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ИСПОЛЬЗОВАНИЕ СИСТЕМЫ CRISPR-CAS9 В ГЕННОЙ ТЕРАПИИ

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CRISPR-CAS9 IN GENE THERAPY

Аннотация. Эта статья об использовании инструмента генного редактирования CRISPR-Cas9 для изменения генома человека. Основная цель статьи – собрать и предоставить информацию о применении этой системы для терапии различных заболеваний, а также рассмотреть биоэтическую сторону данного метода.

Ключевые слова: Генное редактирование, генотерапия, редактирование эмбрионов, биоэтика, CRISPR.

Abstract. This article is about gene-editing tool CRISPR-Cas9 for altering the genome of human. The main purpose of the article is to collect and provide information about application of this system for the therapy of different diseases and identify bioethical side of this method.

Key word: Gene-editing**,** gene therapy, editing of human embryos, bioethics**,** CRISPR.

Introduction

The CRISPR/Cas9 gene-editing system is a simple and powerful tool for editing precise spots on the genome. This technology was adapted from the natural defense mechanisms of bacteria and archaea. It allows researchers to easily alter DNA sequences and modify gene function. Its many potential applications include correcting genetic defects, treating and preventing the spread of diseases and improving crops.

CRISPR (decipherment - Clustered Regularly Interspaced Short Palindromic Repeats) is an acronym for DNA loci that contain multiple, short, direct repetitions of base sequences. Each repetition includes a series of base pairs followed by the same or a similar series in reverse and then by thirty or so base pairs known as "spacer DNA".

The Cas9 protein is an enzyme that cuts foreign DNA. By inserting CRISPR DNA near target DNA, scientists can tell Cas9 to cut anywhere in the genome. Then

they can swap a replacement gene sequence in the place of the snipped sequence. The replacement sequence then gets automatically incorporated into the genome by natural DNA repair mechanisms. When any components are transferred into other, more complex, organisms, it allows for the manipulation of genes, or "editing."

CRISPR-Cas9 has become popular in recent years. Scientist Church notes it is easy to use and is about four times more efficient than the previous best genome-editing mechanism called TALENS.

The first reports of using CRISPR-Cas9 to edit human cells in an experimental setting were published by researchers from the laboratories of Church and Feng Zhang of the Broad Institute of the Massachusetts Institute of Technology and Harvard in 2013. Studies using in vitro and animal models of human disease have showed that the technology can be effective in correcting genetic defects. Examples of such diseases include cystic fibrosis, cataracts and Fanconi anemia, in accordance with a 2016 review article published in the journal Nature Biotechnology.

The merits of CRISPR-Cas9

1) CRISPR-Cas9 system is easier to design and simpler to use because targeting a new locus requires only the redesign of a sgRNA rather than synthesis of a new guiding protein as in "zinc finger" nuclease and transcription activator-like effector nuclease.

2) CRISPR-Cas9 is multiplexing in that multiple loci can be targeted simultaneously if multiple sgRNAs are provided. Moreover, wide-type -Cas9 can be reprogrammed into catalytically inactive Cas9 that when fused to transcriptional modifiers such as VP16 can modulate target gene expression.

Challenges of this technology

1) Specificity of CRISPR-Cas9.

One of the major hurdles to the clinical translation of CRISPR-Cas9 is its off-target effects, which may lead to uncontrollable and unpredictable consequences including malignant transformation. Much effort has been made to reduce the off-target effects, such as modifying Cas9 construction, optimizing sgRNA design and the nickase Cas9 strategy.

2) the need of correcting rather than deleting culprit mutations is that the variations in mutational patterns between individuals of the same disease may necessitate specific for patient design of sgRNAs and donor templates. This personalized requirement poses a big challenge to the scale production of CRISPR-Cas9 gene therapy in the future.

3) Immunogenicity of CRISPR-Cas9 and delivery vehicles

Another layer of hurdles to the in vivo therapeutic applications of CRISPR-Cas9 possible host immune responses triggered by Cas9 proteins or delivery vehicles. Cas9 proteins or peptides are also of potentially immunogenicity given their bacterium origin.

Host immune responses may attenuate therapeutic effects and cause side effects, thus should be minimized or circumvented. Developing nonviral vectors such as nanoparticle- and lipid-based vectors may represent a promising way to circumvent the immunogenicity of viral vectors. Humanizing Cas9 protein is a potential strategy to minimize the immunogenicity of Cas9 peptides. Ways to reduce the immunogenicity of CRISPR-Cas9 components and delivery vehicles are interesting areas for future researches.

4) Suitability of edited cells.

Therapeutic genome editing by CRISPR-Cas9 may alter the suitability of edited cells, which in turn can affect the efficacy and duration of gene therapy. In cases where therapeutic genome editing renders a growth advantage, the number of edited cells needed to salvation the disease phenotype is relatively small and therapeutic efficacy is easier to obtain and sustain. In a study in which CRISPR-Cas9 was used to correct disease-causing mutation in a mouse model of hereditary tyrosinemia, 15 for example, only 0.25% of liver cells were initially genetically corrected, but 33 days later, the proportion of genetically corrected cells reached 33.5%, which was sufficient to rescue the disease phenotype.

There are also cases in which therapeutic genome editing renders a growth disadvantage. If we use CRISPR-Cas9 to inactivate oncogenes in cancer cells, for example, the genetically edited cells will be out competed by their unedited

counterparts quickly because the latter retain malignancy and thus possess a growth vantage over the former. As a result, repeated episodes of treatment and pretty high editing efficiencies would be needed to be therapeutic, which is rather challenging and out of the capacity of current CRISPR technologies.

Application of CRISPR in gene therapy

I. CRISPR/Cas9 was used to correct the genetic defect at the origin of Duchenne muscular dystrophy, caused by deletions of dystrophin gene, in a mouse model and in induced potential stem cells (iPSCs) derived from a patient lacking gene using CRISPR-Cas9 More recently, the new gene-editing CRISPR-Cpf1, has successfully corrected Duchenne muscular dystrophy in both a mouse model and DMD cell models derived from patients .

II. CRISPR and Cancer.

As of 2016 CRISPR had been studied in animal models and cancer cell lines, to learn if it can be used to repair or thwart mutated genes that cause cancer.

The first clinical trial involving CRISPR started in 2016. It involved removing immune cells from people with lung cancer, using CRISPR to edit out the gene expressed PD-1, then administrating the altered cells back to the same person. other trials were under way or nearly ready, mostly in China, as of 2017.

In 2016, the United States Food and Drug Administration approved a clinical trial in which CRISPR would be used to alter T cells extracted from people with different kinds of cancer and then administer those engineered T cells back to the same people.

III. A group of scientists in Oregon has successfully modified the genes of embryos using CRISPR, a cut-and-paste gene-editing tool, in order to correct a genetic mutation known to cause a type of heart defect. The experiments were conducted by biologist Shoukhrat Mitalipov and colleagues at Oregon Health & Science University in Portland on dozens of single-celled embryos, which were discarded before they could progress very far in development.

The team used of embryos that were created for in vitro fertilization, using the sperm of men who had a severe genetic defect. The sperm contained a single copy of

the gene MYBPC3, which confers a risk of sudden death and heart failure due to thickening of the heart muscle known as hypertrophic cardiomyopathy.

In the experiment, the team used Crispr/Cas9 to snip DNA at the location of the defective MYBPC3 gene in the fertilized eggs. Most of the embryos naturally repaired the break in the DNA by substituting the normal version of the gene, which originated in the egg. About two-thirds of the embryos did not contain the mutated version of the gene; and the team also eliminated the risk that some, but not all, of the cells in the embryos contained the edited genes.

In vitro gene therapy

In April 2015, Chinese scientists reported results of using CRISPR/Cas9 to alter the DNA of non-viable human embryos. They tried to correct a mutation that causes beta thalassemia, a lethal heritable disorder. The study had previously been rejected by both Nature and Science in part because of ethical concerns.The experiments resulted in successfully changing only some of the intended genes, also CRISPR sometimes snipped out the wrong place in the DNA and had off-target effects on other genes. The researchers stated that CRISPR is not ready for clinical application in reproductive medicine.

In November 2018 Chinese twins became international news as the first gene-editing babies in the world. He Jiankui and his team had recruited couples with husbands with HIV infections kept under control by helping antiviral drugs. The IVF procedure for these couples would use a reliable process “sperm washing” to remove the virus before insemination, so father-to-child transmission was not a concern.

People inherit two copies of *CCR5*, one from each parent. He chose the gene as a target because he knew that about one percent of Northern European populations are born with both copies missing 32 base pairs, resulting in a truncated protein that doesn’t reach the cell surface. These people, known as *CCR5Δ32* homozygotes, appear healthy and are highly resistant to HIV infection.

In the embryos Chinese’s team edited, the researchers did not attempt to delete these exact 32 base pairs; rather, the group designed CRISPR to cut *CCR5* at the base pair at one end of the natural deletion. The error-prone cell-repair mechanism, which

CRISPR depends on to finish knocking out genes, then deleted 15 base pairs in one of Lulu's copies of the gene, but none in the other. With one normal *CCR5*, she is expected to have no protection from HIV. Nana, according to the data He presented in a slide at an international genome-editing summit held in November 2018 in Hong Kong, had bases added to one *CCR5* copy and deleted from the other, which likely would cripple both genes and provide HIV resistance.

The couples' children could also pass the protective mutation to future generations. The core issue is whether such germline editing would cross an ethical red line because it could ultimately alter our species. The prospect of this irrevocable genetic change is why, since the advent of CRISPR as a genome editor 5 years earlier, the editing of human embryos, eggs, or sperm has been hotly debated.

Bioethical issues

Bioethics is the study of the ethical issues emerging from advances in biology and medicine. It is also moral insight as it relates to medical policy and practice. Bioethics are concerned with the ethical questions that appear in the relationships among biotechnology, medicine and medical ethics, law, and other. It includes the study of values relating to primary care and other branches of medicine. Ethics also relates to many other sciences outside the realm of biological sciences.

Many of the same concerns that have been mentioned on this site in regards to genetic engineering apply to gene therapy using CRISPR. Not every trait or disease has a purely genetic basis. Also, if someone has a gene for a particular disease, in many cases, that only means person may get the disease. Pre-emptively removing a gene that has not been fully characterized may lead to unforeseen adverse effects. Occasionally genes have both "good" and "bad" effects. Additionally, sometimes the same gene may be recruited for different purposes. We need to exercise caution when moving into areas in which our knowledge is still incomplete.

The most pressing bioethics issue is that of safety. CRISPR will not be in the clinic for a long time because it sometimes cuts the DNA in the wrong spot. Off-target cutting can be lethal to cells. Much of the current research on CRISPR is finding ways to ensure accurate editing. Even being off by one nucleotide can wreak

havoc on an organism, so until gene editing becomes more accurate, it will continue to be limited to studying model diseases or possibly for stem cell research.

Another ethical issue is raised by Harvard scientist George Church who points out that once gene editing is able to cure diseases, "some scientists will be tempted to use it to engineer embryos while in vitro fertilization. Researchers have already shown that genome editing can rewrite DNA sequences in rat and mouse embryos. With such techniques, a person's genome might be edited before birth – or, if changes were made to the eggs or sperm-producing cells of a prospective parent, even before conception."

This brings up issues of autonomy and human dignity. Gene editing techniques might allow parents to make genetic decisions for their children. Moreover, as society have seen with the eugenics movement, fear of mental illness or other culturally driven preferences may lead some parents to decide to have their embryo's genome edited without well understanding the complex genetic basis, if there is a genetic basis, behind these traits. This delves into even more fundamental questions on the role that genetics plays in determining our traits.

Conclusion

Recent successful results in the gene therapy explorations in model animals has revealed the promise of CRISPR-Cas9 as a gene therapy tool for genetic diseases. For the final application of CRISPR-Cas9 to the clinic, however, there are still many hurdles to overcome, such as off-target effects, efficiency and specificity of delivery vehicles, translatability of in vivo delivery methods, immunogenicity of delivery vehicles and Cas9 peptides, and fitness of edited cells. With the quick advances in CRISPR technology, scientists can optimistically expect these hurdles to be surmount in the foreseeable future to pave the way for the final application of CRISPR-Cas9 to gene therapies of human.

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