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Genomics & Medicine

December 11, 2015

### **The Future of Human Gene Editing**

With the relative ease of using technologies such as the CRISPR/Cas9, Zinc Finger Nuclease (ZFN), and Transcription-Activator Like Effector Nuclease (TALEN) methods, human gene editing is the reality that we face. The unprecedented rate at which these tools have been created is not only affecting the scientific community, but also society as a whole. The results of these technologies has given rise to many questions, ranging from the accuracy and reliability of the methods themselves to the ethical implications as well. To continue moving forward in this field those questions must be addressed, as they will shape the direction of progress.

Gene editing is a type of genetic engineering where DNA can be inserted, replaced, or removed from a genome using artificially engineered nucleases. The first genomic editing strategy uses DNA endonucleases called zinc finger nucleases (ZFNs). Zinc fingers are transcription factors, which have the ability to recognize specific bases of the DNA sequence. This specificity allows researchers to hand pick which ZFNs to use to target a desired sequence in the genome. Though ZFNs are effective, they're relatively expensive and difficult to make. A more flexible system has been recently discovered; transcription activator-like effector nucleases (TALENs). TALENs are also a transcription factor/nuclease comprised of arrays between 33 to 35 amino acids in length. Each TALEN targets a single nucleotide, making it easy for individual sequences to be targeted. Though larger than ZFNs, TALENs have the ability to do most of what ZFNs accomplish, but cheaper, faster, and arguably better. The newest method added to

the list of genome editors is the clustered, regularly interspaced, short palindromic repeat (CRISPR)/CRISPR-associated-9 (CAS9) system. Unlike ZFNs and TALENs the CRISPR/Cas9 is not human-made, it was discovered as a naturally occurring “self defense” mechanism in bacteria, and functions in two parts. First, the CRISPR. It was found that in bacterial DNA a certain sequence would be repeated many times with unique sequences between the repeats. The sequences found between the repeated regions match the DNA of viruses, specifically viruses that are harmful to bacteria. This is the function of the CRISPR, which is part of the bacteria’s immune system which keeps small excerpts from dangerous viruses around so it can recognize and defend against them. Now it’s up to Cas9 to finish the job. The microbe then copies the DNA in each spacer into an RNA molecule which is then taken up by a Cas enzyme. Together the viral RNA and the Cas enzyme drift through the cell and if they encounter DNA from a virus that matches the CRISPR RNA then the viral RNA latches onto the DNA and the Cas enzymes cut the DNA, preventing the virus from replicating. Essentially Cas9 is an enzyme that cuts DNA, and CRISPR is a collection of DNA sequences that tells Cas9 exactly where to cut. Cas9 could be engineered to cut not just viral DNA but any DNA at a precisely chosen location by changing the guide RNA to match the target. Replacing a mutant gene with a healthy one can be done by adding another piece of DNA that carries the desired sequence. Once a CRISPR system has made a cut, this DNA template can pair up with the cut ends recombining and replacing the original sequence with the new version.

CRISPRs are easier to design because they make a short RNA sequence that is paired to the targeted DNA sequence rather than engineering an entire custom protein. However, it is due to the short guide RNA sequence the CRISPR/Cas system tends to have more “off-target” hits when compared to ZFNs and TALENs.

Though these technologies will undoubtedly play a major role in the future of medicine, clinical testing is still far down the line. Additional research and animal testing is necessary to fine tune the protocols of these technologies. In South Korea farmers have begun to use gene editing techniques, to create ‘double-muscled’ pigs by altering a single gene in the animals. With the demand for pork in Asia rising, breeding more muscled pigs is a unique solution to the problem. Their study focuses on removing the myostatin gene (*MSTN*), which inhibits the growth of muscle cells and keeps the animals muscle size in check. They used TALENs to cut out *MSTN*, and the cell’s natural repair system stitches the DNA back together. Without the *MSTN* gene the pigs show many desired traits, such as leaner meat and higher yield of meat per animal. However, there were some problems encountered as well, such as birthing difficulties stemming from the large size of the piglets, and shorter life expectancies. The team is performing the same experiment using the CRISPR/Cas9 method, and results have not yet been published. Jin-Soo Kim, who led this project, points out a benefit to this type of animal modification; the fast time frame, stating “we could do this through breeding but then it would take decades.”

While animal testing is important, the focus has been turned to human applications. Only one study thus far has been performed utilizing human embryos. Researches led by Jinjiu Huang at Sun Yat-sen University in Guangzhou in China used non-viable human embryos, which could not result in a live birth, for testing. They attempted to modify *HBB*, the gene responsible for beta-thalassaemia, a potentially fatal blood disorder, using CRISPR/Cas9. The team injected 86 embryos and waited two days for the CRISPR/Cas9 system to replace the missing DNA and for the embryos to grow. Of the 71 embryos that survived, 54 were genetically tested and showed that just 28 were successfully cut, and only a fraction of those

contained the replacement genetic material. “If you want to do it in normal embryos, you need to be close to 100%,” Huang says. “That’s why we stopped. We still think it’s too immature.” Additionally, his team also found a surprising number of ‘off-target’ mutations, assumed to be introduced by the CRISPR/Cas9 complex acting on other parts of the genome. This effect is one of the main safety concerns surrounding germline gene editing because these unintended mutations could be harmful. The rates of such mutations were much higher than those observed in gene-editing studies of mouse embryos or human adult cells.

George Daley, a stem-cell biologist at Harvard Medical School, is cautious of the fast pace of this gene editing movement, stating “their [Huang’s] study should be a stern warning to any practitioner who thinks the technology is ready for testing to eradicate disease genes.” The desire to treat patients with gene editing techniques has greatly sped up the development process of these methods such that human treatment is in the very near future. Along with human treatment comes an ethical debate; where do we draw the line with genetic modification?

Currently there are no global regulations on human genome editing. Each country participating in research has its own policies, but there is no universal set of rules stipulating what can and cannot be done on this dividing topic. With this unknown territory becoming more and more integrated into everyday life it is essential to first discuss the benefits and risks of this technology. The International Summit on Genome Editing was held in Washington D.C. earlier this month to just that. Hundreds of scientists from around the world gathered to examine the uses of human gene editing and three major points were agreed upon. First, is that basic research must continue. Basic and preclinical research using gene-editing techniques, such as CRISPR, “is clearly needed and should proceed,” according to a statement released from the summit. This means that scientists and bioethicists endorse the use of these methods in human somatic cells.

However, the summit's second point states that clinical applications should be limited to somatic cells alone. This allows for the modifications to stay in the patient alone, and not get passed on through generations as modifications to the germline cells would promote. While editing germline cells would be useful as a way to avoid inheriting debilitating and life-threatening genetic diseases, the committee writes that it could also be used to enhance human capabilities, and could theoretically allow parents to design their future baby as they see fit. The committee posted, "It would be irresponsible to proceed with any clinical use of germline editing unless and until the relevant safety and efficacy issues have been resolved...and there is broad societal consensus about the appropriateness of the proposed application." The third point from the summit is to keep an open and ongoing forum to keep up with how the research evolves and for continued consensus across the world.

The future of this field is promising, however it is still early in the research and development phase. Modifications and alterations to these methods are continually being made to make them more effective and efficient.

As for the the CRISPR, Huang plans to work out how to decrease the number of off-target mutations using adult human cells or animal models. He is considering tweaking the enzymes to guide them more precisely to the desired spot, introducing the enzymes in a different format that could help to regulate their lifespans and thus allow them to be shut down before mutations accumulate, or varying the concentrations of the introduced enzymes and repair molecules. He also speculates that using other gene-editing techniques might also help, because though the CRISPR/Cas9 is relatively efficient and easy to use, other systems, such as TALENs and ZFNs, tend to cause fewer unintended mutations.

Germ-line genome editing to correct a disease-causing mutation must not create mutations at other sites. In contrast to the zygote-injection strategy, editing of stem cells that can be propagated in vitro enables characterization of the modified stem cells before use in therapy. Spermatogonial stem cells (SSCs) give rise to haploid sperm. Developments in animal models have shown that SSCs can be grown as clones in culture and then transplanted back into the testis to generate sperm. Thus, a potential strategy is to isolate SSCs, use genome editing to precisely correct a disease-causing mutation, perform whole-genome sequencing of clones that have undergone gene correction, and use only the clones that are free from off-target mutations.

Genome editing technologies are the medical treatment of the future. Researchers and doctors have the ability to modify the human genome to prevent and/or treat diseases. As long as an open discussion is maintained on the rules and regulations of this technology, it has the potential to change the world as we know it.

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