Commentary: Jonathan Moore

Will CRISPR be safe enough for the clinic?

Recent results reported by Kosicki et al.[1] from Allan Bradley's lab at the Sanger Institute suggest that CRISPR/Cas9 gene editing can cause far more genetic damage than had previously been realised. The damage discussed in the paper, large deletions of 1 kb or more, arises from the way cells repair on-target cuts, as opposed to the well-described potential of wild-type forms of Cas9 for off-target editing. High fidelity versions of Cas9 therefore offer no prospect of a solution. So how big a problem does this phenomenon represent for the patients who could benefit from CRISP/Cas9-based therapies and the companies trying to commercialise the treatments? The only way we are going to find out is by looking for the large deletions reported by Kosicki et al in other cell types that have been edited by a range of nucleases and guide RNA.

Few things in biology can be more fashionable than CRISPR/Cas9. The technology has seen broad uptake by the research community for generating disease models and studying gene function, but has probably generated the most excitement for its therapeutic, agricultural and industrial potential. While high specificity and predictability are clearly important for most CRISPR applications, they are absolutely crucial for the technology to realise its potential as a basis for therapeutics. Significant effort has been applied to understanding the rules for a Cas9/gRNA complex being able to trigger a double stranded break. However, once high throughput methods of identifying the sites where Cas9 was cutting were found, it was realised that some gRNAs could trigger cutting at hundreds of sites, albeit at low rates. Fortunately, these tendencies were guide-specific, with some guides driving very specific cutting. Moreover, this tendency towards off-target editing could be much reduced by mutating the protein to form high fidelity versions.

The starting point

The starting point for the recent Sanger Institute study[1] was the use of guide RNA targeting introns as a negative control to test the efficacy of guides targeting a gene's exons. The overwhelmingly frequent consequence of double-strand break induction is thought to be repair of the lesion by the error-prone pathway of non-homologous end joining so as to leave a small insertion or deletion. Hence editing with Cas9 targeted by guide RNAs vs introns should hardly ever lead to a gene's loss of function. In contrast, Kosicki et al.[1] report that editing with intron-targeted guide RNAs leads to a loss of gene function around 20% of the time for haploid loci and around 4% of the time for diploid loci (suggestive of independent events on both alleles). Analysis of the damage revealed the cells that had lost expression of the gene of interest after editing with intron-targeted guide RNAs contained large deletions that also removed an exon. The authors note that repair of Cas9-mediated edits will also elevate the rate of inversions and translocations which, together with large deletions, could lead to transcription of cancer associated genes in a small minority of cells. As in the clinical setting billions of cells may need to be edited for therapeutic effect, the likelihood of some receiving a major assist along the road to cancer looks high.

Similar low probability events, made almost inevitable by large numbers, gave the gene therapy field a decade-long knockback when early retroviral vectors designed to treat X-linked severe combined immunodeficiency induced frequent leukaemias[2]. Therefore, the results of Kosicki et al, raise considerable concerns and have ignited much debate. The Kosicki experiments were mostly performed in mouse embryonic stem cells where the Cas9 and guide RNAs were provided in transposon constructs, so it can be argued that this situation represents a special case. However, the authors of the Sanger Centre paper note that they observed this phenomenon in every cell line tested and for every locus examined.

This large scale damage may be a reasonably likely consequence of any genome editing method that introduces double stranded breaks. These findings may have only emerged now because of three factors: the cost of making new reagents to target Cas9 is low so making an intron-targeted control is affordable; the authors were tracking expression of a non-essential gene whose function could be easily assayed by flow cytometry and they made an effort to find out what was going on. Now that we know what to look for, we can test whether these results are specific to fully-functional Cas9 introduced by transposons. It is also important to perform similar experiments using other methods of introducing CRISPR-Cas (eg transfection of RNA/protein complexes) and alternative editing technologies such as zinc finger nucleases and TALENs.

Genome editing methods that don't make double strand breaks appear far less likely to induce the large scale damage highlighted by the Kosicki paper. Versions of Cas9 with mutations in one of the two nuclease domains make single stranded nicks, rather than double stranded breaks, and in the presence of donors for homology-directed repair can introduce targeted mutations with reasonable efficiency. For non-dividing cells, base-editing technologies should provide a much cleaner way to make knockout mutations via introduction of STOP codons. Therefore, even if the findings of Kosicki et al.[1] do give an accurate impression of the frequency of large scale damage induced by gene editing, there may be much safer ways to edit that are already in hand. A critical priority for the field is to see if this is true.

References:

1. Kosicki M, Tomberg K, Bradley A. *Nature Biotechnol.* **36**, 765-771 (2018).

2. Hacein-Bey-Abina, S *et al. N. Engl J. Med.* **348**, 255-256 (2003).

This article was written by Jonathan D. Moore, Chief Scientific Officer, Horizon Discovery Group Plc.