijani P, Lyu P, Makani V, Sarhan WM, Yoo KW, El-Korashi L, Atala A, Lu B: **CRISPR/Cas9 increases mitotic gene conversion in human cells.** *Gene Ther* 2020.

7. Papadakis MN, Patrinos GP: **Contribution of gene conversion in the evolution of the human beta-like globin gene family.** *Hum Genet* 1999, **104:**117-125.

8. Katic I, Grosshans H: **Targeted heritable mutation and gene conversion by Cas9-CRISPR in Caenorhabditis elegans.** *Genetics* 2013, **195:**1173-1176.

9. Wilde JJ, Aida T, Wienish M, Zhang Q, Qi P, Feng G: **Efficient zygotic genome editing via RAD51-enahnced interhomolog repair.** *BioRxiv* 2018.

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Ethics Declaration:

Conflict of interest:

The author declares that he has no conflict of interest.