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Applications in gene editing in cell totipotency

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Abstract. Cell totipotency is that in multicellular organisms, each of cells contains all sets of genetic materials. Therefore, cells have the potential of developing into new individuals. According to tissue culture in plants, this has inspired biologists and they started conducting extensively in the field of cell totipotency for a long time. Moreover, scientists have made significant achievements in terms of the field in cell totipotency from embryonic cell division technology, nuclear transfer, induced pluripotent stem cells and so on. However, based on limitations in the development of technology, mechanisms of differentiation and dedifferentiation in animal cells are still unknown. The emergence of CRISPR technology in the twenty-first century has revolutionized an efficient method to research structures and functions in genes. Because of its durability and efficacy in genome editing, the CRISPR/Cas9 system has recently become one of the trendiest issues. What's more, this kind of technology also had a huge breakthrough in many fields, and it has typically been used to genetically modify pluripotent stem cells, which are then differentiated into specific cell types and used for functional study and even clinical transplantation. This review aims to introduce the general applications of CRISPR and expound its potential breakthroughs and mechanisms in animal cell totipotency, which may guide future research efforts in this area.

Keywords: CRISPR, differentiation, animal cell totipotency.

1. Introduction

In organisms that are multicellular, cell totipotency refers to the fact that each cell possesses all sets of genetic material. As a consequence, cells have the capacity to grow into new individuals. According to recent studies, which show that each plant cells have cell totipotency. That means they can develop into a new and intact plant from a single cell or partial tissue regenerations. Utilizing plant cell totipotency to develop plant cell tissue culture technology and virus-free plants. This not only has a positive effect on the benefiting economy. On top of that, it has wide-ranging implications for scientific research and plays a crucial role in preserving biodiversity by safeguarding endangered plant species. Biologists obtain inspirations from plant cell totipotency, which led to a chain of research in animal cell totipotency. This produced embryonic cell division technology, nuclear transfer technology and induced pluripotent stem cell technology. Besides, this also developed regenerative medicine, animal cloning, stem cell culture and various fields. All of these have huge important in research and medical significance [1].

Based on early studies, scientists have already revealed that the nucleus in animal cells maintains totipotency while they are growing. Furthermore, under the role of some factors in the oocyte cytoplasm, they can help animals to restore the ability which is cell totipotency in order to develop into a new

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individual. In subsequent studies indicate that the result in gene selection is differentiation. During this process, some genes will be labeled due to the combination of methylation, acetylation or some miRNA. On the other hand, some substances located in the cytosol such as MPF can delete that mark. As a result, cells have the ability to restore totipotency. In conclusion, highly divided cells can possess totipotent iPS cells by adding some factors because they enable cells to differentiate for totipotency. Animal cell differentiation and dedifferentiation are extremely accurate and precise processes. Because of technological limitations, scientists have little understanding of the mechanisms of cell differentiation and dedifferentiation, nor do they fully comprehend the structures and regulatory processes of genes relevant to cell totipotency. Because of these constraints, the number of trials using reverse cell development is often modest. Consider Shinya Yamanaka's induced IPS cells. The experiment's success rate was only 1/5000. In recent years, conditions for experimenting with reverse cell development have become an increasingly popular topic. The primary focus of this guide is on totipotent genes and processes. However, the sheer volume of gene data implies that the research will most likely take a long time to complete. Therefore, it is important and expected to develop new technology in order to perform the experiment of reversing cell growth [2].

The CRISPR/Cas9 system is a powerful tool for genetic engineering that is composed of a small guide RNA (sgRNA) and a Cas9 protein that functions as an endonuclease. After the specific identification of the sgRNA, Cas9 protein will arrive at a certain location in the genome. Therefore, it can cut a double-stranded DNA (dsDNA), and later produce a double-strand break (DSB). However, there is a key point. DSB performs restoration by nonhomologous DNA end joining (NHEJ) or homology-directed repair (HDR). Moreover, NHEJ which is dominant is these processes has a high possibility of becoming mutant. Based on that situation, CRISPR/Cas9 acts as an efficient way to perform cutting in target genes. This method possesses a variety of advantages, like being easy to control and having a highly efficient characterization. Functions and impacts of targeted genes can be identified by cutting and breaking some of those genes. With the development of CRISPR technology, this led to a serious set of changes. For example, scientists not only can conduct gene excision of double-strand, but also evolved to single-base excision that does not involve breaks. This progress also means that both DNA and RNA can be altered to validate findings. It greatly furthers our understanding of mechanisms related to potentiation / differentiation within certain animal cells, and it holds great promise for future achievements in research on cell function and behavior [3,4].

This review combined contemporary research with findings from animal totipotency and CRISPR gene editing in order to explore the possible roles in genes and mechanisms of animal cell totipotency. What 'more, it offered guidance and instructions for cloning and tissue culture in the future.

2. Hundreds years of cloning-Cell totipotency

2.1. Performance on cell totipotency under natural conditions

The capability of a cell to fertilize other cells that have already been divided in organisms, such as sperm and spores, is known as cell totipotency. Because when animal cells mature into individuals, they possess all of their genetic material. In light of this, animal cells also possess cell totipotency. However, due to multiple constraints, it is challenging for them to demonstrate such a skill. It's important to note that animal cells that exhibit different totipotent behaviors [5].

Lower creatures like polyps and other coelenterates produce in a manner akin to fungi. The only difference is the size. Highly evolved animals, however, have a far reduced capacity to exercise that capacity when compared to lower organisms. The explanation for this is that the level of cell totipotency in animal cells varies. Strictly speaking, only the nucleus has cell totipotency in animal cells because the nucleus contains all genetic materials. The first experiment on the fertilized egg of an African claw frog was conducted in 1964 by the English scientist Gurdon, who demonstrated that the egg had cell totipotency. Even while the nucleus is capable of doing so, it has very little flexibility.

Scientists' attention is once more attracted by the fact that lower animal cells have the capacity to build organs. Because of that result, scientists are excited about finding cell totipotency's ability in animal

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cells, and start researching stem cells, artificial organs and clones. But there are still a lot of secrets that remain undiscovered. As a result, research on cell totipotency is still substantial and instructive.

2.2. Clone

Cloning exhibits cell totipotency at the highest level. Therefore, it's important to mention the word "clone" here. A cell can reproduce by an asexual process called cloning, which allows it to divide repeatedly. A highly divided cell's ability to clone reveals that the totipotency of an animal cell can be determined. This finding has not, however, received official validation.

From the beginning in the year 5000 BC, humans recognized that they could cultivate corn using other plants. On top of that, Gregor Mendel experimented with plant hybridization in the 1866s. This verified a fundamental theorem of heredity. The first known clone was created in 1902 by German scientist Hans Driesch, who was able to induce a monozygotic twin with a natural clone. After that year, Herbert Weber of the US Department of Agriculture created the term "clone." Hans Spemann's experiment was the primary fountain of inspiration for subsequent cloning. American scientists Robert Briggs and Thomas King established themselves based on the results of these experiments. An embryo developed after inserting the frog's zygote into the ovum's nucleus. For the first time, a human embryo could be reproduced using a nuclear transfer technique. It can also be considered a turning point for clones. The experiment was successful 104 times, and 27 out of 35 of the embryos turned into tadpoles.

At this point, the principal of cloning technology using animal nuclear transfer has been established. Following this, more and more animals have been reproduced using this method, such as the macaque. But successful rates of cloning in mammals are still low [6, 7].

Nuclear transfer technology suggests that early embryos have the ability for cell totipotency, although that capacity is considerably lower than the case for the early ones. Additionally, zygotes have a chemical in their cytosol that can alleviate restrictions on cell totipotency. The reasons for the reduction in cell totipotency that occurs as cells develop and the remedies for the problem. Scientists have struggled with these kinds of problems for a long time. Consequently, this article will expand on these questions.

3. CRISPR technology

3.1. The discovery of CRISPR technology

The discovery of CRISPR technology, as with other important scientific discoveries, can be regarded as a by chance. Francisco Mojica discovered CRISPR while studying an archaea in Spanish. After that, he found an intriguing phenomenon: this archaea contained multiple components. There would be no repetition of these 30-base chunks. Furthermore, the spacing was roughly 36 bases within two repeated bases. That's why Mojica saw this as CRISPR, or Clustered Regularly Inter-Spaced Palindromic Repeats.

Subsequent research investigations have revealed that the CRISPR system was actually part of a far more complex immune system seen in prokaryotes. The purpose of this technique was to combat viral infections. Furthermore, there are two components from the natural CRISPR/cas9 system: the cas protein and CRISPR. The cas9 protein cuts off a segment of the virus's DNA when it infects bacteria, and it will subsequently insert this portion into its own CRISPR region. After this procedure, the RNA will be replicated from the DNA and joined with the cas9 protein. Consequently, ribonucleoprotein (RNP) complexes were developed. This complex has the capacity to prevent bacteria from getting infected. Because of this mechanism, the RNP is able to recognize the DNA in the virus when it re-infects bacteria. Moreover, cas9 works to effectively degrade viral DNA [8].

The roles that distinct CRISPR system components participated in were demonstrated by French scientists Jennifer Doudna and Emmanuelle Charpentier. And such components replicate the mechanism by which bacteria aid in cutting off targeted DNA: the CRISPR system. The discovery by Emmanuelle Charpentier and Jennifer Doudna that gene editing be accomplished by altering the CRISPR system in bacteria and cutting off specific points in DNA molecules is significant.

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3.2. Mechanisms and applications of CRISPR-cas9

With the CRISPR-cas9 system, edition of certain DNA sequences by cutting particular regions of the DNA can be achieved. There are two parts that function as distinct characters in this system. One is being used to cut the cas9 protein, while the other is being utilized to guide the RNA. A complex, RNF was created when these two parts were merged. It can pinpoint the location and erase DNA damage. Cas9 must first be found and then it has to connect with PAM, which is a specific genomic location. Guide RNA will then partially unzip the double-stand DNA after this step is complete. The truth is that in order to combine DNA on a specific sequence, guide RNA needs to be altered. If CRISPR identifies a target sequence, cas9 would be rapidly cleaved. Consequently, unzipping of the double-stranded DNA would result in enabling the target gene to be cut [9].

The DNA damage repair mechanism begins to function operating once the gene is cut. The telomeres created by the two ends of DNA are joined together during this process. Because the targeted gene is difficult to fully repair after deletion, the CRISPR-cas9 system is thought of as a tool for gene deletion. Additionally, the targeted gene's original function will be lost. By rendering one or two of the cleaved domains of the cas9 protein inactive, researchers were able to create a novel type of enzyme. And activate enzymes by using CRISPR targeting. Thymidine can be formed via a cytidine-specific mutation if cas9 is allowed to connect with deaminase. Gene editing will result in the transformation of mutant or aberrant illnesses into normal genes. Stop condon introductions is another way to accomplish that. Scientists do a great deal of experiments to increase gene expression. In one experiment, the transcription fusion factor bound to cas9 after the cas9 protein was rendered inactive. Activators that attach to guide RNA can also boost the expression of a gene. To increase expression, the activators bring transcription factors together. On the other hand, the crab-like domain, which is connected to the fusion expression of cas9 on the complex, allows scientists to gather more components if they wish to suppress gene expression. Scientists are able to observe the distribution of a specific DNA molecule in cells by combining the cas9 complex with a fluorescent protein molecule. This aids in visualizing the genome's three-dimensional structure.

Many fields have seen breakthroughs as a result of CRISPR. The uses of CRISPR technology by present researchers, however, are merely the tip of the iceberg. Although CRISPR research is still in its early stages, we think the technology will also revolutionize other industries.

3.3. Application of CRISPR technology in the study of animal cell totipotency

Considering CRISPR technology has moved forward, multiple other fields have made extensive use of this method of gene editing. Scientists have discovered that totipotent stem cells from animals can be used to generate artificial organs, particularly in therapeutic fields. However, from a legal and ethical perspective. Diseases cannot be treated using artificial organs. Stem cell therapy can be used to treat several illnesses, including chronic conditions. For instance, the treatment of Autoimmune Addison's disease (AAD) utilizing Adrenal Cortical Stem Cells. They can encourage the creation of steroids, which is the cause. Meanwhile, the use of CRISPR technology for organ transplantation offers promise for the medical community [10]. In 2022, YangLuhan, a Chinese scientist, performed the first successful transplant of a CRISPR-modified pig's heart into a human body. It indicates that the person was able to survive for 109 days. According to the latest paper, which was published by Nature, a machine survived for more than two years after receiving a transplant from a humanized porcine donor. In comparison to last year's results, lifetime increases significantly. Data demonstrates that during this time, scientists used gene editing 69 times and added 7 different types of human genes to a machine. This is one of the factors that extends the life of a living organism [11]. Numerous animals have had their genes altered up to this point. Additionally, this technology allows for the creation of new individuals. Black Angus cattle are an excellent example. By using these cows as breeding stock, scientists were able to create hornless cows by gene editing. Therefore, cows no longer are subjected to the pain of having their horns clipped.

With the development of technology and the efforts of researchers, many kinds of diseases can be cured, just like I mentioned above. This gives a lot of hope for both patients and scientists to deal with

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disorders. However, the topic of applying CRISPR gene editing to human embryos has been argued for a long time [12]. This is because once scientists are allowed to reproduce these embryos, it will cause an extremely negative impact on the world. There will probably occur a phenomenon where richer people spend their money in order to make their children better than others. This will make the world much less fair than it is now. The use of CRISPR to alter human embryos is prohibited by law and morality. Despite the fact that solely the UK is authorized to undertake gene editing on human embryos. The process is only permitted for a short period of time, though [13].

4. Conclusions

Animal cell totipotency has been studied for many years by researchers. Scientists' efforts result in the change of macros into molecules such as DNA and RNA. However, due to technological constraints, a complete mechanism and theorem system for gene study remains unattainable. This turned into a bottleneck for scientists and researchers. The discovery of CRISPR-cas9 offers hope for the study of gene expression and functions. Furthermore, by modifying genes, scientists can carry out research on the locations and functions of genes through the genome. In the meanwhile, this essay analyzes the basic workings of CRISPR and its uses in several domains, including CRISPR application settings for potentially successful cell totipotency research. We are hoping that these ideas may help with future CRISPR research.

CRISPR has a significant impact on researching cell totipotency. The expressions of cell totipotency can be increased or decreased by altering expressions of activators and inhibitors in cells.This can be beneficial in a variety of academic domains. For instance, growing mature body cells can increase the totipotency of particular genes. since they are cultivable and can become necessary organs. Moreover, cell totipotency aids in the identification of aging-related modifications to the genetic makeup of genes. Scientists can therefore stop this from happening by blocking alterations in molecules that occur with age.

Author contributions

All the authors contributed equally and their names were listed in alphabetical order.

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