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CRISPR studies muddy results of older gene research

Scientists face tough decisions when the latest gene-editing findings don't match up with the results of other techniques.

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It seemed like the perfect plan.

Jason Sheltzer, a cancer biologist at Cold Spring Harbor Laboratory in New York, was on the hunt for genes involved in tumour growth. He and his colleagues planned to disable genes using the popular gene-editing tool CRISPR–Cas9, then look for changes that reduced the rate at which cancer cells multiply. But they needed a control gene that would yield that same effect.

The literature suggested that the gene *MELK* was ideal: there was ample evidence that it is important in cancer-cell proliferation, and clinical trials are under way to test drugs that inhibit the MELK protein. But disabling the gene using CRISPR–Cas9 yielded no effect. "That threw a monkey wrench into our experiments," says Sheltzer. "It brought everything to a halt."

With that result, Sheltzer and his team joined an expanding club of laboratories that have been forced to re-evaluate and repeat experiments, as the spread of CRISPR–Cas9 uncovers potential errors in data collected using older techniques. On 3 April, Sheltzer's team presented the findings at the American Association for Cancer Research annual meeting in Washington DC. The results have also been published in the journal *eLife*[1].

"There's a whole lot of work to be done, just basically repeating the same screens that people had done" with other methods, says Michael Bassik, a molecular biologist at Stanford University in California. "And, I think it's fair to say, to get much better data."

Scale of the problem

Nathan Lawson, a molecular biologist at the University of Massachusetts Medical School in Worcester, was one of the first to systematically characterize the problem. In 2015, he and his colleagues reported their efforts to compare results from two methods in zebrafish: knocking out genes using a gene-editing technique called zinc finger nucleases, and reducing gene expression using molecular tools called morpholinos. They found that half of the 20 genes they tested yielded different results[2]. An additional trawl through genetic databases and the morpholino literature revealed that 80% of results from published morpholino experiments were not reproducible in genetic mutants[2].

Some zebrafish researchers said they welcomed the paper because it forced the community to confront a problem that had only been noted anecdotally. Others were not so happy. "I got some people who told me I ruined the field," says Lawson.

Similar conflicts have cropped up in other organisms. In the model plant *Arabidopsis thaliana*, the use of CRISPR–Cas9 showed that a protein previously thought to mediate the effects of the plant hormone auxin does not have that function[3]. In fruit flies and human cells, large screening studies have turned up widespread discrepancies between results obtained using RNA interference (RNAi) — a technique that reduces gene expression — and those from genetic mutants[4].

Both methods have their limitations, notes Lawson. RNAi occasionally alters the expression of genes other than its desired target. And meddling with the cell's internal RNA-processing machinery can sometimes affect other cellular systems that involve RNA. CRISPR–Cas9 gene editing, meanwhile, requires breaking strands of DNA — which can trigger other responses in the cell, including cell suicide. And the technique can also sometimes cut DNA at unintended sites.

Back to basics

Conflicting results from RNAi and genetic screens do not always mean that one approach was right and the other was wrong, cautions Bassik. Some cells might respond differently to a genetic change that wipes out expression of a gene, as is often the goal with CRISPR–Cas9, compared to how they respond to reducing the expression to very low levels with RNAi.

But often, he adds, the culprit behind the discrepancy can be tracked back to RNAi's potential for off-target effects. And concerns

about that have had researchers flocking to reproduce old results.

In the case of *MELK*, the CRISPR–Cas9 results are particularly concerning because they could undermine the scientific foundation for a clinical trial. But Sheltzer's team showed only that MELK does not seem to have a role in cancer-cell division, notes Carlos Moreno, a cancer researcher at Emory University in Atlanta, Georgia. It is possible that other aspects of MELK, such as its purported role in making cancer cells more resistant to radiation[5], are still valid, he says.

And many successful drugs have been developed on the back of a faulty scientific hypothesis, he adds. The MELK inhibitors in clinical trials might work through some other mechanism, for instance. "That would be no reason to stop a trial if the trial is showing positive effects," he says. "It's important not to throw the baby out with the bathwater."

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