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The Controversial CRISPR-Cas9 Gene Editing Technology. Unintended Consequences

Researchers Assumed CRISPR-mediated Disruption of Genes Was Turning Them Off – But They Were Wrong

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A new study reveals yet another major unintended effect from the CRISPR-Cas9 gene editing tool – with potentially serious implications for the food safety of gene-edited plants. The study found that CRISPR-Cas9 edits intended to knockout the function of a gene fail to do so. Instead, proteins are still produced from the damaged genes, many of which are still functional. The result could be the production of gene-edited plants that are toxic or allergenic.

This suggests existing CRISPR-edited plants with gene knockouts, such as the non-browning mushroom that has been de-regulated in the US, should be subjected to extensive safety checks, as they could contain new proteins or compounds that pose a food safety risk.

Background

Every living cell contains genes that code for proteins that perform all essential functions that constitute a living organism, such as building the structures of our bodies, digesting food, or sensing the environment. However, many genes, and the proteins they code for, have unknown functions. In humans, one in five genes has an unknown function. One approach that scientists use to try to find out the function of proteins is to mutate (disrupt) the structure of the gene encoding a given protein and then monitor the consequences of this "knockout".

The invention of the CRISPR-Cas9 gene-editing tool has allowed scientists to generate gene knockouts far more easily than was possible in the past. CRISPR-Cas9 makes a double-strand cut in the DNA to disable (knockout) a gene, which, scientists have hitherto believed, makes the proteins it codes for nonfunctional. Scientists can then watch what happens to cells or an entire organism as a result, inferring the lost function from what goes wrong.

Scientists were "terribly wrong"

As explained by an article in The Wire, the Cas9 enzyme searches the DNA, using a "guide RNA" to look for a specific sequence, and makes a cut where it finds a match. The gene, split in two, is repaired by the cell, but often results in varying lengths of DNA base units being deleted or added (indels). The Wire article explains, "Many scientists assume that if a chunk of a gene is missing then the protein that it encodes will not function, or even be produced" – but "In many cases, they would be terribly wrong."

The article highlights the new study, by researchers at the European Molecular Biology Laboratory in Heidelberg, Germany, which shows exactly why. Using HAP1 cells, a human cell line used for biomedical and genetic research, the researchers used CRISPR to make cuts in 136 different genes. In about a third of cases, proteins were still produced from these "damaged" genes – and many of the proteins remained partially functional.

What does this mean for plant gene editing?

London-based molecular geneticist Dr Michael Antoniou explained the relevance of the new study in a plant agricultural context:

> "Hundreds or thousands of gene knockouts via CRISPR have been reported in plants, with even more planned in the future. The new study implies that a third of these claimed CRISPR-mediated gene knockouts were not complete knockouts, but only partial knockouts. In some cases there was no reduction at all in gene expression because an almost fully functional protein was still made.
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> "Worryingly, the most frequent outcomes were truncations of the original protein or proteins with a central deletion within their structure. These mutant proteins may not only partially retain the function of the full-length protein, as reported in the study, but could also gain a novel function, with unknown consequences."

Dr Antoniou continued,

> "In gene-edited crops and foods with CRISPR-mediated gene knockouts of this type, such mutant proteins could give rise to an altered biochemistry which could lead to the production of novel toxins or allergens. Or the truncated protein itself could have toxic or allergenic qualities."

Will systematic characterization of protein expression solve the problems?

The authors of the new study conclude their paper by saying, "Our results imply that systematic characterization of residual protein expression or function in CRISPR–Cas9-generated KO [knockout] lines is necessary for phenotype interpretation."

However, Dr Antoniou said, "In order for plant genetic engineers to do this, they would have to conduct an in-depth molecular profiling of their edited plant in order to get a more complete picture of the consequences of the CRISPR edit, including any risks to the environment or health of the consumer. If they did carry out such profiling, it is likely that unintended differences will be found in the edited plant, making it non-substantially equivalent to its non-GM parent. At this point, a comprehensive toxicity and allergenicity assessment should be required, including long-term animal feeding studies."

But that's something that industry has never done with any GM food or crop. The few long-term feeding studies that have been carried out on GM foods and crops have been the work of scientists working outside the industry.

Study builds on earlier findings

This new study builds on earlier observations by Tuladhar and colleagues, which showed

that in 50% of mammalian cell lines investigated, CRISPR-mediated gene knockouts resulted in an altered genetic code at the intended editing site with the production of a novel mRNA and/or protein.

The new study reveals additional mechanisms through which undesirable outcomes can occur at the intended editing site, with unknown consequences.

Non-browning mushroom should be investigated for safety

The discovery that undesirable outcomes can occur at the intended editing site through multiple mechanisms suggests that existing CRISPR-edited plants with gene knockouts, such as the non-browning mushroom that has been de-regulated in the US, should be subjected to extensive safety checks. The mushroom was engineered by knocking out one of six genes that encode for the browning effect after the mushroom has been cut into.

The developer of the mushroom, Yinong Yang of Pennsylvania State University in the USA, emphasized that it did not need to be regulated since it was free from transgenes (genes inserted from another organism) and only contained "small deletions in a specific gene". However, it is now evident that Yang and the US regulators should re-assess their claims of safety for this gene-edited mushroom in the light of the findings of the new study, as well as that of Tuladhar and colleagues. The simple "small deletion" in a single gene in the CRISPR-edited mushroom that is assumed to be innocuous may have led to the production of new proteins and altered biochemistry that pose a food safety risk. An Internet search has turned up no evidence that Yang has conducted thorough investigations that could show the mushroom to be safe to eat.

The new study also shows that Australia's decision to de-regulate gene-edited organisms of the class known as SDN-1 – involving just this type of gene knockout and the subsequent re-joining of the cut ends of the DNA by the cell's repair mechanism – is not scientifically defensible. This is because even supposedly simple deletions of a few DNA base units, performed with the aim of knocking out a gene, can result in the production of novel toxins or allergens.

Making the CRISPR tool more "precise" won't solve the problems

Dr Antoniou explained that both the findings of this latest study, as well as the earlier observations by Tuladhar and colleagues, show that undesirable effects occur at the site targeted for gene editing after the editing event has taken place; that is, the mutant proteins are produced after the editing event. He said, "These effects are completely independent of the gene-editing tool and the editing procedure as whole. Therefore no matter how precisely the editing event is targeted through future technological developments, the problematic outcomes highlighted by these studies are inevitable. There is no way of avoiding these issues because they arise from the innate properties of the basic molecular biology of gene expression.

"Thus people using CRISPR for gene knockout must do so with extreme caution, not only in the medical sphere but also in an agricultural context."

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