Genoma Engineering using CRISPR-Cas9

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ABSTRACT

As humans we have been dreaming with the day when we will be able to change us and make our body better. Now it is not scientifiction idea or movie, it is reality. We as species discovered a new technology called CRISPR-Cas9 that will change everything forever.

CRISPR-Cas9 is a easy and cheap technology/tool that will help all the humankind to create a whole new world of possibilities in the field of Genoma Engineering. We will be able in the future to change our eye color, change our size or even to cure us from a decease like HIV or anything that could be done with Genoma Editing.

The CRISPR technology has already been using to change the DNAs in the cells of mice, monkeys, others organisms as well. Chineses Scientists showed recently that they could use CRISPR technology to change genes in human embryos. Scientist in Philadelphia showed that they could use CRISPR to remove the DNA of an integrated HIV virus from infected human cells. [1]

Author Keywords
CRISPR-Cas9, Genoma Engineering.

1.- INTRODUCTION

Every cell of in our body contains a copy of our genome. Over 20.000 genes. 3 billions letter of DNA. [2]

We have a huge amount of diversity inherently that is what defines us as a species. We are an incredible successful species, numerically certainly speaking, the world has more than 7 billions inhabitants. [3]

Our success as species is because of our great ability to adapt ourself. In 2013 we as species discovered something that is changing the world and will change dramatically our world in the future, the name of this is CRISPR-Cas9.

Using CRISPR-Cas9 we can now edit our DNA in an easy and cheap way never before discovered, but we need to do it wisely. [2]

The CRISPR technology allows scientists to make changes to the DNA and cells that could allow us to cure genetic deceases. [2]

2.-BASIC CONCEPTS

DNA consists of two strands twisted into a double helix and held together by a simple pairing rule. A pair with T and G pair with C. Our genes shapes who we are as individuals and as species. Genes also have profound affects on health and thanks of advances in DNA sequencing researches had identify thousands of genes that affect our risks to diseases.[2]

Changing genes in living cells is not easy, but recently a new method has been developed that promises to dramatically improve our ability to edit the DNA of any species, including humans.[2]

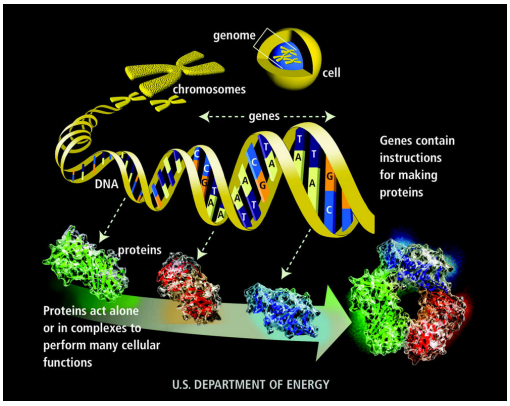

Figure 1 - The genome (inside the cell) contains all of an organism's genetic instructions. Image courtesy of U.S. Department of Energy Genome Programs. [3]

A **genome** is the full set of instructions needed to make every cell, tissue, and organ in your body. Almost every one of your cells contains a complete copy of these instructions, written in the four-letter language of DNA (A, C, T, and G). The human genome contains 3 billion of these "letters" or bases. This means that if your genome were written out on sheets of paper and stacked as books, the tower of tomes would be almost as high as the Washington Monument! [4]

If you think of the human genome as an encyclopedia, the information it contains is divided into 23 volumes, called chromosomes. Each chromosome contains genes - "sentences" of genetic instructions that tell the cell how to make proteins. We know the human genome contains about 20,500 of these genes, but the meaning of much of the remaining text within it is a mystery. [4]

Surprisingly, the human genome is not static. Throughout life, exposure to certain substances - such as X-rays, sunlight, chemicals, and more - can begin to subtly change the genome in some cells. If a cell acquires a set of genomic changes that allows it to grow out of control, invade surrounding tissue, and spread to other sites in the body, cancer develops. A cancer patient is thought to harbor two distinct human genomes - the version contained in normal cells, and an altered one contained in tumor cells. [4]

But ours is not the only genome on the block. All organisms have genomes - not just humans and animals, but also bacteria, fungi, viruses, and other microorganisms that cause diseases. Studying microbial genomes as well as the genomes of their hosts (including humans) can shed light on the nature of infectious diseases. Moreover, analyzing the genomes of our closer relatives - primates, mammals, and vertebrates - and comparing them to our own genome can help researchers determine what parts of the human genome have remained unchanged over time and are therefore likely to be essential. [4]

In 1990, researchers set out to sequence (determine the order of As, Cs, Ts, and Gs in) the human genome. The effort, known as the Human Genome Project, was an international collaboration that concluded in 2003. However, sequencing the human genome was just a first step - now scientists face the challenge of using the tools and knowledge gained from the Human Genome Project to better understand human health and improve disease diagnosis and treatment. [4]

What is CRISPR

CRISPR is an acronym for Clustered Regularly Interspaced Short Palindromic Repeat. This name refers to the unique organization of short, partially palindromic repeated DNA sequences found in the genomes of bacteria and other microorganisms. While seemingly innocuous, CRISPR sequences are a crucial component of the immune systems [7] of these simple life forms. The immune system is responsible for protecting an organism's health and well-being. Just like us, bacterial cells can be invaded by viruses, which are small, infectious agents. If a viral infection threatens a bacterial cell, the CRISPR immune system can thwart the attack by destroying

the genome of the invading virus [8]. The genome of the virus includes genetic material that is necessary for the virus to continue replicating. Thus, by destroying the viral genome, the CRISPR immune system protects bacteria from ongoing viral infection. [6]

3.- STUDY CASES

A.- Change the color of the mouse

Scientists using CRISPR-Cas9 technology already showed that it is possible to change the color of a mouse. [9]

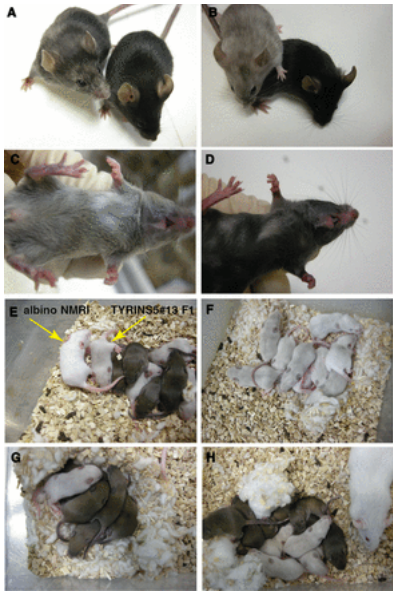

Figure 2 - Alterations in coat color pigmentation in founder animals and their progeny. (A) Coat color alterations of founder mouse TYRINS5#11 (left) with a non-transgenic pigmented littermate. (B) Founder mouse TYRINS5#18 (left) is shown with a non-transgenic littermate: a lighter coat color is observed compared with founder TYRINS5#11, indicating a weaker degree of mosaicism. (C) Founder mouse TYRINS5#35 shows hypopigmented patches in the belly. (D) Founder mouse TYRINS5#60, strongly pigmented and, likely suggesting a higher degree of mosaicism. (E) F1 animals obtained by breeding founder TYRINS5#13 with albino outbred HsdWin:NMRI wild-type mice. A strong reduction of coat color pigmentation is observed in mice carrying the deletion of the Tyr 5′ boundary element. Notably, pigment is not fully absent, as in the case of albino mice (indicated with yellow arrows), but dramatically reduced when compared with pigmented, wild-type animals. (F) All F1 progeny obtained from founder TYRINS5#18 (shown in B) show uniformly reduced pigmentation, indicating that both copies of the Tyr 5′ boundary element were deleted in this founder. (G) F1 mouse derived from founder TYRINS5#19, carrying an inversion of the intervening Tyr 5′ sequence. In this case, the inversion was transmitted to just one individual, showing the same phenotype as those carrying a deletion of the same DNA sequence (panels E, F and H). (H) F1 progeny of the founder TYRINS5#60 (shown in D). The individuals that inherited the deletion of the Tyr 5′ boundary element show a clear and robust reduction in coat color pigmentation. [9]

B.- CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes

Genome editing tools such as the clustered regularly interspaced short palindromic repeat (CRISPR)-associated system (Cas) have been widely used to modify genes in model systems including animal zygotes and human cells, and hold tremendous promise for both basic research and clinical applications. To date, a serious knowledge gap remains in our understanding of DNA repair mechanisms in human early embryos, and in the efficiency and potential off-target effects of using technologies such as CRISPR/Cas9 in human pre-implantation embryos. [5]

Chineses scientists explained that they used tripronuclear (3PN) zygotes to further investigate CRISPR/Cas9-mediated gene editing in human cells. They found that CRISPR/Cas9 could effectively cleave the endogenous β-globin gene (HBB). However, the efficiency of homologous recombination directed repair (HDR) of HBB was low and the edited embryos were mosaic. [5]

C.- First Gene-Edited Dogs Reported in China

Scientists in China say they are the first to use gene editing to produce customized dogs. They created a beagle with double the amount of muscle mass by deleting a gene called myostatin.

The dogs have "more muscles and are expected to have stronger running ability, which is good for hunting, police (military) applications," Liangxue Lai, a researcher with the Key Laboratory of Regenerative Biology at the Guangzhou Institutes of Biomedicine and Health. [14]

Figure 3 - A naturally occurring DNA mutation in the myostatin gene leads to highly muscled whippets, at left, as reported in the journal Neuromuscular Disorders. Scientists in China say they can now engineer the same change into other dogs.

5.- CONCLUSION

Between 1993 and 2005, Francisco Mojica from the University of Alicante, Spain was the first reseacher to characterize what is now called a CRISPR locus, reported in 1993. [12]

He worked on them throughout the 1990s, and in 2000, he recognized that what had been reported as disparate repeat sequences actually shared a common set of features, now known to be hallmarks of CRISPR sequences (he coined the term CRISPR through correspondence with Ruud Jansen, who first used the term in print in 2002). In 2005 he reported that these sequences matched snippets from the genomes of bacteriophage (Mojica et al., 2005). [13] This finding led him to hypothesize, correctly, that CRISPR is an adaptive immune system.

In only few years after the discovery of the CRISPR-Cas9 technology, the world already changed dramatically, a lot of scientific papers were published and a lot of applications of this technology were made. [10]

There are a lot of applications for this technology some we could see with good eyes and others will shock us and some good destroy us.

We can already see in scientific papers, magazines, journals, book, etc that scientist already mapped some color genes [11], did some experiments with humans, mice, dogs, etc and we will still see a lot of discoveries.

Are we ready to "order or buy" a customized dog[14]? Or even, worst, are we prepare to have super babies [15], super soldiers?

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