УДК 582.282.23+575.113
Повшедна І.О. – ст. гр. БХФ-2-15
Київський національний університет технологій та дизайну

ТЕХНОЛОГІЯ РЕДАГУВАННЯ ГЕНА CRISPR/CAS9

Науковий керівник: ст. викладач Звонок О.А.

I.O.Povshedna
Kyiv National University of Technologies and Design

GENE EDITING MECHANISM OF CRISPR/CAS9

Supervisor: O.A.Zvonok

Keywords: CRISPR/Cas9, gene, gene editing technology

The majority of medical therapies available today are directed at managing disease processes, the pathogenic or mis-regulated proteins or molecules associated with disease. However, these pathogenic molecules themselves are typically encoded in or affected by changes in genes or other sequences in the human genome, which encompasses the DNA in all our cells. Gene editing technologies, including CRISPR/Cas9, now offer us the ability to directly modify or correct the underlying disease-associated changes in our genome. Successfully editing or correcting a gene that encodes the dysfunctional or missing protein can in principle result in the expression of a fully normal protein and full correction of the disease.

Gene therapy and other technologies to modify the genome have been in development for many years, and a small number of gene therapies have been approved to treat patients. However, these older approaches have been burdened by challenges to their safety and efficacy and have not yet provided the ability to precisely control a range of different genetic changes.

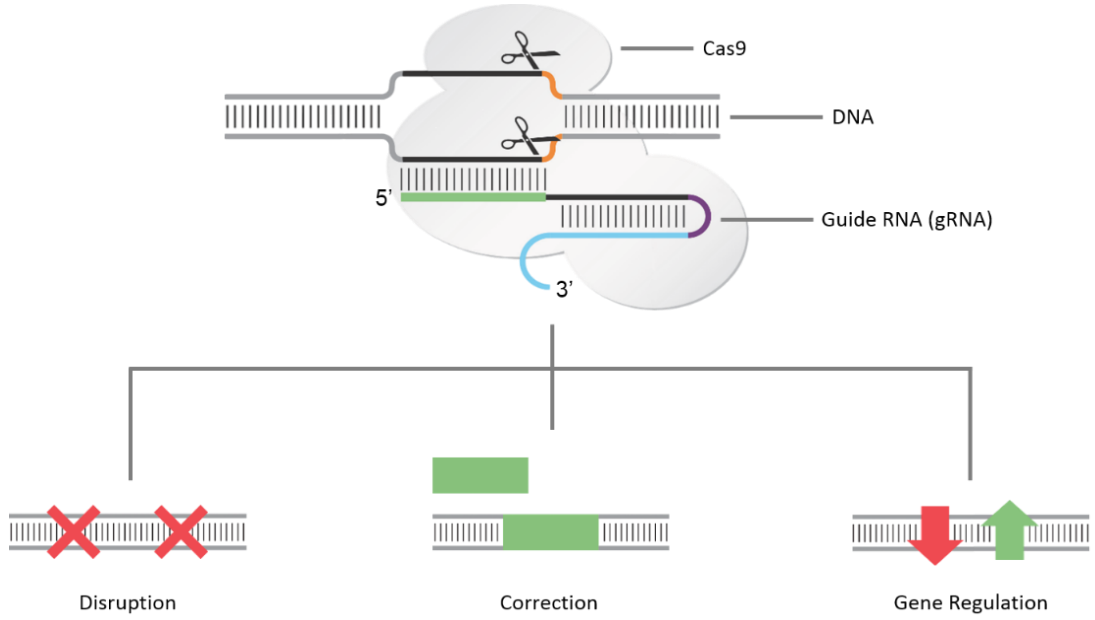
The CRISPR/Cas9 system was first exploited by Danisco in 2008. The company used it to improve the immunity of bacterial cultures against viruses and many food manufacturers now use the technology to produce cheese and yoghurt. Since then the technology has been used to delete, insert and modify DNA in human cells and other animal cells grown in petri dishes. Scientists are also using it to create transgenic animals such as mice, rats, zebrafish, pigs and primates. Between 2014 and 2015 scientists reported the successful use of CRISPR/Cas9 in mice to eliminate muscular dystrophy and cure a rare liver disease, and to make human cells immune to HIV. It is also being investigated in conjunction with pluripotent stem cells to provide human organs from transgenic pigs. Such work is directed towards helping solve some of the shortage of human organs for transplant operations and overcome some of the side-effects caused by organ transplantation such as graft-versus host disease. The technology is also being investigated as a means to genetically engineer insects so as to wipe out insect-borne diseases such as malaria, transmitted by mosquitoes, and lyme disease, transmitted by ticks.

The CRISPR/Cas9 technique is one of a number of gene-editing tools. Many favour the CRISPR/Cas9 technique because of its high degree of flexibility and accuracy in cutting and pasting DNA. One of the reasons for its popularity is that it makes it possible to carry out genetic engineering on an unprecedented scale at a very low cost. How it differs from previous genetic engineering techniques is that it allows for the introduction or removal of more than one gene at a time. This makes it possible to manipulate many different genes in a

cell line, plant or animal very quickly, reducing the process from taking a number of years to a matter of weeks. It is also different in that it is not species-specific, so can be used on organisms previously resistant to genetic engineering.

I believe that CRISPR/Cas9 offers just such an opportunity, particularly to correct DNA changes in somatic (non-germ line) cells in patients with serious disease.

CRISPR/Cas9 is a rapid and easy to use gene editing technology that can selectively delete, modify or correct a disease causing abnormality in a specific DNA segment. "CRISPR" refers to Clustered Regularly Interspaced Short Palindromic Repeats occurring in the genome of certain bacteria, from which the system was discovered; Cas9 is a CRISPR-associated endonuclease (an enzyme), the "molecular scissors" that are easily programmed to cut and edit, or correct, disease-associated DNA in a patient's cell. The location at which the Cas9 molecular scissors cut the DNA to be edited is specified by guide RNA, which is comprised of a crRNA component and a tracrRNA component, either individually or combined together as a single guide RNA (sgRNA). For example, a guide RNA can direct the molecular scissors to cut the DNA at the exact site of the mutation present in the genome of patients with a particular genetic disease. Once the molecular scissors make a cut in the DNA, additional cellular mechanisms and exogenously added DNA will use the cell's own machinery and other elements to specifically repair the cut DNA.



There are more than 10,000 known single-gene (or monogenic) diseases, occurring in about 1 out of every 100 births. Scientists and clinicians are now conducting pioneering research using CRISPR/Cas9 to address both recessive and dominant genetic defects, opening up the potential of gene editing to provide novel transformative gene-based medicines for patients with a large number of both rare and common diseases.