

Unintended changes induced with CRISPR/Cas cause novel risks

New example of specific changes outside the on-target region of the gene scissors

14 October 2021 / Experiments with CRISPR/Cas in zebrafish conducted by scientists at Uppsala University are the first to detect large structural changes at off-target sites. Off-target sites are outside the target site, but can be very similar, which means that the gene scissors can also cut at these sites and cause specific unintended mutations. The publication shows that major DNA changes are possible.

In the study, the scientists examined the genome of zebrafish after using CRISPR/Cas. They found that large structural changes (i.e. large insertions and deletions) had occurred at both the target sequence and at so-called off-target sites. Such large changes had previously only been observed at the target site (i.e. on-target site). Whether other regions of the DNA can also be affected was not known until now.

In various experiments, the scientists injected the gene scissors into zebrafish eggs, and subsequently examined both the genome of the fish that developed from the eggs and their offspring using modern sequencing methods. They found that the efficiency with which changes were induced at the target site was high overall. There were mainly small mutations, and to a lesser extent larger structural changes, such as insertions or deletions.

The examination of possible off-target sites revealed that unintended changes had occurred at three of them. Their analysis showed that mainly small changes, such as point mutations, were induced. However, larger changes were also observed: at one off-target site, for example, 903 base pairs of the DNA were deleted, thus changing a large part of a gene that was not supposed to be changed at all. The major structural changes at on- and off-target regions were found both in the fish that developed from the injected eggs and their offspring.

The results are highly relevant for clinical applications of CRISPR/Cas to avoid unexpected effects that could have major consequences for patients. In plant applications, there have so far been no investigations into whether large structural changes occur at off-target sites. However, it is likely, as it has also been reported at on-target sites. Overall, it is important that the genome of the target organisms is examined in detail after CRISPR/Cas experiments, at both on- and off-target sites to detect unintended changes that may affect other gene sites.

The study was published as a preprint and still has to go through the peer review process.

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