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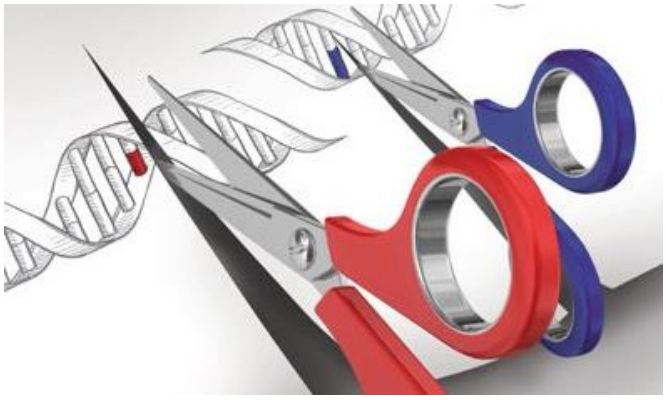
Genome Editing



Genome editing techniques enable the targeted modification of DNA sequences within living cells. They have potential uses in biomedical research, human therapy, agriculture and to help control vector-borne diseases. This POSTnote covers current and future uses of genome editing, how it is regulated and the potential concerns it raises.

Overview

- Genome editing involves manipulating the genetic code at targeted locations within the DNA sequence.
- This has a wide range of applications in biomedical research, human therapies and agriculture, which all have potential benefits for human health.
- There is on-going debate about the extent to which current regulations on genetically modified organisms should apply to the use of genome edited organisms.
- Genome editing raises social and ethical concerns that may affect its future use, including which types of diseases it could be used to treat and whether it is safe to release genome edited organisms into the environment.

Background

Humans have influenced the inheritance of genetic material between generations for millennia, through the selective breeding of animals and plants with desirable traits. In the 20th century, advances in genetic engineering allowed scientists to confer new traits to plants and animals by transferring genes between species (transgenics). However, the new genes are inserted at random locations within the genome, sometimes yielding unpredictable results. Genome editing uses enzymes ('nucleases', see Box 1) to manipulate DNA sequences at one or more specific sites selected by the researcher. It can be used:

- to delete or change the individual bases (A,T,C and G, the letters of the genetic code) in order to disable, repair or modify the function of a gene
- to precisely insert a new gene, thereby conferring a novel trait to an organism.

Such techniques have been used to rewrite selected regions of the genetic code, in order to change the function or characteristics of cells within living organisms such as microbes, plants, mice, monkeys and humans.

How does genome editing work?

Different types of genome editing techniques vary in how they target the nuclease enzymes to specific genomic sites, but all share common mechanisms of action:

- Customised guides recognise one or more specific site(s) within the genome and target the nuclease to the site(s).
- Nucleases are enzymes that act like molecular scissors to cut the DNA at the chosen site(s).
- The cell's in-built repair mechanisms are mobilised to repair the cut. In this repair process, novel DNA sequences added by the researcher may be incorporated into the genome.

Applications of genome editing

The main genome editing techniques are outlined in Box 1. The following section highlights some of their potential applications in humans and animals. Issues related to regulation of genome edited plants will be covered in a forthcoming POSTnote.

Biomedical Research

Cellular & animal models of disease

Many human diseases result from inherited or acquired genetic mutations. A key strategy of biomedical research is to develop cells and animals that carry these genetic mutations and manifest certain features of a disease. These can provide models to study the disease and to test the safety of new therapies. Mouse models have been widely used due to the convenience of their large litter sizes, short life cycle and ease of genetic manipulation. However, significant differences in the size and physiology of humans

Box 1. Genome editing techniques

There are three main genome editing techniques. They all use enzymes (nucleases) to make genetic modifications at one or more selected site within the genome.[1] However each has the potential to cause unintended off-target effects that may limit their use in practice.

- Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs) were the first genome editing systems to be developed. They both target DNA sequences using custom engineered protein sequences that can be complex to design. ZFNs are more restricted in the range of sites that can be targeted than TALENs (see Table below).
- CRISPR/Cas9 is a more recent technique that has become widely used as a research tool because the customised guides are easier and cheaper to make. It consists of a nuclease (Cas9) coupled to a guide sequence (CRISPR standing for Clustered Regularly Interspersed Short Palindromic Repeats).

	Guide design is rapid and inexpensive	Able to target any DNA sequence	Potential to cause off-target effects
ZFNs	×	×	✓
TALENs	×	✓	✓
CRISPR/Cas9	✓	✓	✓

compared with mice may limit their usefulnesss. For example, mice carrying mutations which cause cystic fibrosis in humans do not develop all the symptoms associated with the human disease. Larger animals such as sheep, pigs and monkeys may provide more suitable models for some human diseases. The versatility of CRISPR/Cas9 (Box 1) has greatly extended the range of animals that can be used as models of human diseases.

Early human embryonic development

Understanding the function of genes involved in early embryonic development is important for improving assisted reproductive technologies such as IVF. A UK researcher has been granted a licence to use CRISPR/Cas9 in human embryos donated following fertility treatments.[2] The work aims to investigate possible causes of infertility by identifying molecular signatures (biomarkers) associated with healthy embryos and placental diseases.

Human therapies

One potential use for genome editing is to treat genetic diseases for which existing therapies are ineffective. The implications of genome editing vary with the type of cells it is used to modify: in non-reproductive (somatic) cells editing only affects the patient, whereas changes to reproductive (germ) cells may be inherited by future generations.

Somatic (non-reproductive) cell therapies

Initial efforts to employ genome editing in somatic therapies have focused on genetic diseases of blood cells, such as leukaemia and HIV/AIDS, because of the relative ease of removing, editing and delivering cells back to patients. There are significant technical challenges associated with genome editing of cells within complex tissues and organs. Clinical trials using TALENs (Box 2) and ZFNs are underway and are planned for CRISPR/Cas9. Early results are encouraging and demonstrate the feasibility and safety of using nuclease-based editing in humans.

Box 2. Genome editing as a human therapy

The first reported use of TALENs in a human patient was as an experimental therapy for treating acute lymphoblastic leukaemia (ALL). The new therapy uses immune cells (T cells) engineered to attack leukaemia developed by life science company Cellectis in collaboration with researchers at UCL and Great Ormond Street. The researchers first inserted a specific receptor (CAR) gene into donor T cells to derive UCART19 cells that can hunt and destroy cancer cells displaying a specific surface molecule (CD19). In deriving these cells, TALENs were used to edit two genes in order to:

- stop the UCART19 cells recognising the host cells as foreign and attacking them (by editing a gene known as TCR)
- make the UCART19 cells resistant to a drug used to treat ALL (Campath) by editing another (CD52) gene. This prevents the patient's immune cells from attacking the UCART19 cells, enabling UCART19 to survive Campath treatment and attack the leukaemia.

In 2015, Layla Richards an 11-month old girl at Great Ormond Street Hospital relapsed with an aggressive form of ALL. In the absence of any other treatment options the clinicians sought consent to use the UCART19 cells for the first time in a human patient. Consent was obtained, ethical approval was granted and the treatment was performed under the Hospital's special licence. Within 2 months all signs of the leukaemia had disappeared and she went on to receive a successful bone marrow transplant. More than a year after receiving the treatment Layla remains healthy and shows no signs of the cancer returning. One other patient has been treated under the same licence, and a Phase I trial for up to 10 patients commenced in June 2016 to determine the safety and reproducibility of the treatment. It is hoped that success in such studies would allow UCART19 to become an off-the-shelf therapy available on the NHS within a decade.

Germline therapies

Genome editing of human germ cells (sperm and eggs) and embryos for therapy is prohibited in the UK (see Current Regulation), and some prominent researchers have called for a moratorium on research in this area (see Potential concerns). However, genome editing techniques have the potential to correct heritable diseases caused by individual or multiple faulty genes. In particular, germ cell or embryo editing could be used in the context of assisted reproduction where pre-implantation genetic diagnosis cannot identify unaffected embryos (POSTnote 445).

Animals and agriculture

Disease-resilient animals

Intensive farming practices have contributed to increased prevalence of infectious livestock diseases (POSTnote 392), which reduce farmers' profits and pose problems for animal welfare and human health. There is considerable interest in developing new breeds with inherent disease resilience. Genome editing is being used at The Roslin Institute in Edinburgh to engineer disease-resilient pigs (see Box 3). However some NGOs are concerned that disease resistant livestock might allow further intensification of agriculture leading to potential animal welfare issues.

Animal production of human organs

In the UK there is a shortage of organs donated for transplantation (POSTnote 441). A key objective of regenerative medicine is to create new patient-specific organs and tissues to replace those lost through disease.

Box 3. Genome editing in animals

African Swine Fever (ASF) is a viral disease which affects domestic pigs in Africa, continental Europe and Russia. ASF often results in high and rapid mortality rates in pigs, but is not known to impact human health. There are currently no effective vaccines or treatments available for ASF. In the UK, Defra control measures state that all pigs within infected premises must be killed.

African warthogs do not develop disease symptoms when infected with the ASF virus, but cannot be crossed with pigs using conventional breeding. The different responses to ASF virus are thought to be because domestic pigs and warthogs carry different versions of a gene (RELA) that helps activate the host's immune response to the virus. Researchers at The Roslin Institute have used ZFNs and TALENs to create live pigs which carry the warthog version of the RELA gene.[3] Research is underway to investigate whether these genetic changes will confer ASF-resilience in domestic pigs, which would have agricultural and animal welfare benefits.

However there are significant technical challenges associated with approaches to engineer the complex structure and function of human organs.

One proposed solution is to grow human organs inside animals. The feasibility of making chimeric embryos (comprising tissues from different species) has been demonstrated by injecting rat stem cells into mouse embryos which lacked the gene (*Pdx1*) needed to form a pancreas. The mice developed functional pancreases comprised almost entirely from the donated rat cells. This has prompted on-going research to investigate whether human organs for transplantation can be obtained from gene-edited pig embryos injected with human stem cells.

Controlling Diseases Using Gene Drives

Gene drives can be used to enhance the spread of particular versions of a gene through a population. Normally during sexual reproduction offspring inherit half of their genes from each parent, with a 50% chance of a gene being passed onto the next generation. Gene drives significantly increase this probability, and could be used to facilitate the rapid propagation of desired traits within insect populations to control vector-borne diseases (see Box 4). Concerns over the irreversibility of the gene drive system and the potential ecological impacts have meant that no gene drive organisms have been released to the environment to date (see Potential Concerns).

Regulation

The rapid development of CRISPR/Cas9 and other nucleases has given rise to novel applications such as gene drives, which pose new regulatory challenges (see next section). Other applications of genome editing highlighted in this note are covered by existing regulations:

- **Biomedical research** - genome editing of animals is covered by the Animals (Scientific Procedures) Act 1986. Research on human embryos is permitted only for certain purposes and only under licence from the Human Fertilisation and Embryology Authority (HFEA). UK law prohibits the development of human embryos outside the human body beyond 14 days.

Box 4. Genome editing and gene drives in insects

CRISPR/Cas9 can be used to genetically modify mosquitos in a bid to control the harmful diseases they carry such as malaria (POSTnote 483), Zika virus and Dengue. Strategies include population suppression (by releasing males carrying a lethal gene) and population replacement (using genes that impair the ability of mosquitos to transmit the malaria-causing parasite). Normally, it takes many generations for such gene variants to become widespread throughout a whole population, as there is a 50% chance of inheriting one copy from each parent.

However CRISPR/Cas9 can be used as a gene drive to increase the probability of inheriting a specific gene to almost 100% over fewer generations. This can be achieved by inserting the genes for the CRISPR/Cas9 system into the paternal genome along with the (disease control) gene of interest. In offspring which inherit this genetic construct from their father, the CRISPR/Cas9 system activates and causes the gene construct to be copied and inserted into the maternal chromosome, resulting in two copies of the gene of interest.

One study created a CRISPR/Cas9-based gene drive that accelerated inheritance of a faulty fertility gene required for egg hatching to more than 90% of *A.Gambiae* mosquito offspring.[4] Two copies (i.e. one from each parent) are needed to cause infertility, meaning that mosquitoes with only one copy are carriers, and can spread the gene through a population. Such studies demonstrate a proof-of-concept that CRISPR/Cas9 can create highly efficient gene drives in mosquito populations. Further research is needed to determine their effectiveness for disease control (see Potential Concerns).

- **Human therapies** – UK law prohibits the implantation of an embryo that has been genetically altered in any way (with the single exception of mitochondrial transfer, see POSTnote 431). Somatic cell therapies are regulated similarly to gene therapies and require ethical approval, licencing of clinical trials by the Medicines and Healthcare Products Regulatory Authority, and market authorisation from the European Medicines Agency.
- **GM food and animal feed** - the European Food Safety Authority (EFSA) carries out safety assessments on GM food and animal feed intended for use in the EU. Member States then vote on whether to authorise these products. In the UK the Advisory Committee on Novel Foods and Processes advises the Food Standards Agency on the safety of novel foods, including GM foods.
- **Environment** - UK law implements EU Directive 2001/18/EC to regulate the deliberate release of GM organisms into the environment. UK Ministers may grant licences for experimental uses, advised by the Advisory Committee on Releases to the Environment (ACRE). These assessments are made on a case-by-case basis based on information about the novel traits of a GMO and its potential impact on the environment. EFSA advises on the safety of commercial uses of GMOs on a case-by-case basis with Member States voting on whether to authorise a use and on stipulations such as environmental monitoring.
- The UK will have the option to consider future regulatory options for GM food and animal feed and GM releases to the environment following its withdrawal from the EU.

Potential concerns

There has been considerable media and public interest in genome editing in recent years. For instance, the Nuffield

Council on Bioethics held a public workshop on genome editing as part of its recent inquiry and report in this area.[7] The US National Academy of Sciences (NAS) and National Academy of Medicine co-hosted a summit on genome editing with the UK Royal Society and the Chinese Academy of Sciences in Washington in December 2015.[5] These exercises discussed potential concerns of using genome editing for basic and preclinical research, somatic cell therapy, germline therapy and gene drives.

Safety concerns in basic and preclinical research

Genome editing techniques can produce unintended editing at sites in the genome outside of the gene being targeted (off-target effects, see Box 1).[6] Creating such errors in the human genetic code could have potentially severe consequences to health. Off-target effects can be detected using whole genome sequencing and software can be used to design guide molecules that target unique sequences to minimise the risk of off-target effects. There is a current consensus among the science community that basic research on such concerns should continue subject to appropriate legal and ethical rules and oversight.[5,8]

Somatic cell therapy

Somatic cell therapies raise fewer ethical issues than germline therapy (see next section) because the genetic changes made only affect the individual being treated: they are not inherited by future generations. The Washington summit concluded that because of this, such therapies can be "appropriately and rigorously evaluated within existing and evolving regulatory frameworks for gene therapy, and regulators can weigh risks and potential benefits in approving clinical trials and therapies".[5] There is an on-going dispute over the intellectual property rights for CRISPR/Cas9, but it is unclear whether this will affect the commercialisation of new therapies.

Germline therapy

Use of genome editing to make changes to eggs, sperm or early stage embryos raises additional ethical considerations because the changes made will be inherited by future generations. There is an international consensus at present that no changes should be made to human germ cells or embryos, and the Washington Summit concluded that if, "in the process of research, early human embryos or germline cells undergo gene editing, the modified cells should not be used to establish a pregnancy".[6] UK law currently prohibits the implantation of an embryo that has been genetically altered, but makes an exception for embryos that have been treated by a prescribed method for the prevention of serious mitochondrial disorders (POSTnote 431). The HFEA has yet to issue any licences for mitochondrial disease treatments. In the debate about regulation of such techniques concerns were raised that this might represent a step towards germline modification, whereby parents who could afford such treatments selected 'desirable traits' for their offspring. The Government has made it clear that there are no plans to amend the law to allow germline modification.

Gene drives

A major concern with proposals to use genome editing to control vector-borne diseases such as malaria (Box 4) is the release of gene drives into the environment. This process is thought to be irreversible and could have severe, unpredictable and uncontrollable environmental and ecological impacts. For example, a recent NAS report identified a number of risks to ecosystems and biodiversity, including the risk that the gene drive might transfer to another organism of a different species if released to the environment. It concluded that research to date "is not sufficient … to support a decision to release gene-drives into the environment".[9] However, the report has been criticised for downplaying other potential concerns such as using gene drives for military purposes and possible commercial exploitation in agriculture (for example engineering weeds to be herbicide susceptible) and the implications of this on food security.[10] A House of Lords inquiry into GM insects recommended that "underpinning research is required in order to allow effective monitoring and tracking" of new developments such as gene drives.[11]

Regulatory issues

Modifications involving single bases pose a regulatory challenge because single base changes can occur naturally by random mutation and natural selection. It is currently impossible to discern whether such a change has been made using genome editing or has occurred spontaneously. Such considerations have led to an on-going debate about the extent to which current GM regulations on release to the environment, safety evaluation, traceability and labelling should apply to genome edited organisms used in agriculture and the food industry. NGOs such as Genewatch suggest that the current GM regulations should apply to all genome edited organisms[12] and that any future products containing genome edited ingredients should be labelled as containing GMOs so that consumers can make informed choices. However, the biotech sector suggests that the regulations should only apply to organisms where novel DNA can actually be detected and not to those which could have arisen by natural mutation (such as those involving manipulation of a single base).[13]

Endnotes

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