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Mini Review

Crispr in Assisted Reproduction

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Abstract

The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9) system, a versatile RNA-guided DNA targeting platform, has been revolutionizing our ability to modify, manipulate, and visualize the human genome, which greatly advances both biological research and therapeutics development. Here, we review the current development of CRISPR/Cas9 technologies for gene editing and its application in assisted reproduction. CRISPR can efficiently repair a gene defect in human embryos.

Keywords: *Gene Regulation; CRISPR-Cas9; Embryo Transfer*

Introduction

CRISPR is present in Archaea and bacteria and it was discovered by three principal researchers: Emmanuelle Charpentier, Jennifer Doudna and Francisco Javier Mojica. The most important function of CRISPR is defend the bacterium from virus invasions. This defence system can be transmitted to the offspring. The use of CRISPR as a biotechnological tool has turned out to be one of the most revolutionary discoveries in the field of genetic engineering. In assisted reproduction, CRISPR could be used to modify the genome of embryos and germline eliminating genes or mutations responsible for diseases and thus be able to reverse abnormal embryos that are currently discarded in IVF laboratories. There is a discussion about the correct application of CRISPR within the scientific community. It is necessary to create laws to apply this system in humans without problems.

Evolution of CRISPR

The first discovery of CRISPR is attributed to the researcher Yoshizumi Ishino, who studied in 1987 the iap gene of the alkaline phosphatase isoenzyme in Escherichia Coli. There were 5 homologous sequences with 29 nucleotides that were organized as direct repeats with 32 nucleotides as spacers [1].

In 2005 it is possible to publish the origin of the spacers found between the repetitions. These sequences came from viruses that had previously infected the bacteria [2].

CRISPR / Cas is divided into 2 classes, 5 types and 16 subtypes depending on their mechanism of action and the type of components. CRISPR class 2 and more specifically with the effectors Cas9 and CPf1 are the most used in genetic engineering. Class 2 CRISPR systems consist of three components: target-specific CRISPR-derived RNA (rRNA), target-independent transactivate RNA (RNAtrac) and the effector (Cas9 or Cpf1).

E Charpentier and J Doudna in 2012 studying one of these systems in the test tube showed that it was enough with the Cas9 and the guide RNA to produce a cut in the fragment that we want. They included in the article a very interesting phrase that is "... for the programmable editing of genomes". They intuited that this could be used to edit genomes (Jinek., *et al*. 2012). A few months later Zhang and Church demonstrated for the first time *in vivo*, with mouse and human cells and only using guide RNA and Cas, the genetic material could be edited [3,4].

To explain the function of CRISPR we can differentiate three different stages:

- Acquisition stage: It has the objective of capturing and internalizing the fragments of the invading genetic material (genetic material that we know as proto-spacer when it is part of the genome of the foreign microorganism) to the CRISPR locus.
- Expression stage: In this stage pre-ARNcr is formed, which contains the RNAtracr locus and all the spacers that have been internalized in the first stage separated by the repeats.
- Interference stage: This stage occurs when a new infection of the microorganism occurs. The mature RNAcr is going to bind to the activating RNAtracr sequence, forming a complex necessary to direct the endonuclease up to the sequence of the genetic material that it must degrade [5-7].

CRISPR in Reproduction

Scientists have been able to modify the natural mechanism of action of the CRISPR system for use in genetic engineering. The CRISPR/Cas9 technology allows embryonic genome editing with 3 steps: isolation of zygotes from super ovulated females, transfer of sgRNA and Cas9 mRNA in the zygote and transfer of embryos to pseudo-gestated animals to produce a viable F0 generation.

More than 10,000 hereditary monogenic disorders affecting millions of people in the world have been identified. Some of the examples of gene mutations that manifest late symptoms in adults are BRCA1 and BRCA2, associated with an increased risk of breast and ovarian cancer and MYBPC3 whose mutation is related to hypertrophic cardiomyopathy (HCM). CRISPR/Cas9 is co-injected with spermatozoa in the ovum in M phase during fertilization by ICSI. In this study, the mutations that sperm possessed are eliminated and zygotes are free of disease [8].

Duchenne muscular dystrophy is a genetic disorder produced by the mutation of point C to T in exon 23 of the dystrophy gene which leads to the absence of the complete skeletal muscle protein. This mutation can be corrected by editing the germ line in mice with coinfection of Cas9 mRNA, sgRNA-DMD and an exogenous template of single-stranded oligonucleotides in zygotes [9].

Despite the selection efficiency and the high frequency of HDR, some of the embryos treated with CRISPR/Cas9 have indels induced by NHEJ and therefore are not suitable for carrying out the transfer. The choice of repair of DSBs using NHEJ or HDR may depend on the phase of the cell cycle, with HDR restricted to S and G2 phases when DNA replication is complete and sister chromatids are available as repair templates. The HDR mechanisms were negatively regulated in the early M and G1 phases, favoring the genome edition induced by NHEJ. When the mutations are homozygous in the embryos, the HDR mechanisms based on wild-type alleles cannot be used. It is necessary to look for a new approach for the correction of these mutations [10].

Conclusions

As conclusions we can say that gametes and human embryos employ a different system of response to DNA damage, thus reflecting the evolutionary importance of maintaining the integrity of the germ line. Gametes and zygotes support a greater number of DSBs during meiotic recombination and segregation so that an efficient capacity for genome repair is critical. In the future we can modify the genome of embryos and gametes to eliminate the mutations that produce hereditary diseases.

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