Horizon Scanning Series
The Future of Precision Medicine in Australia

Gene Editing

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1. Abstract

Gene editing is a rapidly developing technology that can manipulate the sequence of a target genome to create or repair gene mutations, including the repair of mutations that are known to cause disease. Pending ethical and philosophical debates, as well as improvements in efficiency and fidelity, gene editing holds the potential to treat and cure a range of genetic, epigenetic and viral diseases. For now, this technology can assist in providing resources with which to assess the role of specific gene mutations in a patient setting and model patient-specific responses to the timing and type of currently available treatments. The role of gene editing in this context is to facilitate the correction of potential disease causing mutations in patient cell lines, thereby providing a patient-specific internal control for precision medicine.

Although there are multiple gene editing platforms available to researchers, the CRISPR–Cas9 system is currently the most widely used due to its simplicity and low cost. Whilst work continues on the efficacy and safety of delivering the required gene editing biomacromolecules, clinical trials are already underway and companies are intensively focusing their efforts to translate gene editing technologies into human therapeutics.

Here, we discuss the role gene editing has to play in precision medicine, highlight recent advancements and identify the gaps in the Australian market that will require investment to ensure that we are internationally competitive in our efforts to see precision medicine reach its full potential for Australian healthcare.

2. Introduction

Gene editing is the process by which changes can be made to DNA sequences in the genome of any organism. At the basic level, gene editing works via engineered nucleases that cut DNA at specific sequences in the genome. After the DNA is cut the natural cell repair process can be utilised to make insertions, deletions or replacements of segments of DNA at the targeted location to correct mutations, create mutations or influence gene expression. This can include the application of gene editing to restore protein function and alleviate disease.

There are four major platforms of gene editing currently available including meganucleases, zinc-finger nucleases, transcription activator-like effector nucleases and CRISPR-Cas nucleases (reviewed in (Yin, Kauffman & Anderson 2017). Briefly, **meganucleases** are engineered from natural homing endonucleases and target natural sites that occur in the genome. However engineering meganucleases to target novel sites is challenging and has limited their translational development. **Zinc-finger nucleases**

are artificial proteins where a fused DNA binding domains dimerise to cut the DNA efficiency. Despite difficulties in engineering effective zinc-finger nucleases, they were first to be used in clinical trials for the treatment of HIV (Pablo et al. 2014). **Transcription activator-like effector nucleases** are easier to design than zinc-finger nucleases and can target longer stretches of DNA but are still more costly and difficult to engineer than the CRISPR-Cas nucleases.

The forth and most revolutionary gene editing platform is the **Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR–Cas)** system. CRISPR uses a programmable RNA to guide the nuclease (Cas9) to find a specific target within the genome. Once the Cas9 cuts the DNA, this system can edit the site by inserting, deleting or replacing DNA sequences (Figure 1). Two distinct systems of DNA repair are utilised by the cell after a Cas9 mediated cut. Non-homologous end-joining simply reconnects the cut ends. This is prone to the formation of small deletions (indels). Homologous DNA repair can use a short provided DNA sequence to repair the region that has been cut based on DNA homology. This mode of activity is the preferred for correction of mutations in precision medicine and methods are available to increase the use of this form of repair. The CRISPR-Cas system evolved in bacteria as a defence mechanism to ward off viral attack (Barrangou et al. 2007). It is simple to engineer, with scientists able to make gene edited cell lines in 1-2 weeks. CRISPR-Cas9 is cost effective, targets DNA with great precision and can also be used to alter transcriptional repression, transcriptional activation and epigenetic modifications. Furthermore CRISPR-Cas technology can simultaneously activate and repress multiple genes at different sites in the genome that are known to be associated with disease (Dominguez, Lim & Qi 2016).

So what does this technology offer precision medicine?

The ability to insert or delete DNA sequences simply and accurately in living cells allows researchers to test the impact of gene function at different stages of disease development. In its simplest form, 'correcting' or 'repairing' a mutation in a patient cell line and then verifying that this is enough to return that cell type to a normal phenotype is invaluable in proving a causative mutation. Generating mutations within cell lines for the purposes of screening compounds allows the development of new therapies. As it is also possible to edit multiple sites on the genome simultaneously, it is possible to model complex age-related diseases such as cancer. The use of CRISPR-Cas9 to correct a mutation in patient tissues provides the long term potential to treat disease at a genetic level. Indeed, gene editing within the fertilised embryo would allow the elimination of disease causing mutations before birth. These many possibilities has seen the use of CRISPR-Cas technology spread rapidly in both the laboratory and commercial sector worldwide. Long-term goals of gene editing are to use this technology to predict individual patient responses to treatments, develop patient specific treatments and ultimately eliminate inheritable disease. Collectively the impact of gene editing may be measured by resulting discoveries in basic research, changes in individual patients drug and treatment strategies and ultimately in the effective slowing or elimination of genetic diseases.

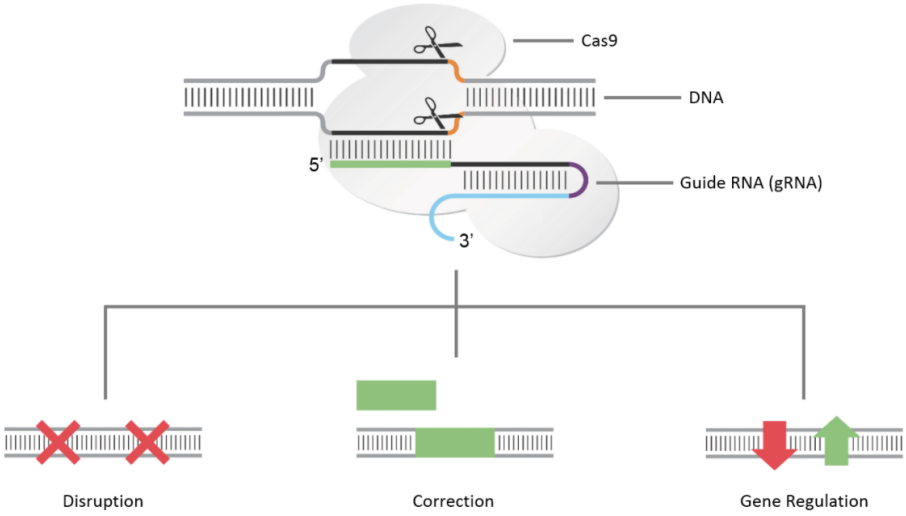

Figure: CRISPR-Cas9 system.

Despite the advantages of CRISPR-Cas9 there are still challenges that currently prevent its wide translation to human trials. One challenge to overcome is the possibility of non-specific DNA editing, referred to as 'off-target' effects, where a gene change occurs away from the gene of interest (Cho et al. 2014; Fu et al. 2013; Lin et al. 2014). For example, off-target edits that may activate or modify cancer-causing genes of cause chromosomal rearrangements are of particular concern. There is a large body of research currently focused on developing methods to identify (Tsai et al. 2015; Frock et al. 2015) and reduce (Kleinstiver, Tsai, et al. 2016; Kleinstiver, Pattanayak, et al. 2016) non-specific gene editing throughout the genome, which will be essential to fully elucidate the safety and efficacy of CRISPR-Cas9 technology for humans.

Less considered are the 'on-target' mutations which may arise as the cells repair the cut DNA. This can lead to new mutations arising. As each cell will respond differently to the gene editing machinery, research efforts need focusing to fully elucidating outcomes prior to translation to human trials. However with the rapid development of improved methodologies for delivering gene editing it is foreseeable that improvements in the fidelity of the system will reduce both off target and on target mutations. Finally a regulatory framework for the use of gene-editing in human trials that addresses ethical, cultural, scientific issues in addition to instilling confidence in the general public will be essential.

3. Applications

3.1. Improved models of disease

A key strategy to understanding disease is to model it in cell lines or small animals. These models allow researchers to study the disease and test the safety and efficacy of new therapies. In some instances larger animals such as sheep and pigs can provide more suitable models for some human diseases. The use of gene editing to create mutations in model animal genomes has rapidly accelerated the pace at which such models of disease can be generated.

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However, model animals are not humans, nor do they contain the associated gene polymorphisms of a patient, many of which will modify the presentation of disease. For this reason, where possible, it is critical to model disease using the patient's own cells. This is now more readily possible with the advent of recent technology that enables the generation of stem cell lines from a patient. Coupled with gene editing, it is now possible to generate a mutation or correct a mutation in a patient cell and use these to model disease. The development of panels of gene mutations in a specific gene will allow the screening of compounds libraries to find new treatments. The correction of proposed disease causing mutations using gene editing will also provide screening platforms for developing or testing patient specific treatments.

3.2. Early human embryonic development

Understanding the function of genes early in embryonic development is important to advance our knowledge of genetic disease. New research shows that it is possible to alter the genome of a human embryo (Ma et al. 2017) however it is illegal to allow these embryos to proceed beyond 14 days in Australian and in the United Kingdom. In the United Kingdom a CRISPR-Cas9 licence was granted to investigate possible causes of infertility by identifying the genomic makers associated with healthy embryos and placental diseases. Despite implantation of a genetically altered embryo is strictly prohibited by law public discussions on gene editing are often attracted to this topic. It will be key to effectively communicate that this is not the goal of gene editing research and to highlight its potential to improve lives using ethical and publically accepted technologies.

3.3. Current clinical applications of gene editing technologies

There a several ongoing clinical trials evaluating zinc-finger nucleases in the treatment of HIV, haemophilia B and Hurler syndrome, an inherited disorder of carbohydrate metabolism that occurs almost exclusively in males. US companies such as CRISPR Therapeutics, Intellia Therapeutics and Editas Medicine are working to translate CRISPR-Cas9 technologies to a wide variety of human diagnostic and therapeutic applications. In Australia, while gene-editing services exist within research institutes there are currently no listed gene editing companies and no clinical trials actively recruiting. In June 2017, Merck was awarded an Australian patent for use of its proxy-CRISPR which claims to enable cutting of DNA in difficult to reach cell locations. Some specific examples of existing or potential applications are listed below

Viral infections: Gene editing is a powerful tool for advancing treatments for viral infections such as human papillomaviruses and HIV/AIDS (White, Hu & Khalili 2015). Human papillomaviruses (HPVs) are cancer causing agents. Worldwide, HPV infections account for more than half of all infection-linked cancers in females (ref). The CRISPR-Cas system has been used to inactivate key proteins and reduce tumour growth in mouse models. HIV/AIDS continues to be a major public health problem around the world. Current antiretroviral therapies inhibit viral replication, thus assist in halting progression of the disease, however the virus can permanent integrate in to the DNA and thus are at risk of viral reactivation despite antiretroviral therapy. Gene editing can potentially play a role in eliminating the integration of viral DNA and reducing the risk of recurrent activation.

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Cancer: In 2016, the National Institutes of Health (NIH) approved the first application of CRISPR-Cas9 technology in a human trial aiming to augment cancer therapies that rely on enlisting a patients T Cells. T cells were removed from 18 patients with several types of cancer, edited using CRISPR technology and then infused back into the patient (ref). Researchers in the UK have also started a safety study with 10 children with cancer using transcription activator-like effector nucleases (ref).

"In 2015, Layla Richards an 11-month old girl at Great Ormond Street Hospital (UK) relapsed with an aggressive form of leukaemia. In the absence of any other treatment options the clinician's attained consent under a special licence to use gene editing (transcription activator-like effector nucleases) for the first time in a human patient. Within 2 months all signs of the leukaemia had disappeared and Layla went on to receive a successful bone marrow transplant. More than a year after receiving the treatment Layla was healthy with no signs of the cancer (ref)"

Future gene therapy for rare diseases: While much work needs to be done before we can use gene editing in clinical trials for inherited diseases, there may come a day where it is feasible to deliver gene editing approaches to existing genetic disease to at least restore partial function to some cells. This aligns with precision medicine in that the individual patient may have a particular mutation that requires editing. An example might be muscular dystrophy. Muscular dystrophies are a group of rare inherited neuromuscular disorders characterized by progressive muscle weakness and muscle degeneration. They are clinically, genetically, and biochemically heterogeneous, with large variations in age of onset and level of impartment. They derive from mutations in various genes important for muscle function and homeostasis. In mouse models of duchenne muscular dystrophy removal of the Dmd gene mutation that causes the disease by using CRISPR-Cas9 partially restore muscle function (Long et al. 2016; Nelson et al. 2016; Tabebordbar et al. 2016). To date, proposed therapies and clinical trials have not be able to address individual differences in gene mutations that may be causing the disease. CRISPR/Cas9 technology enables direct sequence alteration to sites affected in each patient personalising the treatment of muscular dystrophy disorders (Pini et al. 2017).

4. Gaps and the future

Australia is well placed to take market share in the role gene editing can play in precision medicine. Our skilled workforce, depth of clinical and scientific expertise and current focus on innovation (Commonwealth of Australia 2017) align nicely with the currently opportunities in the field of gene editing. While many laboratories have adopted CRISPR and gene editing technologies as a part of their basic research, there are currently no Australian led gene editing trials that are currently recruiting. Imminent tasks for Australia will include, national discussions to ascertain clear goals and sustainable objectives for gene editing that will benefit our healthcare system. National investment and ethical but innovative governance of gene editing will also be key. Furthermore, communication and education of the general public will be imperative.

There is likely to be ongoing ethical and philosophical questions raised by gene editing and the role precision medicine that will be unique to Australia. While the unknown should be carefully governed it should also be embraced. The debates around gene editing human embryos will always attract media attention, thus is it important for science and its regulatory bodies to clearly disseminate how gene

editing can be used to develop more effective treatment strategies for debilitating diseases to which we currently treat.

Australia's efforts can also be placed on establishing a collaborative framework and identifying innovative opportunities to translate this technology to diagnostics and human trials. Equally important will be evaluation of risks, benefits and costs to the Australian healthcare system.

5. Conclusion

We are now many decades into the era of being able to edit genomes. The improvements in the efficiency of systems such as CRISPR-Cas9 have accelerated the pace with which application to precision medicine is likely. Indeed, the capacity to edit the genome in very early development brings not only the prospect of curing genetic disease, but the ethical challenge of whether this should be allowed. What is clear is that gene editing is a technology with many potential benefits to human health. With sufficient ethical and regulatory frameworks, this technology has the potential to significantly contribute to personalised medicine.

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