CRISPR-Cas9 as protection therapy against cardiovascular disease

CRISPR-Cas9 como terapia de protección de enfermedades cardíacas

David Vetcher[1]

ABSTRACT

The use of CRISPR technology for gene editing has started a new era in biology and medicine. CRISPR technologies can modify the genome of any eukaryotic cell in an easy, fast, cost-effectively, and precise way. This new modality offers a promising therapeutic approach for heart disease protection and treatment by rewriting the genetic basis of disease.

Keywords: *CRISPR-Cas9 system, gene editing technology, cardiovascular gene therapy.*

RESUMEN

El uso de la tecnología CRISPR para la edición de genes ha iniciado una nueva era en biología y medicina. Las tecnologías CRISPR pueden modificar el genoma de cualquier célula eucariota especialmente humana, de una manera fácil, rápida, económica y precisa. Esta nueva modalidad ofrece un enfoque terapéutico prometedor para la protección y el tratamiento de las enfermedades cardíacas mediante la reescritura de la base genética de la enfermedad, corrigiendo los errores o defectos genéticos responsables.

Palabras clave: *sistema CRISPR-Cas9, tecnología de edición de genes, terapia génica cardiovascular.*

Revista Argentina de Cardioangiología Intervencionista 2022;13(2):61-67. https://doi.org/10.30567/RACI/202202/0061-0067

INTRODUCTION

In February, 2017 we submitted the article *Impact and opportunity of CRISPR-Cas9 in Cardiology* to the Argentine Journal of Interventional Cardioangiology (RACI)—the official organ of the Argentine College of Interventional Cardiologists (CACI)— that was accepted in May 16 2017, and published in Issue #2 (April-June) of 2017.[1] Almost 5 years ago, neither we nor the editor-in-chief, Dr. Alfredo Rodriguez knew could anticipate the impact this article would have on our readers, especially because it dealt with a scientific area that is almost foreign to our own discipline with its own reading priorities.

Contrary to what we believed, this article drew much attention as other people who are more aware to what happens in other disciplines told us, as there was something new out there that was changing dramatically the world of DNA and RNA biology.

Today, after the 2020 Nobel Prize of Chemistry was awarded to Jennifer Doudna from the University of California at Berkeley (**Figure 1**), and Emmanuelle Charpentier from the University of Umea (Sweden) for the discovery in 2012 of a significant finding. The new and powerful Gene Editing technology CRISPR-Cas9. The first description that showed how to turn the natural machinery inside bacteria and archaea into a programmable editing tool that can be used to cut any DNA chain in vivo. Once again, we want to call the attention towards this system as a promising protection therapy for cardiovascular diseases.

We then anticipated that this gene editing technology, which turned upside down all molecular biology research laboratories, was Nobel Prize science and had started a race to achieve this goal.

Therefore, a new era in biology was inaugurated, one where we now could edit, correct, change, erase, insert or to put it more precisely, use insertion–deletion of specific base pairs, in the DNA cut site, and correct errors in the genes responsible for causing diseases.

We were witnessing a scientific revolution right in front of our eyes with the capacity of changing the genome sequence in all eukaryotic cells, especially human cells in an easy, accurate, and cost-effective way.

The possibilities of manipulating DNA sequences in a predictive way using CRISPR-Cas9 technology have multiple and innovative potentialities, and real applications for medicine. Reducing the risk of cardiovascular disease is its promising objective.

Cardiovascular disease (CVD) has a multifactorial, biologically complex etiology. I will clarify it, CVD is associated with metabolic, genetic, environmental, and behavioral risk factors that, combined, challenge our understanding of their etiopathogenesis.

Unlike cases where there is only a single mutation that causes the disease, cardiovascular diseases are caused by a variety of different mutations. Correcting the single mutation is easier. Breakthroughs in molecular biology make it possible to unravel more and more molecular pathways and genetic causes involved in cardiovascular disease.

Gene editing advances so fast that next-generation technologies are already surpassing CRISPR-Cas9 with a more accurate, second-generation technology called Base Editing. This new technology repairs the genetic defect by replacing only one base pair, thus correcting the mutation at one point. Also, it uses other tools that have recently been created in laboratories to insert

1. Former President of the Argentinian College of Interventional Cardiologists.

✉ Corresponding author: *David Vetcher. davidvetcher@gmail.com*

No conflicts of interest whatsoever.

Received: 05/03/2021 | Accepted: 25/01/2022

and delete longer sequences, complete sentences instead of just letters, to the point of cutting and pasting whole genes.[2]

TERAPUTIC GENE EDITING WITH CRISPR-Cas9

Genome therapies seek to correct genetic errors or defects

The FDA has recently given its approval for clinical studies to be conducted with the objective of developing new therapies to approach cardiovascular disease in the 21st century using CRISPR-Cas9 Gene Editing to treat the leading cause of death worldwide.

Gene editing is a perfect tool for to control and cure cardio-specific genetic causes associated with cardiovascular disease. With the use of these technologies, it is possible to cure diseases that would otherwise require chronic treatment or have no treatment whatsoever (orphan diseases) accessible to pharmacological agents.

The therapeutic use of genes and base editors to treat genetic disorders corrects the cause of the disease directly instead of treating its symptom. It uses a gene editor to introduce a specific mutation to knockout gene activity thereby achieving loss of function. Altering genes associated with several cardiovascular diseases stimulates the generation, development, and accessibility of new safe drugs.

Researchers started proposing ways of correcting genetic disorders 50 years ago in the 1970s. Experiments in Gene Therapy started back the 1990s. The most important landmarks in that line of research were the FDA approval from 2017 of the first treatment for a genetic disorder that causes blindness.

The second approval was for the treatment of muscular atrophy disease (Zolgensme [Novartis]). First-generation gene therapy was Gene Transference Therapy and Gene Editing and, currently, new RNA-based drugs.

The therapeutic action of these drugs would not be that of a vaccine since the immune system is not involved here. However, the concept is similar, one single preventive therapy to provide lasting protection and possibly for life against cardiovascular diseases.

Gene inactivation has the capacity to reach permanent therapeutic benefits without the need for continuous treatment that requires the repeated administration of drugs. For example, with CRISPR there is this possibility of a single therapy by inactivating the PCSK9 gene permanently and, thus, reducing LDL-c for life.

The long-term objective is to be able to use CRISPR-Cas9 in patients and protect them from cardiovascular disease during their entire lives. The key to protecting the overall population could be in our own bodies. CRISPR-Cas9 has a lot to offer. It is possible to think of the benefits associated with the correction of genes associated with the risk of cardiovascular disease.

Using CRISPR-Cas9 to place those same mutations in normal people and protect them against cardiovascular disease. We are at the beginnings of this knowledge. Gene therapy offers new hope for people with genetic diseases. However, there is still a long way to go to reach its full therapeutic potential and make gene editing as safe and effective as possible.

PCSK9 THERAPEUTIC GENE EDITING

The relation between coronary artery disease and LDL-c has been demonstrated by a wide body of scientific information.[3] Research groups fed a group of mice with cholesterol-rich diets and another group with low cholesterol diets. The liver samples of these mice data were analyzed to see what genes seemed to be most affected by the addition of cholesterol to their diet. These genes coded proteins involved in the production of cholesterol.

When the liver detects that not enough cholesterol is entering the body through the diet, it has the capacity to take over, producing cholesterol molecules. And the other way around, if there is enough cholesterol in the body, the liver reduces its production.

These studies revealed that there was a gene that had never before been studied.

It was speculated that this gene could code an unknown protein involved in the production of cholesterol. They were on the look-out. They tracked down the protein that would be called convertase subtilisin/kexin type 9 or PCSK9.[4,5]

PCSK9 was discovered and identified in 2003 thanks to the research conducted with families with familial hypercholesterolemia (FH). They used a virus to take the gene to the liver of mice, which had the effect of increasing the production of the PCSK9 protein. They observed that the LDL cholesterol levels of mice skyrocketed as a response to the elevated protein level.

The more PCSK9 the more LDL cholesterol, which is equivalent to a higher risk of cardiovascular disease. Interest for developing therapies using the new gene editing technology and silencing the disease-causing genes grew.

Dr. Jonathan Cohen, and Dr. Helen Hobbs from the Southwestern Medical Center at the University of Texas published a historic study on PCSK9 in the *New England Journal of Medicine* with the finding that an increased PCSK9 activity caused familial hypercholesterolemia. Reference.[6,7]

They mapped a gene responsible in a particular region of chromosome 1 that had mutations in PCSK9 gene. This established that the patient's mutations that were causing familial hypercholesterolemia were making the protein work better than normal, that is, it was hyperactive. Mutations that make a protein be hyperactive and work better than normal are quite unusual. However, mutations that inactivate a protein are much more common.

Given that hyperactive mutations increased LDL cholesterol, they were expecting to find inactivating PCSK9 mutations in the population that would presumably reduce LDL cholesterol.

They sequenced PCSK9 gene in the 128 people with low LDL cholesterol in whom they identified two mutations without PCSK9 sense. They found 3% carried a copy of one of the mutations. This 3% not only had a 30% reduction in the LDL cholesterol levels, but also a 90% lower risk of coronary artery disease. Their PCSK9 mutations had given them a natural protection.

Several publications establish that CRISPR-Cas9 could work really well. Ding et al. obtained PCSK9 loss of function through the CRIPR-Cas9 system in humanized mouse livers to the point of reducing the LDL cholesterol levels from 35% down to 40%.[8]

Getting to knockout gene activity with CRISPR-Cas9 and reduce cholesterol levels obtaining the effect equivalent to

people who were born with a PCSK9 genetic mutation (in only one of the two copies) and who have very low levels of cholesterol that reduce the risk of CAD in nearly 90%.

If these results are translated into human beings, it would be similar to taking a statin pill every day for the rest of their lives to reduce cholesterol levels. In this case, it would possible to obtain the same therapeutic effect as statins with only one injection of the editing PCSK9 gene.

The PCSK9 protein in the bloodstream could be reduced to a large extent by silencing its genetic source in the liver with RNA interference; this should result in less LDL cholesterol and a lower risk of cardiovascular disease as seen with the PCSK9 mutations that occur naturally.

These people who lacked PCSK9 were healthy, suggestive that a drug targeted at silencing PCSK9 would be safe. This way they were seeking to achieve a similar effect by editing PCSK9 with CRISPR -Cas9 to benefit patients.

The strategy was simple: administer CRISPR-Cas9 to the liver, inactivate the PCSK9 gene permanently, see how the amount of PCSK9 proteins in the bloodstream decreases, how the levels of LDL cholesterol drop in blood (from 35% down to 40 %), and achieve reductions of approximately 90% in the risk of developing coronary artery disease. All that is required is to knockout the activity of the gene.

Mechanism of action

The PCSK9 gene is in chromosome 1 p32.3 and it is mainly expressed in the liver and the small intestine, which play a key role in cholesterol synthesis and regulation. Circulating PCSK9 binds to the LDL receptor (LDL-r) in the cell membranes of hepatocytes and reduces the number of LDL-r receptors of hepatic surface thus increasing the LDL-c plasma levels.

The increased production of PCSK9 degrades LDL receptors; this is associated with a decrease in receptor activity, which eventually results in elevated blood LDL-c levels.

On the contrary, it was demonstrated that lower productions of PCSK9 due to mutations with function loss are associated with lower LDL-c levels and a lower cardiovascular risk. Different groups of investigators started studying this specific gene. They started studying patients with extremely high LDL cholesterol levels that put people at risk of cardiovascular disease, particularly coronary artery disease that eventually causes heart attacks. These patients had high levels that were so high that they had been suffering heart attacks since their childhood. The condition seemed to be hereditary. Thus, they were eventually diagnosed with the disease known as familial hypercholesterolemia.

Familial hypercholesterolemia (FH) is a hereditary lipid metabolism disorder that starts with high LDL-c levels. FH is the most common monogenic disease in humans. In Argentina the study conducted by Corral et al. revealed a prevalence of heterozygous FH of 1:291 in the General Pueyrredon county by implementing clinical criteria for diagnostic purposes.[9]

FH is linked to gene mutation in LDL receptor. This receptor is the main responsible factor for LDL lipoprotein uptake. Such mutations will reduce the receptor's capacity to enter LDL from blood and into the cells reaching increased LDL-c levels from very early stages of development. Exposure to elevated LDL-c levels during their whole lives makes these patients develop coronary artery disease early.

FH carriers under 40 years showed a 100 times higher risk of having a cardiovascular event compared to the overall general population. Due to its high risk and chances of reducing it with the appropriate use of hypolipidemic drugs this condition has been the center of attention of several studies on iPCSK9, and it is considered that these drugs will be a great tool in the therapeutic armamentarium for the management of FH.

The pharmaceutical industry soon became interested and launched a development program around PCSK9. After a decade's work, the resulting drug, *inclisiran*, proved to have a very effective and safe profile reducing LDL cholesterol levels in clinical trials in patients with cardiovascular diseases. The drug seems to be on in the pipeline to being approved by the U.S. Food and Drug Administration.

Several companies decided to inhibit thee PCSK9 protein directly in the bloodstream, thus creating a new therapeutic option, the PCSK9 inhibitors. They created synthetic antibodies that capture PCSK9 and neutralize it following the model of natural human antibody that fight infections. Two of these monoclonal antibodies *Alirocumab* and *Evolocumab*, obtained FDA approval back in 2015.

In the coming years, large clinical trials showed that both drugs reduce risk of cardiac issues in high-risk patients. PCSK9 inhibitors are a new hypolipidemic group that plays a role reducing LDL-c and cardiovascular events. However, the cost-effectiveness analysis of these drugs is still controversial.[10] They have not become so popular as anticipated due to their high cost and administration regimen (drug needs to be injected subcutaneously, every few weeks, for the rest of the patient's life. An unappealing proposal compared to the daily intake of a pill as it occurs with statins, the typical way of reducing cholesterol levels.)

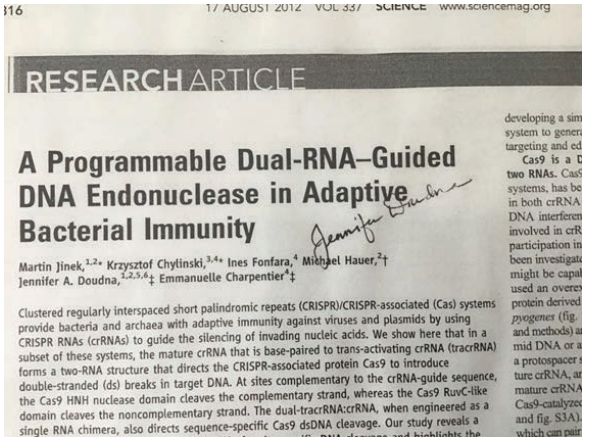
It is necessary to define what population of patients will benefit the most from PCSK9 inhibitors before adding a new drug to their treatment regime. The LDL-c threshold is a determining factor regarding drug indication, sparing this pharmacological resort for the group of highest-risk patients with the highest LDL-c levels. Its safety and efficacy profile has been demonstrated in clinical trials that support their use although long-term evidence is still scarce. Despite these problems, PCSK9 is widely considered a successful story in genetics: only twelve years passed between the discovery of the gene and the approval of drugs for patient use.

GEN *ANGPTL3* INACTIVATING MUTATIONS

There is another gene called *ANGPT3* located in chromosome 1. Protein coding occurs in the liver and is secreted into the bloodstream where it knocks out an enzyme that metabolizes triglycerides. When this gene is the carrier of inactivating mutations that silence protein activity, it explains the low levels of triglycerides and LDL and HDL cholesterol.

The members of a family with this beneficial hereditary condition—a hypobetalipoproteinemia with very low levels of LDL cholesterol, triglycerides, and HDL cholesterol—did not have a functional ANGPTL 3 protein due to their natural mutations (that geneticists call knockouts). This had a protective effect against coronary artery disease.

They had perfect health, no harmful effects of any kind because they did not have a functioning *ANGPTL3* gene. Unlike the PCSK9 in which mutations occur in 3% of the population, the *ANGPTL3* mutations are rarer. They reduce risk of coronary artery disease by one third. Also, there is



816 17 AUGUST 2012 VOL 337 SCIENCE www.sciencemag.org

RESEARCH ARTICLE

A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

Martin Jinek,[1,2]* Krzysztof Chylinski,[3,4]* Ines Fonfara,[4] Michael Hauer,[2]† Jennifer A. Doudna,[1,2,5,6]‡ Emmanuelle Charpentier[4]‡

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. We show here that in a subset of these systems, the mature crRNA that is base-paired to trans-activating crRNA (tracrRNA) forms a two-RNA structure that directs the CRISPR-associated protein Cas9 to introduce double-stranded (ds) breaks in target DNA. At sites complementary to the crRNA-guide sequence, the Cas9 HNH nuclease domain cleaves the complementary strand, whereas the Cas9 RuvC-like domain cleaves the noncomplementary strand. The dual-tracrRNA:crRNA, when engineered as a single RNA chimera, also directs sequence-specific Cas9 dsDNA cleavage. Our study reveals a

developing a sim system to gener targeting and ed Cas9 is a D two RNAs. Cas9 systems, has be in both crRNA DNA interferen involved in crR participation in been investigat might be capal used an overex protein derived pyogenes (fig. and methods) a mid DNA or a a protospacer ture crRNA, a mature crRNA Cas9-catalyze and fig. S3A). which can pair

Figure 1. *Jinek M, Chylinski k, Fonfara I, Hauer M, Douda JA, Charpentier E. A programable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 2012;337(6096):816-21.*

evidence that these mutations minimize the risk of type 2 diabetes. A quadruple impact result: less cholesterol, lower triglyceride levels, less cardiovascular disease, and less risk of diabetes. Several companies have been actively developing therapies with ANGPTL3 to recreate these natural mutations, and to this date, their drugs have worked well in clinical trials reducing LDL cholesterol and triglyceride levels.[11]

HIV GENE

Yet another gene—HIV CCR5—is in the pharmacological target through inactivating mutation (deletion of both copies of the CCR5 gene). HIV binds to the surface protein CD4 of lymphocyte T to enter the cell. However, it also needs an additional factor to infect them, a surface protein called CCR5. A new way of fighting the virus is that, since CCR5 is completely gone from the surface of T cells, it brings high resistance against infections caused by the immunodeficiency virus.

CHAGAS DISEASE

It is possible to stop Chagas disease by genetically altering parasites in the laboratory like the trypanosome that causes the disease. Sequencing and gene studying *Trypanosome cruzi* is a way to eradicate the parasite and prevent trypanosomiasis through a new gene therapy. Chagas is a neglected diseases that constitutes a complete challenge for genetic research.[12]

There is this hypothesis of a crossed reactivity process among the parasite-dependent factors and the genetics of the host that triggers the disease. HLA gene studies indicate the existence of susceptibility to the infection and/or development of chagasic cardiomyopathy associated with patients' genetic components that create new opportunities to treat Chagas disease.

CARDIAC AMYLOIDOSIS

The genetic variation or hereditary transthyretin amyloidosis (hATTR) is caused by a protein called transthyretin. The offensive protein is produced by the liver only from a gene called TTR located in human chromosome 18 that is exported into the bloodstream. It is a rare, genetic disease caused by a mutation in the gene that codes transthyretin that prevents it from folding correctly and precipitates in the form of amyloid fibers.[13]

In some patients, transthyretin makes up lumps called amyloid while in blood to eventually be deposited in organs like the heart and peripheral nerves. As lumps build up in the organs, they can create potentially life-threatening issues. The heart becomes thickened by these amyloid lumps, the only recourse is heart transplantation. Other than that, there is no other way to treat cardiac amyloidosis. All that's left is treating its heart failure.

Transthyretin does not seem to play any essential role in the body. Therefore, if the TTR gene is silenced by RNA interference, there should not be any serious side effects. If the production of transthyretin is cut from the source, there is no transthyretin in blood meaning that no amyloid lumps will be found. Over time, lumps that are already in the heart can be eliminated. Even if that was to happen, treatment could stop or even revert the progression of the cardiac signs associated with hATTR amyloidosis thus preventing disease worsening. Pharmaceutical industry decided to develop drugs. After the data of the APOLLO clinical trial demonstrated that Patisiran would have the capacity to reduce the production of transthyretin and improve the patients' quality of life. It was approved by the FDA. It was the first RNA interference (RNAi) drug.[14]

HYPERTROPHIC CARDIOMYOPATHY

It is a hereditary disease that has a specific mutation in the MYBPC3 gene that causes the reference disease.

The team of reproductive biologist Shoukhrat Mitalipov from the Oregon Health & Science University in Portland, United States recruited a patient with severe hereditary hypertrophic cardiomyopathy injecting him with CRISPR-Cas9 and a synthetic DNA with the normal MYBPC3 sequence in the zygotes. In some of the resulting embryos, the patient's mutating MYBPC3 gene had corrected.[15]

In theory, genetically modified embryos could have been implanted in a mother's uterus, which, upon being born, would be free from the father's disease. However, embryos were destroyed within 2 weeks after fertilization, after the study, to guarantee that everything was ethical. Mitalipov thought that it would take 5 to 10 years before genetic editing of embryos would be ready for use to prevent diseases in babies.

By 2017, there were no scientific barriers to take genetically modified embryos from pregnancy to birth. Human embryo editing was already feasible (many researchers had already tried). The only thing that stopped the first genetically modified baby from being born was that no researcher was willing to cross that line. The case appeared on YouTube: *Dr. He Jiankui that his team have produced the first genetically modified babies. Twin girls Lulu and Nana, the first citizens of the CRISPR generation.* No one knew whether it was a historic scientific achievement or a fiasco. It turned out to be a hoax.

The main concern was that the capacity to modify the babies' genes would be within our reach. The objective was to make sure that it was not done prematurely and, if it were to be done someday, it should be done in a transparent, ethical, and safe way. An exciting direction is the use of CRISPR-Cas9-based technology in additional, non-cardiologic applications that we will report on herein as additional information.

Scientists are looking for genetic cures for diseases such as cystic fibrosis, Duchenne muscular dystrophy, sickle-cell anemia, and HIV by lowering the levels of infection or making them undetectable. Also, Alzheimer's and Parkinson's disease. Also, they're administering more effective inhibiting drugs against cancer with approval to conduct clinical trials whose objectives are cancer-involved genes like sotorasib, manufactured by Amgen. This drug deactivates the genetic mutation KRAS G12C that codes a protein responsible for lung, colon-rectal, and pancreatic cancer, type 1 hereditary tyrosinemia, human reproduction, and in the development of Covid-19 or ARDS diagnostic kits based on the CRISPR-Cas9 system.

Also using base editing researchers (Dr. Francis Collins, director of the U.S. National Institutes of Health, and Prof. David Liu from Harvard University) have successfully cured progeria in mice. This rare but devastating genetic syndrome is caused by mutation in a gene that codes a protein called Lamin A that plays a structural role in cell nucleus and causes fast premature aging in children.

To conclude, George Church, a remarkable Harvard geneticist had already anticipated: remember the word CRISPR. It allows us to change our relationship with nature. CRISPR can be really used in humans to change DNA. It actually allows us to change human evolution if we ever wanted to. It will make it possible to take human genome engineering to an unprecedented scale. The revolution has begun.

SUPPLEMENTARY DATA

Gene editing

It consists of double-strand breaks at the DNA target site. Tool used is CRISP-Cas9. Editing refers to a process through which a text is modified to eliminate errors, insert new fragments, erase words or paragraphs to achieve the desired writing. Editing is the act through which the writing or a text is modified. The same concept that we apply here can be used with the genetic code. The double DNA helix is broken, and a physical tear is created. However, the cell has the machinery to repair it and preserve the thread of life. It would be like a word processor for your DNA. You can think of it as a cursor you used in Microsoft Word. Wherever you make the cut on the DNA that is the equivalent of a cursor in the genome word processor. Right there you can write a new word.

CRISPR

It stands for Clustered Regularly Interspaced Short Palindromic Repeats. Term was first coined by Spaniard Francisco Mojica.[16] The first description of CRISPR was a new family of repetitive prokaryote sequences. In nature, the CRISPR-Cas9 system is used to cut viruses in bacterial and archea genomes. It is an immune system that provides bacteria with immunity against viruses, thus protecting the former from the latter. It is an adaptative immune response that makes them resistant to viruses. A record of past viral infections is stored in the bacterial genomic DNA. That information recorded is used to fight repeated infections by the same viruses.

When there is a new invader, bacteria can store part of the invading DNA in their own DNA (in the spacer, between repetitions). When the invader returns, bacteria make a copy of that spacer handing it over to protein Cas9. The latter looks for a match with the base sequence (of the virus) according to the instructions contained in the guide RNA molecule. Then, it makes a cut at that same spot. Nature invented CRISPR. How old is CRISPR? It is millions of years old. Cas proteins already participated capturing the sequences of invading viruses.

At the laboratory it has been used since 2013 in different ways: it can remove unwanted genes or insert new DNA at the location of choice, introduce specific changes in specific positions, correct errors in the genes responsible for causing diseases. A study conducted by Argentinean researcher Marraffini demonstrated that CRISPR-Cas9 targets a specific sequence of the DNA molecule. It was the first time ever researchers saw the potential application of this technology.[17]

Cas9 is a protein specialized in cutting DNA (molecular scissors). It can break strands in the nucleic acid chain. It is directed to a precise location by guide RNA. It looks for the specific sequence and then moves on to cut it. It carries the copy, looks for a match, and then cuts.

Guide RNA

It is a short, synthetic, personalized, programmable RNA, designed in vitro that is built with a specific base sequence of the desired target. Afterwards, it is released into the cells through various mechanisms. Its purpose is to direct the Cas-9 enzyme towards its target (GPS effect) into the DNA and then indicate the direction of the locus of interest where the cut should be made. It can be programmed, and easily exchanged for different sequences. Then, it is used to find and cut. A part of the sequence (the first 20 base pairs) should match that of the target DNA site. If it is necessary to change direction in the DNA these 20 base pairs are replaced with the wanted sequence. Any DNA can be cut out by just changing that small RNA part. This can be done at the lab in a test tube but also inside the cell.

CRISPR-associated Cas 9

It is a gene editing tool. Right now, it is the easiest method for genetic manipulation. Also, it is versatile, precise, and effective. It works by cutting any target double-strand DNA fragment with a matching target sequence. CRISPR-Cas9 has the capacity to treat cancer, muscular dystrophy, sickle cell anemia, recognize any pieces of DNA inside a cell and organism. It is basically a universal tool. It is often compared to a molecular pair of scissors or razor to cut DNA.

This gene selection system consists of two basic components in its machinery, a non-specific endonuclease enzyme known as Cas9 that interacts associated with CRISPER and a guide RNA. The Cas9 protein and a (guide) RNA are assembled in a unit that scans any double-strand DNA molecules with which it comes into contact. It works the same way as a modern word processor by correcting, inserting words or deleting others to program cell codes. If placed in the nucleus of a human cell, the machine will scan the 46 chromosomes of human genome.

When it meets the target sequence, it takes the first 20 pair bases with which the guide RNA is designed and tests whether the pair base sequence designed in the RNA matches the DNA base pair sequence at that location. If there is a perfect match (or an almost perfect sequence sometimes) the machine will cut out both DNA strands where the change is to be made, like some sort of GPS navigator that locates an address and the site where a change is to be made.

Natural cell repair processes make the cut end meet again. However, the repair process is prone to errors, and often inserts or eliminates a letter from the base pair. It has multiple potential applications to genetically enhance crop seed genomes, insects, and genetic models. Also, in medicine to develop experimental therapies for humans. If you can cut out a gene from a (target) cell and create a rupture at the site of interest, then you can change that gene. It is a useful tool that allows us to change our relationship with nature. And, it allows us to change human evolution if we really wanted to. That is just how profound this is.

Base editing

It only corrects mutations at one point where the toxic version is different in a single letter only. Disorder is the result of a single incorrect base letter in a gene. It is a new second-generation genetic editing technology based on CRISPR-Cas9—a completely new way of gene editing—a more precise form fresh out of the laboratory in 2016. It makes it possible to use a base editor to correct a single disease-causing mutation provided that the correction involves a change from C to T (cytosine base editor) or from A to G (adenine base editor).

It has been a tremendous breakthrough in the field of precision gene editing. It allows the substitution of a single DNA base pair in the genome for a different base pair making the double-strand break unnecessary, which prevents it from causing unwanted mutations associated with the cut. A total of 30% of all known genetic problems are due to specific mutations

DNA

Deoxyribonucleic acid is the master blueprint for life. The mechanism of heredity that passes genetic information from generation to generation. It is the chemical molecule that carries the genetic instructions in living beings written in 4 letters designated by the first letter of their chemical names, A T G C. It consists of two strands that twist around each other making up a double helix. Every living being has its own unique DNA that determines what that being will be, a plant, an animal, a man or a rat. Specific sequences code specific genes that, in turn, give instructions to assemble specific proteins. However, if a mutation mixes up the letters, the instructions can be confusing and produce a different version of the protein causing a disease.

RNA

Ribonucleic Acid. It often carries genetic information from the DNA to ribosomes, the cellular factories that assemble proteins. It is a chemical cousin of DNA. Like DNA, it has four letters (known as bases) and can create pairs with matching letters in the DNA. Unlike DNA it has a single strand. RAN letters make it possible for Cas9 to find a unique sequence in DNA. Human Genome it is like a book where each chromosome is a different chapter, and every gene is like a sentence. If we compare the genome with a thick encyclopedia, CRISPR would be the tool to find a specific word in that encyclopedia capable of deleting or changing it. Every human cell has a collection of 46 large DNA molecules known as chromosomes included inside the cell nucleus. Genetic information is distributed among the 46 different chromosomes, a total of approximately 6400 million base pairs of the DNA sequence, which together make up the human genome.

These chromosomes are 23 pairs of chromosomes. (Diploid). These chromosomes are numbered from 1 to 22, ordered by decreasing size (chromosome 1 is the largest of them all). The 23rd pair of chromosomes determines whether a person will biologically be a male or a female and comes in 2 versions: a larger X chromosome and a smaller Y chromosome that have different genes. With some exceptions, females have two X chromosomes in every cell while males have one X and one Y chromosome. Chromosomes within each pair are mostly identical (except for X and Y chromosomes). However, occasional, they have imbalances in base pairs of DNA sequence across the strands.

These imbalances—also known as variants—are responsible for making each person unique. One of the chromosomes of each pair is inherited from the person's mother, and the other chromosome from the father. The variants coming from the mother are the ones that make a person look like the mother in all kinds of traits like physical appearance, health, and risk of disease. Similarly, the variants inherited from the father create similarities with the father. This mixture of variants from the mother and the father makes the person a true hybrid of both patents. Due to this chromosome pairing, most genes in the genome are present in two versions, two copies, one from the mother, and one from the father.

Mutation

It is a particular error in a gene that makes its coded protein change an amino acid in one position for another one eventually altering its function.

Genetic Code

Instructions contained in a gene that tell the cell how to make a specific protein.

Human Genome Project

It is an international effort led by the U.S. National Health Institutes. Its objectives were to determine complete base pairs of DNA sequences in the 23 chromosome pairs and identify and map accurately the location of all the genes. Launched back in 1990, it was declared completed in 2003. A total of 20 000 protein-coding genes were identified in the human genome. The cost of the project was $3 billion. Dr. J. Craig Venter founded the company Celera Genomics in 1998 with the intention of sequencing the human genome as a private effort parallel to the Human Genome Project.

The commercial model of this alternative source was to sell access to the patented Celera sequence database to all those researchers willing to pay thousands of U.S. dollars in fees every year. Celera's competition with the U.S. federal government with the intent of making money was controversial. However, since sequence data was so useful, researchers used this system provided by Celera to track data and find exactly what they needed.

Technological Revolutions in Biology

- In the 1980s: 1985 (K. Mullis) Polymerase Chain Reaction, known as PCR.
- In the 1990s: High-performance DNA Sequencing.
- In the 2000s: Gene Editing Technology.

REFERENCES

1. *Vetcher DJ. Impacto y oportunidad de CRISPR-Cas9 en Cardiología. Rev Arg Cardioangiol Interv 2017;8(2):62-6.*
2. *Musunuru K. The CRISPR Generation: The Story of the World's First Gene-Editing Babies. Editorial Book Baby edición 21 octubre 2019 ISBN 978-1-54398-637-2*
3. *Goldstein JL, Brown MS. A century of cholesterol and coronaries: from plaques to genes to statins. Cell USA 2015;161:161-72.*
4. *Abifadel M, Varret M, Rabès JP, et al. Mutations and polymorphisms in the proprotein convertase subtilisin kexin 9 (PCSK9) cause autosomal dominant hypercholesterolemia. Nat Genet 34 (2003), pp 154-156.*
5. *Abifadel M. Identification and characterization of new gain-of-function mutations in the PCSK9 gene responsible for autosomal dominant hipercolesterolemia. Atherosclerosis 2012:223:394-400.*
6. *Horton J, Cohen J, Hobbs H. Molecular biology of PCSK9: its role in LDL metabolism. Trends Biochem Sci 2007;32(2):71-7 doi: 10.1016/j.tibs.2006.12.008*
7. *Cohen JC, Boerwinkle E, Hobbs H. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N Engl J Med USA 2006;354:1264-72.*
8. *Ding Q, Cowan CA, Musunuru K, et al. Permanent alteration of PCSK9 with in vivo CRISPR-Cas9 genome editing. Circ Res 2014 Aug15;115(5):488-92 doi: 10.1161/CIRCRESAHA.115304351 Epub 2014 jun10*
9. *Corral Pablo, Geller AS, Polisecki EY, et al. Unusual genetic variants associated with hypercholesterolemia in Argentina. Atherosclerosis 2018;277:256-61.*
10. *Recomendaciones para el uso de inhibidores de PCSK9. Documento de posición de la SAC. Rev Arg Cardiol Vol 85 Supl 6 Dic 2017.*
11. *Mutaciones inactivadoras del Gen ANGPTL3 Genetic and Metabolic Determinants of Plasma Levels of ANGPTL8. Oldoni F, Kozlitina J, Hobbs HH, Cohen JC. j. atherosclerosis. August 2021 331 DOI: 10.1016/*
12. *Coura JR, Vinas PA. Chagas Disease: a new worldwide challenge. Nature 2010;465(7301 Supp):S6-S7.*
13. *Merlini G, Bellotti V. Molecular mechanisms of amyloidosis. N Engl J Med 2003;349(6):583-96.*
14. *Alnylam Pharmaceuticals. Clinical trials. Patisiran (hATTR AMYLOIDOSIS). APOLLO- Phase 3 study.*
15. *Ma H, Marti-Gutierrez N, Park SW, et al. Correction of a pathogenic gene mutation in human embryos. Nature 2017;548(7668):413-9. DOI: 10.1038/nature23305 PMID: 28783728.*
16. *Mojica FJ, Diez-Villaseñor C, Soria E, Juez G. Biological significance of a family of regularly spaced repeats in the genomes of Archaea, Bacterial and mitochondria. Mol Microbiol 2000;36:244-6.*
17. *Marraffini LA, Sontheimer EJ CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. Science 2008;322(5909):1843-5.*