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Articulos de Revision

Crispr, a tool for genome editing

Crispr, una herramienta para editar genomas

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Abstract: The article focuses on the use of the new tool, CRISPR (short palindromic repeats grouped at regular intervals), which makes it possible to edit the genomes of living beings more precisely than other techniques; this paper mentions works related to the halting of angiogenesis, cancer, Kaposi?s sarcoma in immunodeficiencies, Parkinson?s disease, regeneration and genetic modification in humans, all of these investigations have in common the use of the CRISPR tool. The ethical complications involved in using this technology on the DNA of human embryonic cells are also discussed, which according to different criteria, could lead to the generation of ?improved? human beings, i.e. not only without susceptibility to degenerative or incurable diseases, but also modified in physical aspects that would not necessarily be linked to any pathology.

Keywords: CRISPR, genome, DNA, technology.

Resumen: El artículo se centra en la utilización de la nueva herramienta, CRISPR (repeticiones palindrómicas cortas agrupadas a intervalos regulares), la cual permite editar los genomas de los seres vivos de manera más precisa que otras técnicas; a lo largo del artículo se mencionan trabajos relacionados con la detención de la angiogénesis, cáncer, Sarcoma de Kaposi en inmunodeficiencias, Parkinson, regeneración y modificación genética en humanos, todas estas investigaciones tiene en común la utilización de la herramienta CRISPR. También se comenta las complicaciones éticas que conlleva utilizar esta tecnología en el ADN de células embrionarias humanas, que según diferentes criterios, podrían llevar a generar seres humanos ?mejorados?, es decir no solo sin susceptibilidad a enfermedades degenerativas o incurables, sino también modificados en aspectos físicos que no necesariamente estarían ligados a alguna patología.

Palabras clave: CRISPR, genoma, ADN, tecnología.

The name CRISPR is resonating in various scientific arenas, but what is it really and why is it currently being studied and used? In principle, the acronym CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats, so CRISPR is the name given to a locus (locus = position of the gene or other DNA sequence) in the bacterial chromosome where genes are found, with which a powerful tool has been created that allows the DNA of any organism to be deliberately manipulated. The name CRISPR now also identifies a technique for changing the genome [1];. The name CRISPR therefore refers to a section of DNA in bacteria and archaea, and also to the technique that uses genes from this section as a tool in genome editing.

These repeated sequences that are now known as CRISPR were identified many years ago. A group of Japanese scientists studying Escherichia coli [2] ; and then the Spaniard Francisco

Mojica experimenting with Haloferax mediterranei (archaea) discovered these sequences [3]. CRISPR functions as a defence system in these microorganisms, equivalent to acquired immunity in animals [1, 4]; A bacteriophage (virus that infects bacteria), when it infects a cell, inserts viral DNA into the bacterial DNA, precisely at the site known as CRISPR. By this mechanism, the bacteriophage attacks are recorded over time, creating an infection file, and the inserted viral pieces are transferred to the progeny from generation to generation to protect them against the same viruses that caused the first infection. The defence of these prokaryotes is mediated by genes at the CRISPR locus, which is why such simple organisms immunise themselves against reinfection by viruses.

The CRISPR locus functions as a registry of DNA pieces taken from the genome of the bacteriophage that previously infected the bacterium. The associated genes (Cas) are those that generate proteins involved in the creation of the archive, Cas9 (CRISPR-associated 9) is the endonuclease that destroys the DNA of the reinfecting bacteriophage, the specificity of the system is provided by the crRNA (CRISPR RNA) that recognises by base pairing the nucleotide sequences of the phage DNA, the tracrRNA (trans-activating crRNA) serves to assemble the crRNA-DNA hybrid in the active site of the Cas9 [1]. Therefore the efficiency of the system, i.e. the active form of Cas9 endonuclease, occurs when there is a complex between the components: crRNA, tracrRNA and target DNA (Figure 1).

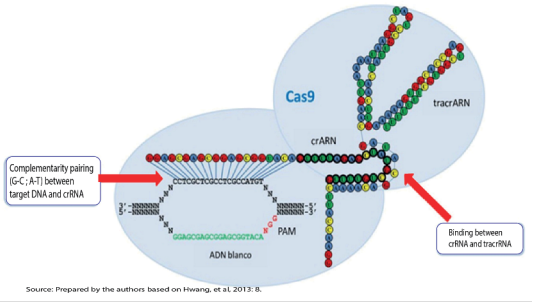

Figure 1
Schematic representation of a natural complex of the crispr/cas9 system on a DNA target.

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The image above shows the natural complex of the CRISPR/Cas9 system on the target DNA. The crRNA interacts with the complementary strand of the target site on the DNA (green) which harbours a PAM sequence (proto-spacer adjacent motif (red NGG), which is an essential targeting component necessary for the nuclease to cut the invading viral DNA and not the bacterial DNA. The folding of the tracrRNA and crRNA molecules shown in the image were predicted according to the Mfold 20 [5] software package and the association between the two (tracrRNA and crRNA) is based on Jinek?s model [6]. Thus, tracrRNA is a longer strand of bases and provides a hairpin loop structure, and serves to assemble the crRNA-target DNA hybrid at the Cas9 nuclease active site; it is important to note that although the CRISPR complex is hybridised to one strand of DNA, it is able to cut both strands.

Thus, Jennifer Doudna and Emmanuelle Charpentier (winners of the 2015 Prince of Asturias Award[1]) took advantage of these characteristics to design the CRISPR/Cas 9 technology that is revolutionising genetic engineering. This system is currently used to cut out areas of interest in the DNA and introduce desired sequences that would produce desired genotypes or phenotypes; They designed a sgRNA (single guide RNA) that has the same characteristics as tracrRNA and crRNA, this sgRNA directs the nuclease towards the target DNA with the same precision as a bacterial crRNA and tracrRNA would, to obtain a sgRNA with specificity against the genomic target of choice, it must be designed with base complementarity, i.e. G-A and C-T (Watson and Crick?s rules), When the sgRNA and Cas9 are introduced into the eukaryotic nucleus where the target DNA is located, it will be cut in both DNA strands, generating a DSB (double stranded break). This type of cut is repaired by the NHEJ (nonhomologous end-joining) system, which is not precise, causing the DNA to undergo insertion or deletion of bases (indels) around the DSB, which could in some cases lead to loss of function of the target DNA [1]. Gene inactivation is used to generate knockout organisms, in which the function of the blocked gene is studied, or genes of interest can be inserted into the DSB to modify some function or structure in the desired organisms and, for example, to produce a biotechnological product of interest.

The CRISPR/Cas9 system is a powerful, easy-to-use and highly specific tool for the eukaryotic genome [7], especially for human cells [8].

1. Current research using the CRISPR system

So, there is currently a range of research using the CRISPR tool, and here are some of the most current and controversial examples:

1.1. Adeno-associated virus (AAV)-CRISPR/Cas9 to downregulate vegrfr2 expression and block angiogenesis in vitro

Angiogenesis is a natural process that refers to the formation of blood vessels in body tissues, in some cases this process can be stimulated to heal wounds, or improve heart problems, and its slowing or inhibition is used as a treatment for cancer and other diseases.

Abnormal angiogenesis is a component of many diseases, including macular degeneration, diabetic retinopathy, arthritis, tumour growth and metastasis. The purpose of the following research in Japan was to examine whether the AAV-CRISPR/Cas9 system could reduce the expression of VEGFR2 (vascular endothelial growth factor receptor 2) in the vascular endothelium of human cells in vitro, the results showed that this system reduced the expression of VEGFR2 by 80% and also completely blocked the activation of VEGF (vascular endothelial growth factor) by Akt (a type of protein serine-threonine kinase, also known as protein kinase B, involved in metabolism, cell proliferation, cell signalling and apoptosis), and also reduced the proliferation, migration and tube formation of human retinal microvascular endothelial cells9. Thus, the AAV - CRISPR/Cas9 system proved to be a potential tool to inhibit pathological angiogenesis and thus improve the status of diseases where blood vessel formation is a major factor in pathogenesis.

1.2 Application and optimisation of CRISPR-cas9 by genetic engineering in salamander (Ambystoma mexicanum)

The axolotl is a type of salamander that has a regenerative capacity; if it loses a limb, a new one is generated within weeks, and even complex tissues such as bones, nerves, parts of the brain and spinal cord are regenerated; it is an animal model for understanding regeneration mechanisms. Scientists claim that the key lies in its genome, but as the New York Times, quoted by the newspaper UNAM Global, points out, the salamander has 32 billion base pairs [10], ten times more than the human genome (3 million base pairs), so studying its genome is still a challenge.

Researchers in China described an optimised protocol for creating genetically modified axolots using the CRISPR/Cas9 system. With this protocol, individuals harbouring a homozygous frameshift mutation can be obtained, allowing analysis of phenotypes in this generation [11]. In other words, genomic manipulation of the salamander is essential for understanding the development and physiology of regeneration, this protocol can potentially be applied to other animal models, especially organisms with a well-determined transcriptome (mRNA transcribed in certain circumstances, globally) but lacking a well-characterised genome (set of genes and their arrangement in the cell).

1.3 CRISPR/Cas 9 in cancer therapy: hopes and challenges

The term cancer is used to describe a group of diseases in which an excessive and uncontrolled process of cell division in the body is observed. Cancer results from three processes: proliferation of a group of cells (tumour or neoplasm), invasion of this group into adjacent tissues and metastasis to more distant tissues and organs, and is caused by abnormalities in the genetic material. The CRISPR/Cas9 technique has proven to be a potential tool for discovering new genetic and molecular targets and interactions in cancer therapy, providing insight into how tumours respond to drug treatment, and can be used to create immune cells and oncolytic viruses for immunotherapy [12].

Cancer is the second leading cause of death worldwide and while the different mechanisms involved in abnormal cell growth are still being studied, the CRISPR system is being used to study the unknown mechanisms and create possible cures for this diverse set of diseases with molecular mechanisms that are similar in some ways and different in others.

Research in Australia used CRISPR/dCas9 (a variant of the described CRISPR/Cas, which works by the same mechanism) in a panel of human melanoma and triple-negative breast cancer cell lines (does not express genes for estrogen receptor, progesterone or human epidermal growth factor receptor 2 HER2/neu); this research suggests that PTEN (phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase-enzyme that acts as a cancer suppressor through its lipid-phosphatase activity) expression is lost in different types of cancers, with even small changes in PTEN activity affecting susceptibility and prognosis. This may provide an alternative therapeutic approach for aggressive cancers that are refractory

to current treatments [13]. Thus, the study of the different proteins involved in cancer initiation, promotion, transformation or progression may provide new therapeutic targets and a better understanding of mechanisms to generate new therapies.

1.4 Reduction of Kaposi?s sarcoma-associated herpesvirus latency

Kaposi?s sarcoma-associated human herpesvirus type 8 (KSHV) is the etiological agent of Kaposi?s sarcoma (KS), which is a cancer that generates abnormal tissue in the form of patches that grow under the skin in different areas such as the mouth, nose, throat, etc. This cancer defines the diagnosis of AIDS in people infected with HIV-1 or in immunosuppressed transplant patients; currently there is no effective treatment for KS, so the survival rate is low; KSHV establishes a latent infection in the host and constitutes a major challenge for the treatment of KS, the CRISPR/Cas9 System is being used for the study of a latency-associated nuclear antigen (LANA), which is absolutely necessary in maintenance, replication and segregation of the episomes (DNA molecule capable of replicating autonomously in the host cell cytoplasm or when physically integrated into the host cell chromosome) of KSHV during mitosis, making LANA an ideal target for CRISPR/Cas9 editing.

A study in Nebraska, USA, was the first to demonstrate the feasibility of using the KSHV LANA-targeted CRISPR/Cas9 system with an adenovirus delivery system to interrupt KSHV latency in infected epithelial and endothelial cell lines, laying the groundwork for a viable strategy in countries where patients have high viral load readings, and could also be used against other tumorigenic viruses [14]. It is important to study the infectivity of adenoviruses and their association with CRISPR, for example, the effect is lower when working with B cells of the immune system due to low infection efficiency.

1.5 CRISPR/Cas9 as a protector of neuronal death in a Parkinson?s disease model

Parkinson?s is a fatal disease with neurodegenerative progression caused by genetic and/or environmental factors. These patients present a movement disorder due to chronic progressive destruction of the nervous system in several areas of the brain; proteins (Lewy bodies) are deposited in the area of the substantia nigra (SN) producing apoptosis in the dopaminergic neurons in this area, and the corpus striatum (CS) does not receive any signal from the SN to react to movement; if the problem continues, atrophy due to disuse is produced.

It has been suggested that inflammatory responses contribute to the disease, research conducted in 2003 in the US studied affected dopaminergic neurons, indicating that they accumulate neuromelanin (oxidised and polymerised dopamine). Microglia (which support neurons and remove cellular debris) uses phagocytosis to internalise neuromelanin molecules, becoming activated and producing inflammatory mediators and molecules toxic to surrounding neurons, Prolonged exposure of microglia to neuromelanin may provide amplifying damage that accelerates the disease, and the research revealed that there is a specific receptor expressed by microglia in this process, called RAGE

(transmembrane receptor for advanced glycation end products), which may be used as a therapeutic target in the treatment of Parkinson?s disease, and is also involved in other diseases such as diabetes, atherosclerosis and Alzheimer?s disease [15]. In February 2019, research in Korea used this therapeutic target, researchers generated umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs) that secrete secrete sRAGE (soluble receptor) which prevents the binding of this very receptor to its ligand in the cell, the production of these cells was achieved using the gene editing method, CRISPR/Cas 9. These UCB-MSC cells were transplanted into the corpus striatum of an animal model (mice with Parkinson?s disease induced by rotenone); the results indicate that neuronal death was reduced and movement improved, so this therapeutic approach based on UCB-MSCs could be a treatment strategy for Parkinson?s disease [16]. Thus, thanks to CRISPR technology, such research is possible, enabling new treatments for neurodegenerative diseases to be outlined.

1.6 Genetic modification in humans using CRISPR

In 2018, scientist He Jiankui announced to the world the birth of two genetically modified twin girls using CRISPR technology. The newspaper SINC (science is news) in November 2018 published an article indicating that researchers at the Southern University of Science and Technology (SUSTech), in China, announced that they had created two twin girls modified with the CRISPR genetic cut-and-paste tool; one day after the announcement, the University itself issued a malpractice complaint to the director of the study, the scientists eliminated the CCR5 gene with the aim of creating resistance to HIV, smallpox and cholera [17]. CCR5 is a co-receptor that HIV needs to fuse its membrane with the host cell (dendritic cells and CD4+ T lymphocytes) and introduce its genetic material (RNA), so deleting the CCR5 gene does not transcribe the CCR5 protein and therefore HIV does not enter the cell and the person would be immune to the virus. However, this type of manipulation could have unintended effects that could become evident in the years to come.

Molecular biologist Lluis Montoliu, from the National Biotechnology Centre, declared to the SINC newspaper that this modification carried out by He Jiankui and his team does not serve to correct a genetic anomaly in the embryo that could produce a serious and/or incurable disease, but on the contrary it is an experiment in genetic improvement that aims to create people with different capacities [17]; Therefore, in this case, the aim is to create a new lineage of human beings who will theoretically be immune to HIV; the ethical implications of this experiment are various, since in reality not only the genome of the girls was altered but also that of their offspring, and in reality what is really wanted is for the therapies developed to be effective and, above all, safe. According to a report by the US Department of Health and Human Services, the scientist He Jiankui indicates that, ?In this increasingly competitive global search for gene editing applications, we hope to stand out?, but to date there has been no formal publication of the experiment in any scientific journal [18]. The

experiment has been developed ignoring the concerns of the international scientific community regarding the manipulation of human germ lines.

Discussion

CRISPR positions us a step ahead in the field of gene editing and in the last 5 years it has been intensively applied in humans, animals, plants, bacteria and viruses, however this research opens ethical, social and economic debates due to the possible impact on a global level.

This technology is mostly being used to correct various genetic defects in adults, therefore errors in the genetic material would not be passed on to the next generation; the use of this technology is simple; with bioinformatics programmes and molecular biology techniques, the use would be expanded, in addition to the above, it is considered a relatively inexpensive technique, therefore it could be the promise for those sick people with incurable and disabling problems.

CRISPR can be applied to various pathologies such as leukaemias, cardiac, renal, hepatic problems, cancers, etc. However, there is a possibility that it could be used for eugenic purposes or to design humans with different abilities.

If diseases can be cured, conditions such as old age, poor eyesight, baldness, among others, could also be improved. As John Harris points out in his book Superman and Wonder Woman: The Ethical Dimensions of Human Biotechnology, quoted by Bergel, ?We stand on the brink of a new revolution of awesome power. The molecular biology revolution will give us unprecedented scope. It will allow us to manufacture new life forms on demand, life forms of all kinds. The choice before us is not whether or not to use this power, but how and to what extent? [19] . In this sense, there are various answers to ?when?, ?how? and ?to what extent?, which would depend on each country, as each nation has its own laws, regulations and ideologies that in the end would be the basis for determining the reach of this research that aims to ?improve? the human race, with this anticipating ethical problems.

One might think that gene editing is more ethical than pre-implantation diagnosis as it involves the repair and not the destruction of the embryo, so the question arises: How ethical is it to generate perfect humans? [20] On the other hand, will this technology be accessible to all and without restrictions? Will it generate social inequality? Are there laws governing genetic manipulation in all countries and are they sufficient?

Future professionals must be trained with clearly identified competences and ethical-social commitment to respond to emerging problems, as Johann Wolfgang von Goethe rightly said: ?Knowledge increases doubts?.

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