CRISPR Applications in Oncology Research

Søren Hough
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In 2012, Drs. Emmanuelle Charpentier, Jennifer Doudna and Feng Zhang determined that a bacterial immune system could be repurposed as a gene editing tool. This system, commonly referred to as CRISPR (clustered regulatory interspaced short palindromic repeats), has broad application in both the study and treatment of disease. It allows precise nucleotide-level edits of the host DNA — be that in zebrafish, mice or even human cells.

CRISPR Holds Promise, But Comes with Caveats

CRISPR also presents an exciting opportunity to knock out or even correct genes *in vivo*. Yet there are still plenty of hurdles in the way. Perfecting the process of delivering CRISPR into the body (using viruses and/or nanoparticles) to target specific tissues is a major impediment to therapeutic development. Similarly, it is still unclear whether it's more efficient to deliver DNA, RNA or a pre-assembled RNA/protein complex (RNP) into the host. Each of these options comes with its own advantages and disadvantages and scientists are still trying to evaluate the best path forward.

CRISPR remains an imperfect system. Its high level of specificity belies the fact that it suffers from off-target effects (like siRNA and similar techniques). Although some papers have suggested ways around this, including non permanent editing with inactive Cas9 (dCas9) fusion proteins and high-fidelity SpCas9 variants, there are no clear answers on how to completely ameliorate the phenomenon. To add to the complexity, researchers continue to debate the tolerability and toxicity of Cas9 once delivered to cells.

The other roadblock to CRISPR research is how cells natively handle double-stranded breaks. It turns out that cells are more likely to use nonhomologous end joining (NHEJ) than homology-directed repair (HDR). Because NHEJ happens at a much higher (and inversely proportional) rate to HDR both *in vitro* and *in vivo*, it can be hard to introduce precision edits in models.

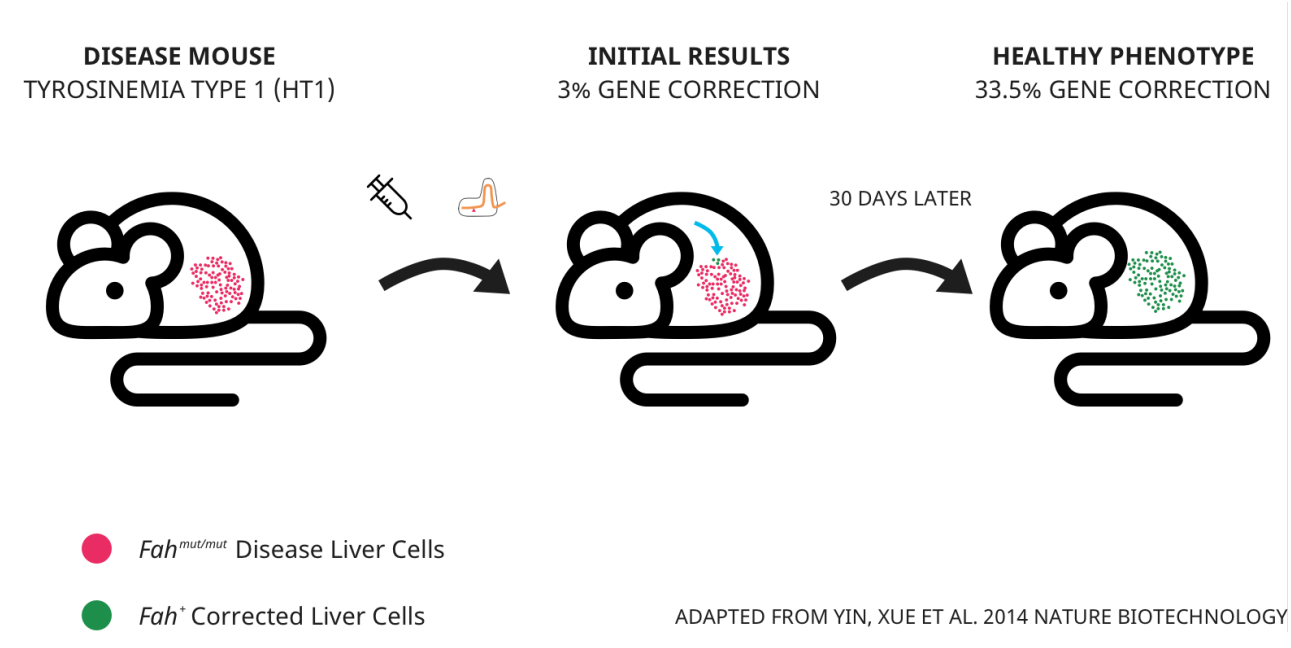
In the days since CRISPR was first used for gene editing, many iterations of the technology have attempted to improve HDR efficiency. Some of these include inhibitors designed to target essential proteins in the NHEJ pathway, asymmetric DNA donors and varied combinations of component delivery. Though some of these options show promise, they remain under development.

CRISPR As a Therapeutic Tool

For now, these limitations mean CRISPR is best suited to treat simpler diseases. More specifically, the system can be used to either knock key disease genes out via NHEJ or to make small corrections that meet the basic threshold for disease phenotype reversal. A good example of this is the paper by Yin et al. 2014 where the first ever adult mammal was cured of a disease using CRISPR.

In this study, the research team treated hereditary tyrosinemia Type 1 (HT1) by targeting the *Fah* gene in mice. The diseased mice had a mutated copy of *Fah* which caused cytotoxic protein build-up in their liver cells. The team designed their CRISPR system to include a donor template (necessary for HDR) and an sgRNA targeting *Fah* with the intention of correcting a single nucleotide in the *Fah* locus.

Yin et al. then used hydrodynamic injection to deliver their system into the liver. It should be noted that this delivery method generally leads to low rates of editing efficiency (~3%). Despite this, the regenerative nature of the liver and the fact that corrected cells lacked cytotoxic *Fah* protein build-up meant that over a 30-day period, the liver repopulated with corrected *Fah* cells and a healthy outcome was achieved.



This was a stunning proof-of-concept for how we might seek to edit genes for therapeutic purposes. Indeed, the paper demonstrates the promise of CRISPR-based medicine. However, it should be noted that this study is removed from cancer research and certainly from clinical trials in several ways.

For one, tyrosinemia is a monogenic disease, making it easier to treat than its more complex polygenic counterparts (like cancer). Moreover, this study benefited from the fact that corrected cells were selected for. This is not always the case, particularly in cancer treatment where tumor cells generally have a survival advantage. The benefit of selection meant the liver could repopulate with just 3% corrected cells. This isn't common to other diseases where a 30% editing rate or higher could be necessary.

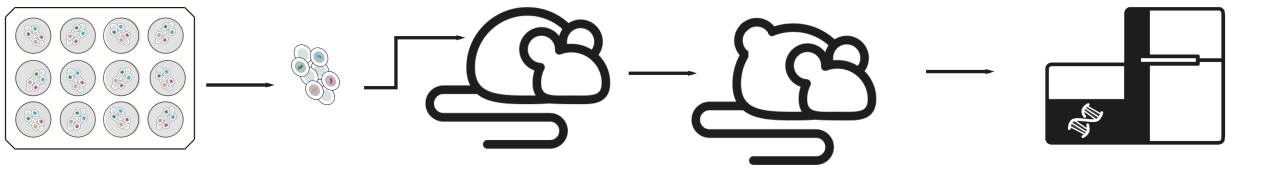
The other caveat to this study is that targeting the liver is more difficult in humans than it is in mice (via hydrodynamic/tail vein injection). Options for combinatorial delivery via nanoparticles and adeno-associated viruses (AAVs) are under investigation as possible avenues for treatment. If tissue-specific and non-invasive methods emerge, CRISPR use *in vivo* may become more viable.

Applications of CRISPR in Oncological Research

Nevertheless, the black box of cancer and tumorigenesis is an excellent place to apply CRISPR technology both in the lab and in the clinic. Researchers can now design CRISPR libraries with large pools of sgRNAs. These guides can target a panel of candidates to identify possible oncogenes ("Which sgRNA halts the growth of or shrinks a tumor?") and tumor suppressors ("Which sgRNA induces tumor growth?").

Digging through genes to determine the Achilles Heel of well-known cancer pathways can also present opportunities for targeted cancer treatment. This is invaluable when dealing with cancers which are unresponsive to small molecule drugs, or for addressing cancers which tend to relapse due to insufficient drug efficacy. Some scientists are already using the broad capability of CRISPR to investigate these possibilities.

As an example, imagine a scientist is performing a CRISPR screen. They have designed thousands of sgRNAs targeting a panel of possible tumor suppressors. To do this experiment, the investigator would pair the screen with what's known as a xenograft assay.



In a xenograft assay, cells that have been infected are injected subcutaneously into immunocompromised mice. The expression levels of the sgRNAs in the resulting tumor can then be recorded to determine which edited gene led to the phenotype. This can be further confirmed via deep sequencing (Song, Li et al. 2016).

In this hypothetical experiment, the scientist may see that out of the panel of genes they screened, an sgRNA targeting BRCA1 (sgBRCA1) is significantly upregulated in the tumor. The investigator might use other known tumor suppressors, such as p53, as positive controls to verify the screen worked. They can then explore the function of BRCA1 as a potential tumor suppressor by designing additional sgRNAs and experiments.

CRISPR can also be used to generate disease models. This can be done *in vitro* and *in vivo* with relative ease as compared with older methods. One review by Mou et al. outlines several studies where mouse models were created using somatic and germline editing. These models included "point mutations, deletions and complex chromosomal rearrangements," all made possible by precise CRISPR targeting using plasmid and viral vectors.

Another potential application of CRISPR research is drug validation. Many cancer therapeutics on the market today aren't fully understood. Studying currently available medicines could improve dosage and treatment options. Further, developing these drugs as a combination therapy with CRISPR and/or RNAi may present a more robust set of treatment options for next generation medical care.

Direct Therapeutic Use of CRISPR in Cancer

Direct therapeutic use of CRISPR in cancer models also shows promise. A study by Liu et al. in 2016 suggests the use of biological "logic gates" in managing on/off switches for cancer pathways. In that paper, the team showed that they were able to reduce tumor size in mice by managing these signaling systems with CRISPR. The group still has questions about its viability in clinical setting due to the specificity of delivery (getting the treatment exclusively to tumors) and the specificity of CRISPR itself (the ramifications of off-target editing).

Therapeutic approaches also include *ex vivo* techniques. A group led by Edward Stadtmauer, MD, at the University of Pennsylvania is reprogramming immune cells to attack tumors. The study is set to use patient-derived T cells, edit them with CRISPR (including specific knockin and knockout modifications) and then reinject the patients. The Penn group are using a method which gives the T cells a specific chimeric antigen receptor, or CAR, so that they can more easily locate and kill cancer cells. Ethical and safety concerns remain around off-target effects (even in *ex vivo* studies like CAR T cell modification), but this shift into the clinical space will set a precedent for cancer treatment in the future.

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Leigh Brody and Søren Hough contributed to this Resource.