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Advances in Gene Silencing Technology

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Abstract. Gene silencing is an important means of regulating gene expression in an organism, and this technique can reduce or block the expression level of a target gene. There are many types of gene silencing techniques, among which the more widely used are CRISPR/Cas9, TALEN, RNAi and so on. In recent years, with the gradual improvement and popularization of biotechnology, gene silencing techniques are slowly becoming known and applied in many fields such as gene research, disease treatment and breeding of new plant varieties. However, not all gene silencing can achieve the desired effect, and there are many reasons affecting the outcome of gene silencing. The target gene is not the only gene that determines the phenotype and there is no significant effect after knocking out the target gene. The technique also has a certain off-target rate, which can also lead to operational failure. This paper describes the principles and applications of three gene silencing techniques and compares the advantages and disadvantages of the three gene silencing techniques in order to select the most suitable gene silencing method from multiple perspectives. The current problems of CRISPR/Cas9 technology are summarized to provide certain ideas for the future development and research of CRISPR/Cas9 technology.

Keywords: genetic silence, CRISPR/Cas9, TALEN, RNAi

1. Introduction

Gene silencing, also known as "gene silencing", is an important form of gene expression regulation in living organisms, where the inhibition of gene expression results in reduced or no gene expression. It was first discovered by R. Peebolte in 1986 during experiments on transgenic plants, and has since been found in eukaryotic organisms such as nematodes, fruit flies, mice and zebrafish. In recent years, gene silencing has become a hot topic of research in various biological fields, and a variety of gene silencing techniques have been applied to the study of gene silencing. Gene silencing mechanisms can be used in plants to regulate gene expression and protect against viruses, while genes can determine the phenotype of individuals and is vital in the development of diseases.

Knockout is one of the techniques commonly used in gene silencing, which is a technique that uses homologous recombination to integrate an exogenous gene into a defined locus on the genome of a target cell, thereby achieving a targeted modification of a gene on a chromosome. This process produces insertions or deletions that may result in code-shifting mutations, thereby eliminating gene expression. Common knockout technologies currently available are CRISPR/Cas9, TALEN, RNAi and others.

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Over time, gene knockdown has slowly shown some problems: knockdown may cause cells to proliferate slowly or stop growing or dying, and further research on these genes is not possible; knockdown is difficult and sometimes the selected gene cannot be accurately knocked down, requiring repeated operations, which not only leads to cell damage, but also results in a waste of resources; sometimes knockdown of a gene does not achieve the desired Sometimes knocking out a gene does not achieve the desired effect, and some phenotypes are not determined by a single gene.

This article provides an overview and comparison of three common knockout technologies (CRISPR/Cas9, TALEN and RNAi), point out the advantages and disadvantages of the three knockout technologies and their specific application pathways, and provide clues on how to choose the right knockout technology.

2. Working principle of gene silencing technology

2.1. CRISPR/Cas9

CRISPR/Cas9 is an auto-adaptive immune system that was first identified in bacteria and archaea. As early as 2009, it was shown that the CRISPR/Cas9 system defends against invading viruses and exogenous DNA by recognizing exogenous RNAs by the Cas9 protein and then interfering or cleaving to prevent their expression [1].

The CRISPR/Cas9 system applied to gene knockdown is based on the principle that single-stranded guide RNA (sgRNA) recognizes the target gene sequence and directs Cas9 nucleic acid endonuclease to efficiently cleave the DNA double-strand, achieving a double-strand break and stimulating the cell to repair it by NHEJ or HR. Usually cells will use the more efficient NHEJ method to repair the DNA double strand, and base mismatches that occur during the repair process, i.e. insertions or deletions of clips, can cause code-shifting mutations that result in target gene loss of function and achieve knockout.

2.2. TALEN

TALEN technology is a novel molecular biology tool, a modified restriction endonuclease. the TALEN system is obtained by modifying the cellular genome through the binding of the DNA of TALE and the cleavage site (Fok I) in an artificially modified restriction endonuclease. the DNA binding domain of TALE consists of a number of repetitive amino acid sequence modules, each of which typically Each module typically contains 33-35 amino acids, with variable amino acid species at positions 12 and 13, and determines the specificity of the module for recognition of the target site [2,3]. The target gene DNA is specifically cleaved by binding of the DNA recognition domain to the target site and by the formation of a dimer by the cleavage domain of FokI. During NHEJ repair, the double-strand break results in the loss of function of the target gene due to random base addition or deletion, thus achieving gene silencing.

The repeated amino acid sequence module of TALE's DNA-binding domain can bind to a single base and can select target DNA sequences for modification, which is a useful tool for genome modification.

As technology advances, other gene silencing techniques have slowly emerged, but TALEN's flexibility and precise targeting cannot be surpassed [4].

2.3. RNAi

RNAi is a more commonly used post-transcriptional level of gene silencing, a universal defence mechanism to stabilize the genome of eukaryotes against invasion of exogenous genes by homologous double-stranded RNA (dsRNA) [3]. After artificial introduction of dsRNA with homology to target genes, dsRNA can be cleaved into several siRNAs by the action of Dicer enzymes, and the antisense genes in siRNAs guide and synthesize RNA induced silencing complexes (RISCs) with target-mediated ability, which cleave target mRNAs. The RISC cleaves a region of the target mRNA molecule that is complementary to the siRNA antisense gene, and ultimately achieves gene silencing through enzymatic degradation. RNAi can be induced by siRNAs ranging from 21 to 23 nucleotides to double-stranded RNAs of several hundred nucleotides, but longer double-stranded RNAs are significantly more effective than shorter double-stranded RNAs in gene silencing [4].

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Because a small amount of double-stranded RNA can block gene expression and this effect can be transmitted to the next generation of cells, the RNAi response process can also be used for gene knockdown. In recent years, more and more gene knockdowns have been performed using RNAi, a simpler and more convenient method [4-6].

3. Advantages and disadvantages

In the following, the advantages and disadvantages of these three gene silencing techniques are compared in terms of ease of experimental design, efficiency, off-target effectiveness, flexibility and application.

3.1. Design simplicity

siRNA is the simplest, followed by CRISPR's sgRNAs, while TALENs is labor intensive. sgRNAs for CRISPR targeting systems are more restrictive, as sgRNAs be designed for DNA near to PAM. TALENs also need to be used in pairs, so the design effort is more complex.

3.2. Efficiency

The efficiency of each system depends on many factors, so it is difficult to directly compare the efficiency of RNAi, CRISPR and TALENs. Inefficient knockdown of CRISPR and TALENs simply means that more clones need to be screened to find monoclonal cell lines where the gene is completely silenced. However, inefficient knockdown of RNAi indicates less gene repression and a less pronounced phenotype.

3.3. Off-target effects

For TALENs, the chances of another site having two opposite target sites are low, requiring both TALENs to bind independently. The off-target effect of CRISPR is reduced by using Cas9 nickelase, which is modified so that Cas9 can only cut one strand of DNA. Double-stranded cleavage requires sgRNAs with two opposing DNA strands. RNAi can cause significant off-target effects. A single siRNA can potentially inhibit hundreds of off-target mRNA transcripts [7].

3.4. Flexibility

CRISPR can be easily used for gene knockdowns, activation, suppression, and base editing. To achieve a gene knock-in, you simply need to provide a repair template. Cells will proceed through homologous directed repair pathways, incorporating new sequences using repair templates. The CRISPR system modifies Cas9 as an enzyme that can't cleave DNA and fuses it to various effector proteins to affect genes. TALENs can also fuse with effector proteins to further expand its versatility. RNAi, on the other hand, can only be used for gene knockout [8].

3.5. Applications

Which gene silencing method is applied depends on the specific project objectives. Studying the function of a gene, knocking it down with CRISPR or TALENs can lead to more significant phenotypic changes than knocking it down with RNAi. However, if knocking out the gene leads to cell death or results in reduced cellular fitness, then it may be more appropriate to use RNAi. Study mutations associated with inherited diseases, and CRISPR or TALENs will be able to introduce them into genes. To do a high-throughput screening project, CRISPR or RNAi systems can be easily scaled up to each target sequence. The size of the TALEN libraries and their repeating elements，the design and cloning of TALEN libraries is more labor intensive and costly to create.

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4. Gene silencing technology application

4.1. Specific applications of CRISPR

CRISPR/Cas9 can be used for the correction of genetic disorders and is effective in the treatment of genetic disorders caused. tabebordbar et al. restored myotonic dystrophy protein expression in a DMD mouse model by adenoviral (AAV) delivery of the CRISPR/Cas9 system, deleting exons containing the original mutation, and the treated mice were able to restore some muscle function defects.

CRISPR/Cas9 has shown promising results in the fields of phytoremediation, functional studies of plant genes, and breeding of novel plant species [9].

4.2. Specific applications of TALEN

Research published in Nature Plants, the research team has developed a technique for targeting genetically stable point mutations into plant mtDNA, calling it TALEN GDM, or TALEN-GDM. This study is beneficial for targeted mitochondrial genome editing, which can generate fertile plants with mtDNA-specific point mutations.

4.3. Specific applications of RNAi

RNAi can be used in genetic research, using RNAi to block the expression of certain genes to investigate whether they have a specific role in the proliferation and differentiation of embryonic stem cells.

RNAi has also made a prominent contribution to medicine. in 2002, Lee et al. showed that the use of siRNA to inhibit some genes of HIV, such as vif, tat and p24, prevented HIV from replicating in cells. in 2003, McCaffrey et al. found that designing siRNAs for HBV genes and transfecting plasmids resulted in the inhibition of HBV in mouse livers. was inhibited.

5. Existing issues and future prospects

5.1. DCas9

DCas9 is obtained by inhibiting the activity of the RuvC and HNH structural domains of the Cas9 nuclease, after which the dCas9 system only has the ability to bind genomic sequences but not to cleave on them. dCas9 is a CRISPR/dCas9 system that generates gene activation or repression when fusing an activating element or a repressor, respectively. As the discovery and application of dCas9 matured, the CRISPR-dCas9 system emerged as a new tool for scientists in molecular biology for the study of activation and repression of gene expression [10].

5.2. Off-target issues in CRISP/Cas9 systems

A major drawback of the CRISPR-Cas9 system is off-targeting, which means that genes similar to the target gene, or genes adjacent to the target gene, can be cut out at the same time, which can cause serious side effects for patients. And some researchers have found that the average off-target rate of potential off-target sites when the CRISPR-Cas9 system acts on human cells is around 40%. Therefore, studying and exploring ways to reduce the off-target rate is one of the more important tasks today.

However, researchers believe that there are several ways to avoid off-targeting in the future. First, the specificity of the bootstrap sgRNA can be improved to increase accuracy; the amount of Cas9-sgRNA can be reduced and controlled to avoid off-targeting; the Cas9 protein can be modified to improve the specificity of the CRISPR-Cas9 system to reduce off-targeting; and the "off switch" of the Cas9 protein can be used to avoid CRISPR-Cas9 off-targeting. "to prevent the CRISPR-Cas9 system from accidentally cutting normal DNA or RNA.

If all these approaches can be applied, and if they are proven in practice to significantly reduce the off-target rate or to have zero off-target, then CRISPR-Cas9 gene scissors can be truly applied to the clinical treatment of disease.

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6. Conclusion

Gene silencing techniques have an important role in living organisms and have been widely used in biology-related fields in recent years. CRISPR/Cas9 has a promising future in the biological field. The high efficiency and precision of CRISPR/Cas9 has made it one of the popular gene silencing techniques currently available. With continuous in-depth research, it has been found that gene silencing technology should not only focus on practicality, but also more on safety. At present, gene silencing techniques still have a certain failure rate and safety issues, which may be related to individual differences in experiments and operational proficiency. Individuals' congenital hidden defects, mindset, experimental environment and operational failure can all have an impact on the gene silencing phenomenon. However, the safety of gene silencing technology is currently under-reported and lacks objective evaluation data; operational failures are mostly attributed to issues such as off-target rates and improper manipulation, which should be more thoroughly studied and discussed. In the future, one can address the current technical problems and safety issues by further exploring the field of gene silencing technology; improving gene silencing technology so that it can be fully applied in clinical and production settings.

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