CLINICAL UPDATE

CRISPR-Cas: Revolutionising genome engineering

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The ability to permanently alter or repair the human genome has been the subject of a number of science fiction films, but with the recent advent of several customisable sequence-specific endonuclease technologies, genome engineering looks set to become a clinical reality in the near future. This article discusses recent advancements in the technology called 'clustered regularly interspaced palindromic repeat (CRISPR)-associated genes' (CRISPR-Cas), the potential of CRISPR-Cas to revolutionise molecular medicine, and the ethical and regulatory hurdles facing its application.

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Within the repeating As, Cs, Gs and Ts of the human genome is the blueprint for all the organs and tissues of our bodies, from skin and hair to the complex neuronal pathways that make up the brain. This information is contained in six billion base pairs of DNA that reside in the nucleus of every nucleated cell, half the information from our mothers and half from our fathers. Since the launch of the human genome project more than 20 years ago, understanding of DNA and its various interactions that make us who we are has increased exponentially.

It has also been discovered that even small, seemingly insignificant changes in the sequence of our DNA can have drastic consequences. The search is on for tools that will allow these mutations to be changed to correct the DNA sequence, in much the same way as one might correct a spelling error in a manuscript before publication – editing the genome, if you will.

Unpacking the basics

The term 'editing' implies a precise and predictable process by which the genome might be changed, and for the past 10 years – at least – the search for the best tool to do this has been on.

Identification of a tool to change the genome started as whisper, but during the past 5 years it has grown to a deafening roar and at last there seems to be a technology with genuine potential to revolutionise the field of genome engineering. Clustered regularly interspaced palindromic repeat (CRISPR)-associated genes (CRISPR-Cas) have emerged as a novel class of sequence-specific endonucleases with unparalleled flexibility, cost-effectiveness and ease of application.

This technology is derived from a bacterial pathway that allows the organism to detect and degrade invading genetic material. Although there are three naturally occurring types of CRISPR-Cas technologies, only the type II system has been proven to be relevant to the genome engineering field so far. Initial work by Jinek *et al.*[1] has seen the progression of CRISPR-Cas from an interesting bacterial phenomenon to a potentially useful molecular tool.Later work by Cong *et al.*[2] ushered CRISPR-Cas to the forefront of the gene-editing technology race.In 3 years, CRISPR-Cas has outshone, outpaced and outperformed the majority of the other gene editing technologies, including the broadly applied zinc finger nucleases and transcription activator-like effector nucleases.

Acknowledging flaws

This is not to say that CRISPR-Cas has been without its problems. One of the major considerations is the predictability and accuracy of targeted outcomes of the DNA cleavage events, and in the earliest iterations of this technology CRISPR-Cas was a dismal failure.

Early estimates of unintentional and potentially catastrophic cleavage events suggested that the sequence specificity for targeting might be off by as much as much as 25%, with DNA double-strand breaks occurring at sites with as little as 75% sequence homology to the targeted locus.[3] In a field reliant on absolute precision with zero tolerance for error, this was an early setback for the technology.

However, diligent investigation has yielded important results, and in early 2016 two research groups from the Broad Institute identified four novel mutations in the Cas9 protein that rendered Cas9 totally reliant on 100% sequence homology to facilitate cleavage.[4] These groups used next-generation sequencing of the whole genome to establish that no unintended breaks had been introduced at other sites – so-called 'off-target' effects. This has potentially delivered the Holy Grail for gene editing, a technology that is now precise and accurate with no potentially mutagenic side-effects, and with this quantum leap in technology we can finally start looking to clinical applications.

Therapeutic applications of CRISPR-Cas

Genome editing has already broadened our ability to investigate the contribution of specific genes and mutations to disease by facilitating the creation of accurate cellular and animal models. This is certainly where CRISPR-Cas9 has cut its teeth, while several groups have also worked to improve the targeted activity of CRISPR-Cas9 and its overall safety.

This is by no means where the application of gene editing ends. A particularly attractive application is correction of underlying mutations to treat diseases, particularly in conditions that have proved to be refractory to traditional therapies.

Genome editing-based therapy can be accomplished in a number of ways, including correction or inactivation of deleterious mutations, introduction of protective mutations, addition of therapeutic transgenes and disruption of viral DNA.

Work is already underway across the globe to translate the potential of targeted gene therapy into viable clinical applications. This includes the recapitulation of the Δ32 mutation in the CCR5 gene, rendering cells immune to HIV;[5] the correction of deleterious mutations in the DNA that result in a number of inherited diseases, including

cystic fibrosis, Fanconi's anaemia, β-thalassaemia[6] and Duchenne's muscular dystrophy;[7] the integration of exogenous DNA that returns function to mutated proteins, where the mutations are too large to allow simple correction; the integration of protective genes that confer therapeutic potential to specific cell types; and therapeutics that would allow us to target and eliminate viral DNA such as HIV, reducing the burden of latent viral infections.[8] These studies have produced promising results, and the number of indications under investigation for gene editing is growing exponentially. Gene editing, and in particularly CRISPR-Cas technologies, look set to revolutionise the treatment of a wide variety of diseases in the near future.

Ethical questions

As with any progressive idea, the implementation of genome engineering is not without ethical and regulatory concerns.

Many of the breakthroughs in the genome engineering sphere have been viewed as huge scientific triumphs, but the translation of these technologies from the bench to the bedside is not without ethical, legal and social issues requiring vigorous debate.

First, can we predict the ultimate consequences of gene editing on the evolution of the human race? Mutation is the backbone of the evolutionary process, and developing a technology that introduces novel mutations while repairing others could have a profound effect on the direction of human natural history. The only answer to this question seems to be that time will tell. We should, however, make informed decisions around the types of genome editing we wish to undertake.

Second, should we allow embryonic/germline engineering, or only permit somatic cell engineering? The genome engineering community has spent much time debating this point, and at the end of 2015 reached a consensus at a meeting in Washington that involved the global community. Genome engineering for research purposes should be allowed in both somatic and embryonic cells, but with important ethical concerns and the concerns around safety, genome engineering for therapeutic applications should be restricted to somatic cells.

Moreover, human beings have the right to health, and as such it is imperative that we find ways to ensure that genome engineering as a therapeutic modality is accessible to all people and not only to those who can afford it.

Along with the ethical concerns around genome engineering, this rapidly evolving field also presents novel regulatory questions. As more and more clinical applications are developed, so the burden on developing processes for their evaluation by various regulatory boards will increase.

How will trials select control groups? Will it be ethical to engineer healthy individuals? How will we compensate for unknown side-effects? What will the threshold be for acceptable off-target mutations? These and other questions will need to be answered before gene editing can be applied in a therapeutic setting.

The future

In conclusion, despite a number of important ethical and practical concerns, genetic engineering remains set to shape the future of molecular medicine.

This technology has the potential to fundamentally alter the way we treat diseases that have a genetic/genomic component, and with careful consideration, continued discussion and improved sharing of information on a global scale, CRISPR-Cas looks as if it may become one of the most valuable medicinal tools of the 21st century.

CRISPR-Cas was only discovered 3 years ago, and while navigating the road to its clinical application is likely to require a great deal of effort, one can only imagine what the landscape of genome engineering will look like in another 5 or even 10 years.

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