Main Submission

CRISPRs to Treat, Understand, and Prevent Disease

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Genome editing technology has the potential to revolutionize healthcare. Development of the clustered regularly interspaced short palindromic repeats (CRISPR) technology has advanced targeted genome editing through engineered nucleases. Referring to CRISPRs, National Geographic states, "No scientific discovery of the past century holds more promise..." (1). In this review, the future perspectives of CRISPR genome editing in health care will be discussed; specifically, how this tool may be used to treat, understand, and prevent diseases.

Since its characterization in 1990 (2), CRISPR/Cas9 technology has revived the field of gene therapy due to its affordability, simplicity of use, accuracy, reproducibility, and application to a wide array of cell types (3). This technology improves upon earlier gene therapies, such as transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs). These earlier methods depended on protein/DNA recognition for target specificity, whereas the CRISPR/Cas9 technology uses ribonucleotide complex formation to bind to its targets, permitting more simplicity in target design and access to diverse genomic locations (3).

CRISPR systems are adapted from bacterial immune systems. The original CRISPR/Cas9 system is composed of Cas9, a single polypeptide nuclease that cleaves DNA, and a single guide RNA (sgRNA) that guides Cas9 to the genomic target site. Double-strand cleavage occurs near a protospacer adjacent motif (PAM) sequence, often one to five nucleotides downstream of the target. The cell responds by imperfectly repairing the break, producing either insertions or deletions (indels) at the target DNA site. If that target is a gene, the indels result in the knockout of the gene. In addition to producing knockouts, this tool may be used to create knock-ins, providing additional possibilities for gene therapy (3).

There are two categories of future use for CRISPRs to treat or prevent human disease: 1) changing somatic cells to treat an individual, and 2) changing germline cells so a disease is not inherited. In both categories, cells are extracted from a patient and genes are edited *ex vivo* using CRISPR/Cas9 techniques before being re-implanted back into the person.

As an example of treating somatic cells, the first human CRISPR/Cas9 trial is underway to treat immune disorders, like metastatic non-small cell lung cancer. In this trial, CRISPR/Cas9 is used to knock down the gene coding for a protein called PD-1 in cancer patient immune cells. Knocking down PD-1 activates the body's immune system so it can fight the cancer (4).

Plans are underway to use similar approaches to treat other diseases. HIV genomes integrated into human immune cells have been deleted using CRISPR/Cas9 technology. The hope is to completely eliminate the HIV virus and its effects in patients. Rather than knocking out a gene, cells extracted from patients with sickle cell disease will be treated with CRISPR/Cas9 methods to replace defective genes with functional ones (5).

With germline cells, CRISPR/Cas9 can modify the genome of cells involved in sperm and egg production, thereby affecting successive generations. Currently, CRISPR/Cas9 is being used to produce transgenics in model organisms, from plants to primates, for a wide range of applications including agriculture and health research (6). For example, in our own research, we are using CRISPR/Cas9 to develop models of heart disease in zebrafish. Models like ours, and others, can then be used to study the molecular mechanisms and physiological impact of the genomic alterations. These models can then act as a tool for high-throughput screening to eventually develop new therapeutics or gene editing strategies. Such precision therapies will protect patients from harmful side effects and reduce the rising cost of disease worldwide (6).

While generating transgenic model organisms is common, 40 countries currently oppose germline modification in humans (7). Many researchers are concerned that these interventions are dangerous, as we may not

witness the side effects until years later (7). We share these concerns; we need to ensure CRISPR/Cas9 knockout efficiency is very high (and hence the risk of "off-target" effects is very low) before approving its use in humans. The level of acceptable efficiency remains a subject of debate and involves determining whether to treat terrible diseases now with the possible risk of unanticipated effects later. To minimize such risks, researchers are improving the CRISPR/Cas9 efficiency of precise genome editing. Even now, CRISPR specificity is being improved by developing CRISPR/Cas9 nucleases with modified PAM specificities (8) and engineered sgRNA structures (9).

CRISPRs modify the genome with great efficiency and safety, and will therefore revolutionize genetic medicine. Approaches are likely to evolve as CRISPRs become applicable to wider targets and more diverse genome editing functions, including large insertions, deletions, and specific base-pair changes, essentially fixing disease mutations. While the technology exists to modify the human genome, there are incredible ethical issues that must be addressed to maximize the positive impact of the technology (1). Through the application of CRISPRs to treat, understand, and prevent disease, healthcare advances will reduce the global burden of diseases and people will lead longer and healthier lives. ■

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