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CRISPR / CAS9 SYSTEM: USE OF GENETIC ENGINEERING IN HEALING CANCER AND OTHER GENETIC DISEASES.

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INTRODUÇÃO

The emergence of a new therapy that uses genetic engineering to do genome editing via DNA cleavage promises to revolutionize future treatments for cancer and other genetic diseases . The CRISPR system is associated with a Cas 9 endonuclease guided from an RNA sequence. More genetic diseases are caused by the loss of global patterns of methylation or hypermethylation of specific loci. So, when we make this association of the Cas 9 endonuclease with the guide RNA and introduce, in vitro, in other cells, this system, CRISPR/Cas9, reaches specific locations in the genome, causing double strand break. After this cleavage, the organism's intrinsic molecular machinery, responsible for correcting errors in the genome, is activated and used to alter mutated DNA sequence, correcting the error. The CRISPR/Cas9 system, even with a short discovery time, has become a very efficient tool, capable of repairing and modifying DNA sequences, but the technique has been suffering from out of target effects, where the CRISPR/Cas9 system ends up accidentally attacking stretches of DNA that are it is not of interest. Its weaknesses and limitations are being studied so that CRISPR therapies can be used in clinical therapy safely and effectively

CRISPR IN CLINICAL APPLICATION

The rapid technological development of genome editing needs attention suitable for improving preclinical and clinical trials to assess toxicity, out-of-the-box effects target and other possible side effects. Several attempts have been made to use CRISPRs to correct mutated genes. One of these studies was performed in a mouse cancer model with the Pten and p53 genes mutated. Mice were transfected with a vector carrying CRISPR designated through a tail vein to reach 20% of the hepatocytes to transform through the blood which also successfully corrected the β-catenin gene mutation frequently involved in cancer with CRISPR. Burkitt's lymphoma is a cancer caused by mutations in the cMyc gene and almost all patients suffering from it have Epstein-Barr virus (EBV) infection. Recently, CRISPR/Cas9 system is used against EBV, generating load reduction viral and tumor proliferation. Furthermore, recent studies demonstrate the successful editing of the Trp53 tumor suppressor gene in Arf - / - Eμ Myc lymphomas. Overexpression of the Myc gene is responsible for several types of lymphoma cancer. Subsequent studies show Mll3 as another important suppressor gene disruption of tumor via ex vivo CRISPRs in acute myeloid leukemia. The successful application of TALENs genome editing tool in a one-year-old girl with leukemia provides the basis for the use of more efficient CRISPRs applications in clinics. In addition to cancers, CRISPRs are also used to correct several genetic diseases. An inherited eye disease called Retinitis pigmentosa, which causes cells to break down photoreceptor cells resulting in gradual loss of vision, was recently successfully edited in iPSC for the RPGR gene, which in a healthy individual is responsible for protein production involved in normal vision, giving new hope to blind patients with retinitis pigmentosa. Another X-linked recessive disease, Duchenne muscular dystrophy (DMD), is caused mainly by a frame-shift mutation in the dystrophin protein, essential for the muscle function and very suitable for genome editing, despite its size is too large, 79 exons. It does not require the entire gene to be expressed and with few changes in the sequence that cause the disease, can bring about improvements in muscle functioning. Thus, an exon skipping technology could be applied with proven success in the mouse model of Duchenne muscular dystrophy. The exon skipping technology with CRISPR/Cas9 opens the door to the treatment of various other diseases such as Ataxia telangiectasia, congenital disorder of glycosylation and Niemann-Pick type C caused by splicing errors. A well-known experiment at SUN-YATSEN University in China on embryos humans to treat the gene that causes thalassemia in human embryos shows that only some embryos in 80 received a corrected form of the gene copy. These examples of successful clinical application with CRISPR define the future technology, but they still need to carry out several clinical and pre-clinical trials to determine the side effects on the patient's health, the immunogenic responses to carriers of vectors and possible genome-wide disadvantages as an off-target result.

CRISPR/CAS9 CHALLENGES IN CLINICAL THERAPY

There are some obstacles that limit the commercial therapeutic application of CRISPR. The gene editing capability of Cas9 is of paramount importance. The breakdown of dsDNA by Cas9 is followed by two natural pathways present in the cell; Non-homologous final union (NHEJ) and homology-directed repair (HDR). NHEJ is naturally the preferred pathway for gene correction in nature, but it is error-prone and causes unwanted mutations, so it is not suitable for the application of CRISPR as therapeutic agents. HDR, on the other hand, is accurate and error-free, but it is not the naturally preferred path for correcting DSBs, so it requires finding more ways to make HDR favored over NHEJ in a natural environment, to efficiently translate the benefits of CRISPRs to clinics. NHEJ is predominant in G1, S, and G2 phases, while HDR in late S and G2 phases. These two pathways were manipulated by researchers for genome editing using CRISPR in mammalian cells for the first time. The editing frequency achieved by the NHEJ ranges from 2% to 25%, which causes high-efficiency deletion of the intermediate sequence. The HDR pathway uses donor DNA as a template to repair DSB in a 'copy and paste' manner using the homologous recombination mechanism. By providing engineered donor DNA, precise modifications, small or large, can be made to the genome. The CRISPR Cas9 genome editing capability using the HDR pathway is not fully developed. As the NHEJ pathway is imperfect, causing InDels to form at the cleaved site, it can lead to a weft shift mutation, resulting in malfunctioning proteins or nonsense mutation mediated transcript decays, thus causing gene disruption. The specificity rate increases with the increase in HDR-mediated repair of DSB induced by CRISPR Cas9. Therefore, using HDR is more favored. However, NHEJ competes with HDR to rectify DSBs. The use of the DNA ligase IV antagonist Scr7 inhibitor (a key enzyme involved in the NHEJ repair pathway) increased genome editing efficiency by up to 19-fold, preventing NHEJ. G2 nuclease cell cycle synchronization has been shown to increase HDR efficiency while reducing unwanted NHEJ events. Likewise, replacement of normal Cas9 with Cas9 nickase (Cas9n) activates HDR with low off target potentials. However, the recombination efficiency is low (1 in 106 - 109 cells), limiting the large-scale applications of HDR in the gene targeting assay. The specificity and precision of the gene editing process following site-specific genomic cleavage by RGENs depends on the nature of the donor DNA. If the foreign DNA has greater than 400 bp homology to the target, it will lead to more efficient introduction of precise nucleotide substitutions or deletions, endogenous gene tagging and targeted transgenes. The specificity of CRISPRs is also in question and many clinical laboratories are concerned about their off-target effects and ways they can minimize these off-targets and develop clinical trials to measure them. Several advances are made in the delivery of nucleases to target cells ex vivo and in vivo, but there is still a need to improve delivery systems to realize the CRISPR therapeutics dream. In addition to specificity, a possible key to success is the isolation of mutant cells (with DNA of interest) from a diverse population of cells. The evolution of the host-pathogen interaction is an ongoing process. The natural development of CRISPRs in bacteria and archaea as adaptive immune responses took hundreds of years to combat invading pathogens. Recently, some study groups have identified anti-CRISPR proteins in viruses that can destroy bacterial CRISPRs with all their memory records of invading viruses and therefore exposing them to the greatest threat of virus attacks. Such findings emphasize the need to develop more accurate and more effective clinical trials for therapeutic CRISPRs

Conclusões

The CRISPR/Cas9 system, even with a short time of discovery, has become a very efficient tool, capable of repairing and modifying DNA sequences. In the field of genetic engineering, the ability to identify a specific location in DNA and modify it has always been a challenge, but this system has made it possible to manipulate genes to create a diversity of mutations in different organisms and cells. It is also worth mentioning its use in the discovery of possible targets for the treatment of pathologies and correction of phenotypes. CRISPR/Cas9 holds great promise for the development of diverse biotechnological therapies, with its easy genetic manipulation capability, efficiency and wide application. However, we must not forget the potential weaknesses or limitations of this system until it becomes accepted by medical practice, and thus, its application for the benefit of society is possible.

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