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15. Genome Editing: A Novel Tool for Disease Resistance

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Abstract:

The traditional healthcare system is at the doorstep for entering into the arena of molecular medicine. The enormous knowledge and ongoing research have now been able to demonstrate methodologies that can alter DNA coding. The techniques used to edit or change the genome evolved from the earlier attempts like nuclease technologies, homing endonucleases, and certain chemical methods. At the molecular level, alteration of the DNA of interest can be executed with the implementation of different gene editing technologies which includes CRISPR/Cas9 (Clustered regularly interspaced short palindromic repeats which are associated with protein 9), TALEN (transcription activator-like effectors nuclease), and ZFN (zinc finger nuclease). These technologies are beginning to be used for new approaches in a variety of areas including research, medicine, agriculture, biotechnology and have the potential to be used in pest control. These new technologies pose challenges for regulators who will find it harder to distinguish between genetic changes in organisms generated by conventional breeding, gene editing, or natural mutation.

Keywords:

CRISPER/Cas 9, TALEN, ZFN

15.1 Introduction:

Genome editing is a technique that can be used to make changes to a cell's DNA (the term 'genome' simply refers to all of the DNA in a cell) (Gaj et al., 2013). DNA sequences contain four different components of nitrogenous bases are represented by the letters A, C, G and T (Komor et al., 2016). Making changes to a cell's DNA has the potential to affect how that cell functions.

Genome editing is used to make precise genetic modifications for various purposes, such as studying the gene function, biological mechanisms, pathology of diseases and to correct mutations in genes that cause one of the 10,000 disorders that result from mutations in a single gene (monogenic disorders) and to prevent such disorders from being inherited (WHO,2019., Ormond et al., 2019).

The intended changes include target gene mutagenesis, insertion and replacement, suppression or activation of the gene expression, and target chromosomal rearrangement. Gene-editing technologies use proteins, called enzymes, to cut targeted areas of DNA within a genome. Cells repair these cuts but if no instructions are provided for the repair, the repair process can make mistakes, resulting in altered DNA sequences. If specific DNA repair information is provided, however, the cell will use this to repair the cut in the way it is instructed.

The use of this process provides an opportunity for researchers to modify the genome, by providing slightly different repair information from what was there before. In this way, it is possible to use gene editing to change a version of a gene from one that causes disease to one that does not (for example gene variants that contribute to Parkinson's disease (Soldner et al., 2016) or genetic metabolic disorders (Hao et al., 2014), or choose the version of a gene that confers better resistance to disease in agricultural plants and animals (for example resistance to powdery mildew in wheat (Wang et al., 2014). It is also possible to use the technique to modify genes without introducing foreign DNA sequences.

For example, gene editing can be used to switch off genes (Maeder and Gersbach, 2016) in laboratory-grown cells to identify their function (Shalem et al., 2014), or to switch off genes that are causing disease, such as in animal models of Huntington's disease.While technologies to make cuts in DNA have been known since the 1970s, using them in a controlled and accurate way, and in organisms whose genome is poorly understood, has been a major hurdle.

However, in the last 10 years, researchers have identified, or created, proteins that permit gene-editing technologies to make gene edits in specific areas of DNA, rather than introducing these changes randomly into the genome. Also, advances in very rapid genome sequencing now mean that genome DNA sequence information for any species can be quickly assembled (Shendure and Ji., 2008), opening up the way for widespread use of gene editing approaches.

Three main classes of endonucleases, including zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas9 system are known as the main tools in genome editing.

15.2 Genome-Editing Techniques:

The recent expansion and advancements in the field of biotechnology provided us with information and insight into the biochemical and molecular mechanisms to edit DNA and, thus, modify downstream pathways.

To date, multiple biotechnologies have shown promise for clinical use, but the field of genome-editing technologies is rapidly evolving and improving. The new techniques seem promising, but the earlier ones have also been updated and improved.

The diverse array of genetic outcomes made possible by these technologies is the result, in large part, of their ability to efficiently induce targeted DNA double-strand breaks (DSBs). These DNA breaks then drive activation of cellular DNA repair pathways and facilitate the introduction of site-specific genomic modifications (Rouet et al. 1994; Choulika et al. 1995).

This process is most often used to achieve gene knockout via random base insertions and/or deletions that can be introduced by nonhomologous end joining (NHEJ) (Figure 15.1) (Bibikova et al., 2002).

Alternatively, in the presence of a donor template with homology to the targeted chromosomal site, gene integration, or base correction via homology-directed repair (HDR) can occur (HDR) (Figure 15.1) (see Figure 15.1 for an overview of other possible genome-editing out comes) (Bibikova et al. 2001, 2003; Porteus and Baltimore 2003; Urnov et al., 2005).

Indeed, the broad versatility of these genome-modifying enzymes is evidenced by the fact that they also serve as the foundation for artificial transcription factors, a class of tools capable of modulating the expression of nearly any gene within a genome. Representative genome-editing techniques are discussed below (Figure 15.1).

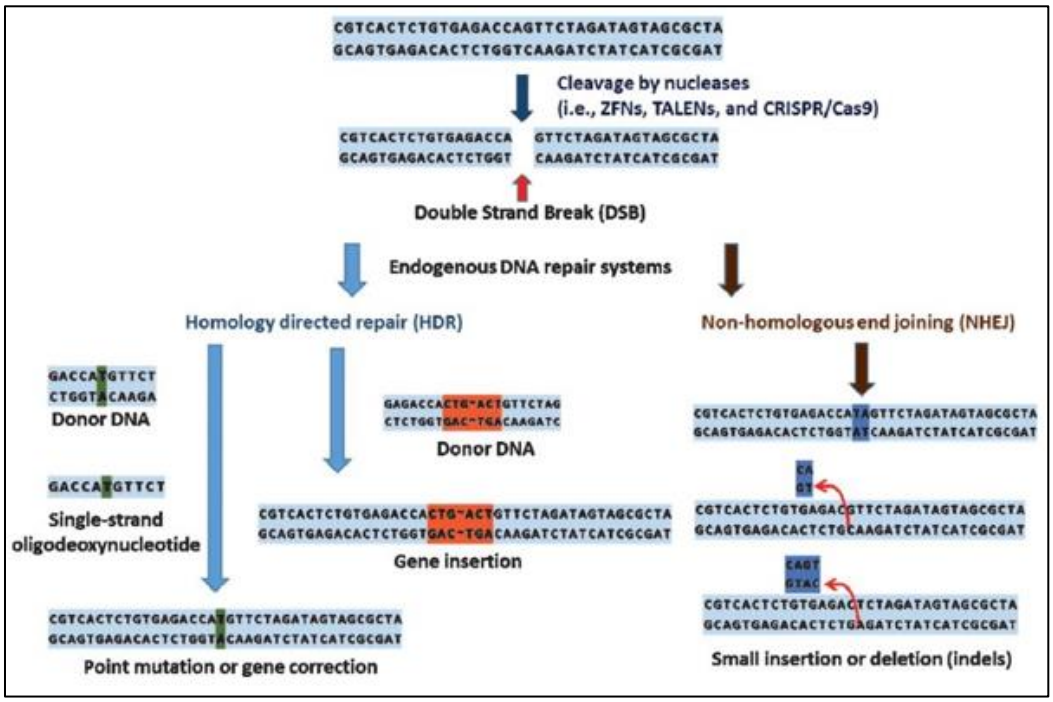

Figure 15.1: Genome-Editing Techniques

15.2.1 Zinc-finger nucleases (ZFNs):

ZFNs use a bacterial DNA cutting enzyme (Kim et al., 1996) that has been combined with proteins called 'zinc fingers', which can be customized to recognise a specific section of DNA (Corbyn., 2015). In 2005, this technology was first used to edit DNA in human cells (Urnov et al., 2005). ZFNs are small (one-third of the size of TALENs and much smaller than CRISPR) so they are easier to package inside delivery vehicles, such as viruses, to enable them to reach their targets in cells for genome editing-based therapies (Chen and Goncalves, 2016).

15.2.2 TALENs:

TALENs (transcription activator-like effector nucleases) again use a DNA-cutting enzyme combined with proteins from bacteria [Joung and Sander., 2013] that target areas of DNA, in a similar way to the zinc finger proteins.

TALENs can be designed with long DNA recognition sections, and therefore tend to have lower unintended off-target cut sites, which can occur when parts of a genome have an identical or near-identical sequence to the target site [Gupta and Munsunuru, 2014., Kim and Kim ,2014].

15.2.3 CRISPR:

Bacteria possess an immune system which recognises invading viral DNA and cuts it up, making the invading virus DNA inactive. This type of immune system is known as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) [Marraffini., 2015].

In 2012, it was discovered that by modifying this mechanism, it was possible to target and cut any DNA sequence and edit genomes [Jinek et al.,2012].

In 2013, this technology was modified further so that the target DNA is bound and blocked, rather than cut, allowing a gene to be turned off without altering the DNA sequence [Qi et al., 2013, Cheng et al., 2013., Esvelt et al., 2013].

In 2014, a further advance allowed the blocking enzyme to be reactivated, enabling a way to turn genes on and off using chemical triggers, or blue light. In 2016, researchers further improved on the performance of CRISPR by allowing for editing of single DNA letters [Komor et al., 2016].

CRISPR, unlike ZFNs and TALENs allows for many DNA sites to be edited simultaneously and easily [Cong et al., 2013]. It is also the most affordable and programmable genome editing technology. While much more accurate than earlier genetic modification technologies, there can still be unintended off-target effects, although these are detectable and new research is rapidly improving the technology's accuracy [Kleinstiver et al., 2016]. For simplicity and consolidation, an overview of genome-editing techniques is presented in Figure 15.2.

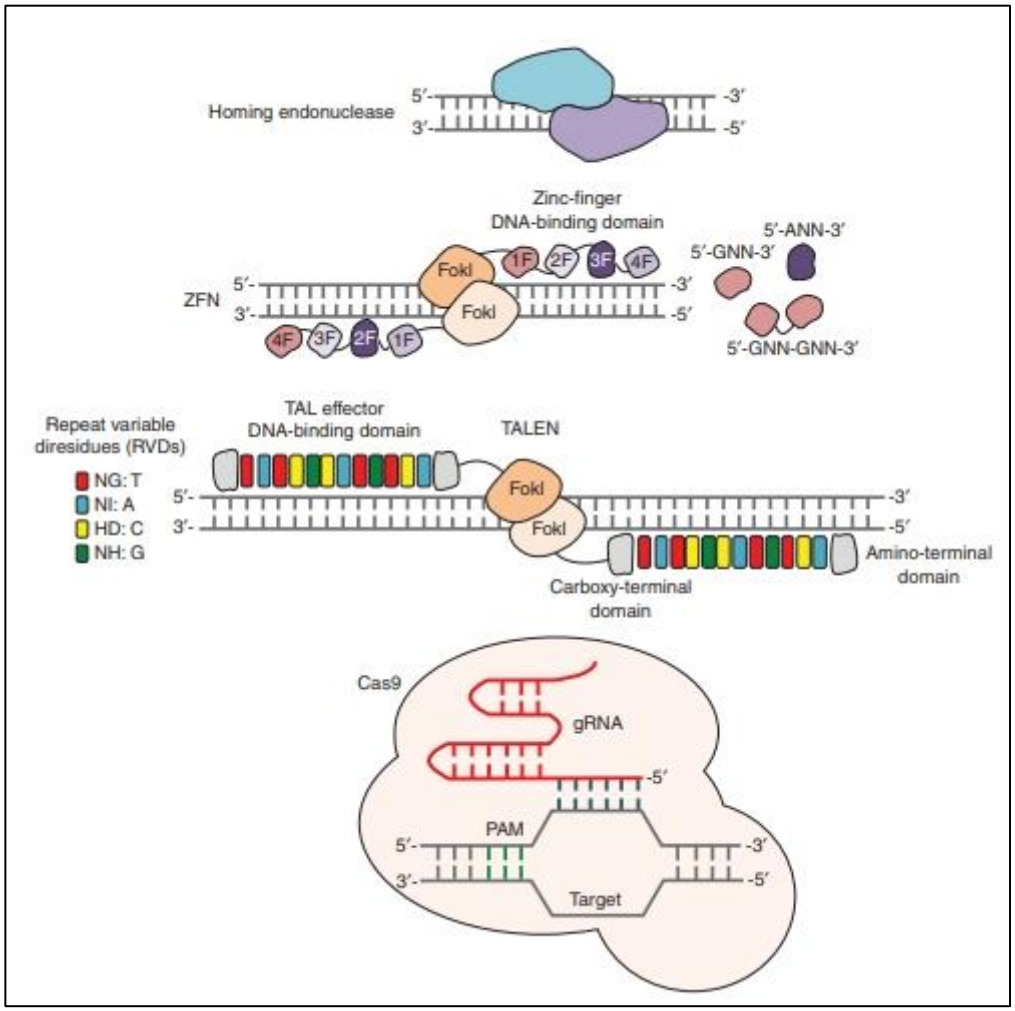

Figure 15.2: Clustered Regularly Interspaced Short Palindromic Repeats

Figure 15.2. Genome-editing technologies. Cartoons illustrating the mechanisms of targeted nucleases. From top to bottom: homing endonucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector (TALE) nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9).

Homing endonucleases generally cleave their DNA substrates as dimers, and do not have distinct binding and cleavage domains. ZFNs recognize target sites that consist of two zinc-finger binding sites that flank a 5- to 7-base pair (bp) spacer sequence recognized by the FokI cleavage domain. TALENs recognize target sites that consist of two TALE DNA-binding sites that flank a 12- to 20-bp spacer sequence recognized by the FokI cleavage domain. The Cas9 nuclease is targeted to DNA sequences complementary to the targeting sequence within the single guide RNA (gRNA) located immediately upstream of a compatible protospacer adjacent motif (PAM). DNA and protein are not drawn to scale.

15.3 Agricultural Applications of Genome Editing:

In agriculture, the new gene-editing technologies make it possible to modify a range of agriculturally important organisms easily, cheaply, and if desired, without introducing foreign DNA sequences (Woo et al., 2015, Voytas and Gao, 2014).

15.3.1 Food Production:

In the US, researchers have used gene-editing technologies on agricultural crops such as maize (Shukla et al., 2009), soybean (Curtin et al., 2013), sorghum (Jiang et al., 2013), and developed a rice resistant to bacterial blight (Li et al., 2012). In commercial development, the common white button mushroom has been modified by CRISPR at Penn State University to prevent them from becoming off-colour by targeting a gene that produces an enzyme that causes browning (Waltz, 2016).

Further, DuPont Pioneer have used CRISPR to produce a higher-yielding waxy corn variety (Servick., 2016) and Calyxt Plant Sciences Inc. have produced soybean lines that are low in polyunsaturated fats, using TALENs (Haun et al., 2014). Chinese researchers have similarly used TALENs and CRISPR to modify a range of agriculturally important plants and animals, including maize (Liang et al., 2014), rice (Miao et al., 2013, Shan et al., 2013), and wheat (Shan et al., 2013). They have also used the techniques to develop goats with longer coats (for Angora) and more muscle (for increased meat yield) (Wang et al., 2015). Elsewhere in the world, researchers have used the techniques to modify barley (Denmark) (Wendt et al., 2013); wheat (India) (Upadhyay et al., 2013); and to study allergenic milk protein production in cow embryos cultured in the laboratory (New Zealand) (Wei et al., 2015).

15.3.2 Animal Health and Welfare:

In the US, hornless dairy cattle have been produced using gene editing to avoid the need for painful de-horning and to prevent animals injuring each other during transport. Using TALENs, the genetic code that makes dairy cattle have horns has been substituted for the one that makes Angus beef cattle have none (Carlson et al., 2016). The University of Missouri has also bred the first pigs resistant to Porcine Reproductive and Respiratory Syndrome by suppressing the production of a protein within the pigs that the virus uses to help it spread (Whitworth et al., 2016). African swine fever is a highly contagious disease that kills up to two-thirds of infected animals. In Scotland, ZFN has been used by the University of Edinburgh to modify a gene in pigs to the version of the gene found in warthogs, to produce pigs that are potentially resilient to the disease (Lillico et al., 2016).

The university has also used gene editing to modify chicken genes so they don't spread bird flu by introducing a gene that produces a 'decoy' molecule that interrupts the replication cycle of the bird flu virus, thereby restricting its transmission. In China, TALENs have been used to add a gene that is found in mice into cattle to improve tuberculosis (TB)- resistance. The modified cattle have immune cells that are better at slowing the growth of the disease and are less susceptible to developing the internal symptoms of TB (Wu et al., 2015).

15.3.3 Pets:

In China, CRISPR has been used to create micro-pigs which are approximately half the size of their non-modified counterparts, which can be sold as pets (Larson., 2015).

15.3.4 Biocompound Production:

By using gene-editing technology to manipulate biological pathways, new materials are being developed, such as algae-derived porous silica-based particles for drug delivery (Delalat et al., 2015), CRISPR modified silkworms to produce spider silk, algae-derived lipids for biofuels (Nymark et al., 2016), and microbial production of pharmaceuticals and commodity chemicals such as ß-carotene (Li et al., 2015), L-lysine (Cleto et al., 2016), and mevalonate (Jakociunas et al., 2015).

15.4 Challenges:

Despite its great promise as a genome-editing system CRISPR/Cas-9 technology had hampered by several challenges that should be addressed during the process of application. Immunogenicity, lack of a safe and efficient delivery system to the target, off-target effect, and ethical issues have been the major barriers to extend the technology in clinical applications.[52] Since the components of the CRISPR/Cas-9 system are derived from bacteria, host immunity can elicit an immune response against these components. Researchers also found that there were both pre-existing humoral (anti-Cas-9 antibody) and cellular (anti-Cas-9 T cells) immune responses to Cas-9 protein in healthy humans. Therefore, how to detect and reduce the immunogenicity of Cas-9 protein is still one of the most important challenges in the clinical trial of the system (Charlesworth et al., 2020).

Safe and effective delivery of the components into the cell is essential in CRISPR/Cas-9 gene editing. Currently, there are three methods of delivering the CRISPR/Cas-9 complex into cells, physical, chemical, and viral vectors. Non-viral (physical and chemical) methods are more suitable for ex vivo CRISPR/Cas-9-based gene editing therapy.[54] The physical methods of delivering CRISPR/Cas-9 can include electroporation, microinjection, hydrodynamic injection, and so on. The main limitation of electroporation method is that it causes significant cell death (Zhang et al., 2020). Microinjection involves injecting the CRISPR/Cas-9 complex directly into cells at the microscopic level for rapid gene editing of a single cell. Nevertheless, this method also has several disadvantages such as cell damage, which is technically challenging and is only suitable for a limited number of cells (Fajrial et al., 2020). The hydrodynamic injection is the rapid injection of a large amount of high-pressure liquid into the bloodstream of animals, usually using the tail vein of mice. Although this method is simple, fast, efficient, and versatile, it has not yet been used in clinical applications due to possible complications (Lino et al., 2018).

Viral vectors are the natural experts for in vivo CRISPR/Cas-9 delivery (Behr et al., 2021). Vectors, such as adenoviral vectors (AVs), adeno-associated viruses (AAVs), and lentivirus vectors (LVs) are currently being widely used as delivery methods due to their higher delivery efficiency relative to physical and chemical methods.

However, the limited virus cloning capacity and the large size of the Cas-9 protein remain a major problem. Furthermore, CRISPR/Cas-9 gene editing has been challenged by ethics and safety all over the world. Since the technology is still in its infancy and knowledge about the genome is limited, many scientists restrain that it still needs a lot of work to increase its accuracy and make sure that changes made in one part of the genome do not have unforeseen consequences, especially in the application towards human trials (Kotagama et al.,2019).

15.5 Bioethical Issues and Genome-Editing Techniques:

Genome-editing tools are powerful in terms of their potential to not only bring biotechnological revolution in the field of crop development and human pathology but also, in the wrong hands, lead to abuse and misuse in multiple ways, including manipulation of germline genetics. Genuine bioethical concerns have been raised by many experts (Krishan et al., 2016).

While time will be the actual judge of these technologies as boon or bane, still the methods can impact the human race probably in the most nuclear ways, and our incoming human race may be victimized in ways we do not yet understand (Krishan et al., 2018).

Principal concerns apart from illegal germline mutation include the morality, the eugenics helping the fittest to survive, ongoing clinical debates about informed consent, religious debate, the possible rise of clones, designer babies, and possibly superhumans.44–46 Moreover, the current literature also rules in the possibility of genome editing as a future weapon of war (Fraser and Dando,2001). While the quest for a healthy baby and right of best possible treatment choice have been acknowledged in many societies, the approaching biotechnological revolution seems imminent and undeniable.

The pressing need, therefore demands a harmonious and regulated translation of needed aspects of genome-editing-related technologies for molecular medicine and other non-clinical crop and food industries. This will need consensus in public opinion, debates among experts, involvement of biotechnologists, opinions of bioethical experts, regulatory frameworks within legislatures, and final guidelines and oversight for the finally allowed limited application.

15.6 Conclusion:

It can be concluded that the technologies of gene editing provide a great platform for researchers to develop a therapeutic agent to treat the disease that is mainly generated through genetic disorders.

With the help of such innovative technologies, researchers can develop new genomes that can be used in the treatment of different kinds of diseases especially cancer. The process is executed by cutting the DNA sequence with specific enzymes and thus this phenomenon is referred to as nuclei engineering and through this process, the DNA can be modified and can be further inserted into the gene of interest.

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