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In vivo reprogramming: A new approach for tissue repair in chronic diseases

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Review Article

Abstract

Medical researchers and biologists have long been fascinated by the possibility of changing the identity of cells, a phenomenon known as cellular plasticity. Now, we know that differentiated cells can be experimentally coaxed to become pluripotent (cellular reprogramming). Recent studies have demonstrated that changes in cell identity are not limited to the laboratory, but also the tissue cells in live organisms are subjected to this process, too (endogenous cellular reprograming). Nowadays "reprogramming technology" has created new opportunities in understanding human chronic diseases, drug discovery, and regenerative medicine. This technology have enabled the generation of various specific cell types including cardiomyocytes, pancreatic beta cell, and neurons, from patient's cells such as skin fibroblasts. Reprogramming technology provides a novel cell source for autologous cell transplantation. But, cell transplantation faces several difficult hurdles such as cell production and purification, long-term survival, and functional integration after transplantation. Recently, in vivo reprogramming, which uses endogenous cells for tissue repair, has emerged as a new approach to circumvent cell transplantation. Up till now, in vivo reprogramming has been practiced in the mouse pancreas, heart, brain, and spinal cord with various degrees of success. In this review, we summarize the progress made, therapeutic potentials, and the challenges ahead in this emerging research area.

KEYWORDS: Cellular Reprogramming, Chronic Disease, Guided Tissue Regeneration, Cellular Reprogramming Techniques

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Introduction

The ability to modify and return a cell to pre-differentiation conditions is a new concept in biology and medicine research. This concept was first taken into account in cloning studies conducted by Gurdon et al. on Xenopus laevis[1] and later by Campbell et al. conducted on sheep.[2]

In these studies, unknown factors found in the cytoplasm of the oocyte cell were used to convert somatic cells to near-embryonic ones; the cells formed eventually became viable organisms. In 2006, Takahashi and Yamanaka realized that by applying a combination of several transcription factors to somatic cells, they could be returned to pluripotent state. This cells are structurally and functionally close to the fetal state. These cells were called induced pluripotent stem cells.[3]

Takahashi et al. revealed the molecular basis of cloning, and it became clear that when the nucleus of a cell is inserted into the oocyte cell, it actually puts the nucleus in an environment that contains transcription factors and, more generally, in the same intracellular conditions as the fetal state. In this condition, the genetic material inside the nucleus is

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reprogrammed and the resulting cell can create a complete organism.[4] Using this method, Yamanaka reprogrammed skin fibroblasts into inducible stem cells and provided a solid basis for the production of other body cells.[5]

Of course, it is worth noting that in other studies carried out in the following years, researchers observed that using just one transcription factor, cells evolutionary closely together, such as fibroblasts and muscle cells, as well as B-lymphocytes and macrophages, can be converted to each other.[6,7] But, the importance of the researches by Yamanaka, Takahashi, and their colleagues was to reprogram the cells to a more fundamental state that would allow researchers to produce a wide range of cells.[3-5] In the following years, studies showed it possible that cells such as neurons be produced from skin fibroblasts, directly and without passing through the stemness stage.[8-10] This approach of converting cells is called as "transdifferentiation". This conversion method was examined both in the culture medium and in animal models, and achieved acceptable results.[11-19] Altogether, a new research field named "cellular reprogramming" and "in vivo reprogramming", which is one of the most powerful branches of cell reprogramming, has been founded.

Recently, in vivo reprogramming has attracted the attention of many researchers worldwide.[20] The importance of this method lies in its high potentials for clinical application and medical use. In fact, this method of treatment was actually found in response to this question: "Can tissues or cells inside the body be converted directly to another ones?"

One of the first studies on this area was performed on pancreatic cells. Since these cells were flexible enough in transforming into developmentally close cells.[12] In the next step, the method was also tested on cardiac and neural tissues. These experiments showed that it was possible to transform the cardiac and neuronal cells by applying a cocktail of transcription factors.[13-16] In the present review, we discuss the advantages of the traditional method of cell therapy and in vivo reprogramming method, as well as examples of researches done in the chronic diseases field of study. Finally, we discuss the therapeutic potentials of this method and also the challenges ahead.

Traditional cell therapy techniques

Years ago, when reprogramming technology had not existed, cell therapy approach was carried out at university laboratories or even in the clinic. In these researches and clinical trials, pluripotent stem cells such as embryonic cells,[21] or multipotent ones such as bone marrow cells, were transplanted to patients suffering chronic diseases.[22,23] These studies have been well considered by Kim and de Vellis for neurological diseases,[24] Segers and Lee for heart tissue repair,[25] Fadini et al. for vascular diseases,[26] and Branski et al. for wound healing,[27] in their reviews. Despite all the valuable efforts of outstanding researchers in this field of study, traditional therapies with their limitations have illuminated the need for a new method to overcome these barriers.

In vivo reprogramming as a novel therapeutic approach

So far, most of the works related to the replacement of deaths cell due to diseases have been concentrated on cell transplantation; but there are lots of limitations in cell transplantation. First of all that stem cells transplanted to the site of injury are not viable, and in most cases they die. Second, these cells stimulate the immune response, and are destroyed by the immune system before being able to differentiate to the required at the site of damage.[28-30]

To solve this problem, in vivo reprogramming method can be used; it means that instead of producing stem cells in the culture medium, and then transplanting them

into the area of injury, cells that are found at the site of the injury can be converted to the desired ones to repair the damaged tissue. In this method, using viral vectors, transcription factors could be targeted to special cells in the area and convert them to another type of cell that is needed. Another limitation of traditional cell transplantation method is the complexity of the process of differentiating cells into desired cells. As we know, the niche around the cells of a tissue, contains growth factors and chemical messengers that provide an exclusive environment to minimizes the probability of unwanted cell production in the area.[31] Therefore, we can take advantage of this exclusive condition by performing a process of reprogramming inside the body and tissue. This article focuses on the history of in-vivo reprogramming in chronic diseases and its progress up to now. Since studies in this field have been conducted mostly on chronic diseases including pancreatic, heart and nervous tissue damages, in the present article, we review the articles on in vivo reprogramming with emphasis on in these three tissues.

In vivo reprogramming in pancreatic cells

The first attempts to transform cells in vivo to produce beta cells from other cells in the pancreas was carried out in 2008 by Zhou et al.[12] In their study, the transcription factors that had specifically expressed in beta cells of the pancreas were injected into the pancreas, and the conversion of exocrine cells of this tissue into beta cells were examined. The researchers eventually introduced synchronous injection of three Ngn3, Pdx1, and Mafa transcription factors as the most efficient way to make this conversion. In their research, reprogramming was carried out directly, meaning that the extracellular cells were transformed into beta cells without passing through the embryonic stages (pluri/multipotent stages). These beta cells were active (insulin and growth factors were secreted). Interestingly, by applying the same factors in the culture medium, the cellular transformation observed in vivo, was not observed, probably due to the absence of in vivo agents in culture medium.[12]

Following the success of Zhou et al. project, further studies were done in this field. They showed that cells that do not secret insulin can be reprogrammed into insulin secreting beta cells by using transcriptional factors and cytokines.[32-34] Despite the successful results, effectiveness of this method in mice was relatively low, which, according to a recent study, can be referred to hyperglycemia and its inhibitory effect on reprogramming of exocrine cells.[35]. It has also been shown in several studies that pancreatic cells have an intrinsic ability and flexibility of converting to each other.[36] For example, in a study by Thorel et al.,[37] it was observed that even when diphtheria toxin was continuously injected into mice in toxic doses for beta cells, insulin production continued, and mice survived. By tracking the source of beta cells, they found that these cells derived from alpha cells of the same tissue. A remarkable point in this study was that they used any transcription factor, and concluded the intrinsic flexibility of pancreatic cells as the main reason for this phenomena.[37] The question that now arises is: "Are the cells of other tissues also able to do this, or it is an exclusive feature of pancreatic cells?"

On the way to answer this question, further steps were taken in the development of in-vivo reprogramming. So, the next step was to look at the conversion of cells that were developmentally close to pancreatic cells. The liver cells were selected to answer this question. They tried to convert these cells via the three above-mentioned transcription factors. Surprisingly, the conversion has occurred and some duct structures that expressed beta cell markers were developed in

the liver. This newly produced cells eliminated the symptoms in animal models diabetes.[38] Another study by Ariyachet et al. also found that intestinal cells could be converted to insulin-secreting cells.[39] These results led the researchers to conclude that the evolutionary close tissues had a potential for in-vivo reprogram to each other. All this together encouraged these scientists to continue their path.

The efforts of researchers in this field to date have focused on finding more efficient methods to convert different somatic cells into beta cells. For example, some researchers are focused on finding more efficient cocktail of transcription factors, while others have been working with creative methods to find other ways to reprogram these cells without expression of transcription factors.[40,41] It is worth noting that these researcher's efforts for reprograming mesoderm cells continued, and recently Rezvani et al.[42] and also Song et al.[43] have shown that myofibroblasts can be converted into hepatocytes to treat hepatic fibrosis.

In vivo reprogramming in cardiac tissue

As discussed in the previous section, one of the challenges facing in vivo reprogramming is the conversion of evolutionary distant cells. In contrast to the pancreas, in cardiac tissue, fibroblast cells and cardiomyocytes come from different progenitors, but the source of both of them is the mesodermal embryonic layer; the advantage of in-vivo reprogramming in the cardiac tissue is the presence of abundant sources of cardiac fibroblasts which migrate to the lesion area after heart damage. This feature led the researchers to test this hypothesis whether cardiac fibroblasts could be converted to cardiomyocytes at the site of the damage?

In 2009, Takeuchi and Bruneau designed an experiment to respond to this question. They reprogrammed embryonic mesoderm tissue into cardiac tissue using transcription factors GATA4, Tbx5, and Baf60c.[44] In 2012, Inagawa et al. observed that non-myocyte cells could be converted to induce cardiomyocytes by applying a cocktail of transcription factors including Gata4, Mef2c, and Tbx5 (GMT).[45] Interestingly, when this process was done in vivo, is was more efficient than in vitro, and also the transcriptome of converted cells was very similar to cardiomyocytes. On the other hand, these cells were also active electrophysiologicaly similar to cardiomyocytes.[13,46]

After this study, other studies were also carried out to increase the efficiency of the GMT cocktail.[47] As an example, the addition of Hand2 transcription factor to GMT (GHMT) improved the efficiency of reprogramming, and improved cardiac activity. GHMT also produced various heart cells, including ventricle, atrium, and conductive tissue.[48,49] Then, other studies using the same method on rat's heart were performed with different sets of transcription factors.[50-53] By introducing miRNAs and their widespread use in cell reprogramming, after much effort, in 2015, Jayawardena et al. demonstrated that miRNAs also could play an important role in cardiac cells reprogramming.[54] In vivo reprogramming itself led to angiogenesis and increased blood flow to newly converted cells, but it was not enough. Therefore, the researchers used angiogenesis stimulator, and they were surprised to find that these stimuli significantly increased the efficiency of in-vivo reprogramming.[13] Mathison et al. showed that another factor that influenced angiogenesis, the vascular endothelial growth factor, also had a positive impact on the efficiency of GMT on in-vivo reprogramming.[55]

One of the major problems in heart stroke is fibrosis, which is caused by fibroblast secretions. In studies of cardiac in vivo reprogramming, cardiac fibrosis significantly reduces, which can be due to the release of anti-collagen agents by induced cardiomyocytes, or because of reduced secretions of fibroblast cells that had not completely reprogrammed, or perhaps both of them.

Cardiomyocytes are not the only cardiac cells that are needed, but also conductive cells that are damaged, or their amount and location is abnormal. Kapoor et al. showed that the Tbx18 transcription factor present in the cardiomyocytes cells culture media converted these cells into pulse producing cells. This experiment was done in vivo on guinea pig model of bradycardia, which returned heart rate to the normal state.[56] It should be noted that more knowledge is needed on the expression of genes in different cells of the cardiac tissue to be capable of converting existing intact cell to desired cell in vivo; but with increasing knowledge of this process, in vivo reprogramming is not very far from minds.

In vivo reprogramming in neural tissue

Recently, in vivo tissue reprogramming of neural tissue has attracted the attention of scientific community. The first symposium on in vivo reprogramming was held in 2014 at the annual meeting of the Neuroscience Association in Washington, DC, which showed the attention of researchers in neuroscience to this research and therapeutic approach.[57]

Cardiac tissue has a poor restorative potential, but in some regions such as sub-ventricular zone and dentate gyrus, as well as certain areas of amygdala, there is an intrinsic potential to produce new cells.[58,59] These newly generated cells can repair minor damages to the tissue.[60] This reparative potential can be used to make it easier to convert neural cells with only one or two transcription factors. In the neural tissue, just like the cardiac tissue, there are cells that are some supportive cells, which they collectively called glia. These cells have some features of precursor cells.[61]

Researchers in the field of in-vivo reprogramming whom focused on neural tissue repair have focused on these cells for conversion purposes. The first attempts on in-vivo reprogramming of neural tissue were done to convert the evolutionary close cells. In a study by De la Rossa et al., in vivo reprogramming of cells from one layer of the cerebellum to the another layer cell was carried out by the Fezf2 transcription factor in the mouse embryo.[62] Similar studies have been carried out by other researchers to convert cells of other layers of the fetal brain.[63] It should be noted that the conversion of adult neurons to each other in the early stages of embryonic life is much simpler, and this is more complicated in adults. Another study was carried out to convert astrocytes to primary neurons by Niu et al., whose aim was to transform glial cells to functional neurons in the brain using a transcription factor called Sox2.[15]

Researchers in this field,[64-66] including Dehghan et al.,[64] along with the factors of transcription, have paid attention on growth factors such as brain-derived neurotrophic factor (BDNF), fibroblast growth factor (FGF), and Noggin, as well as small molecules such as valproic acid. The newly generated cells from these studies integrate into brain circuits, and they are electrically active. In a study by Su et al., they converted spinal cord astrocytes to interneurons on the injury site.[19] These interneurons also were capable of integrating into local circuits, and were functionally active. It was also found that only Sox2, and then Ascl1, were sufficient for this conversion.[67,68] Effects of Sox2 on cell reprogramming is not limited to the conversion of glia to the neurons, but can also be useful in pericyte to neuron conversion.[69] MicroRNAs (miRNAs) have also play a major role in the in-vivo reprogramming of glial cells to the neuron.[70] For example, Ghasemi-Kasman et al. showed that using miRNAs, astrocytes could be converted to neuroblasts, and then to neurons.[71]

Recently, many studies have been conducted to reprogram glial cells to the damaged neurons of various diseases such as Alzheimer's disease, multiple sclerosis (MS), etc. These studies suggest that it is possible to replace damaged cells with functional ones

which can integrate to the brain circuits using this approach.[17,64,72] It can be concluded from these studies that using various factors and environmental conditions, as well as body needs, it is possible to produce special types of cells in the body via in vivo reprogramming process.

Therapeutic potentials of in-vivo reprogramming

Many researchers around the world have sought to use in vivo reprogramming strategy for diseases treatments. Efforts of Zhou et al.,[12] Niu et al.,[15] Rezvani et al.,[42] and Song et al.[43] to treat pancreatic and hepatic diseases, by in vivo reprogramming method, are highly appreciated. Besides, efforts of Ma et al.,[47] Song et al.,[48] Ieda et al.,[50] and Li et al.[53] have been focused on treating cardiac diseased with this method. Guo et al. also looked after the capability of this approach in brain injury models. They sought to convert the reactive astrocytes, which accumulated in the region after the onset of the damage or lesion, to the neurons in the adult mice brain.[17] Dehghan et al. also looked for a way to replace the damaged oligodendrocytes with new ones in the animal models of MS.[64] Applied efforts of neuroscience researchers in this area are discussed in detail in Li and Chen review article.[61]

Challenges ahead for in-vivo reprogramming

In-vivo reprogramming is a novel way of treating diseases. This method can replace new cells in the damaged tissue without the need for cell transplantation. Obviously, cell transplantation is complex and relatively invasive. Although the ability of this method to produce new cells in the body has been well documented in the studies described above, but there are many challenges ahead of this method to prove its ability to treat human diseases.

However, human knowledge is growing, and new emerging technologies will be available to solve these problems and challenges. Researchers now reprogram the cells by artificial increasing in the expression of a number of transcription factors, but their works are still blinded, and they have little knowledge. With the advancement of genomics and proteomics, and in general genomics knowledge, it was hoped to achieve more specific, higher-performing transforming factors. Now, we know that by altering the epigenetic state of the cells, we are able to remove possible obstacles of reprogramming.[73]

However, with the development of epigenetic knowledge, it is possible to find some drugs that will be capable of cell reprogramming, without the induction of gene expression. Of course, new technologies of gene editing such as clustered regularly interspaced short palindromic repeats (CRISPRs) are also powerful tools for cell reprogramming. Technologies of single cell manipulation that asses the genetic material and proteins of a cell individually, including single cell gene sequencing and single cell RNA sequencing, can also be effective in completing our knowledge. These new techniques introduce us the general conditions of the cells, so that we can come up with suitable strategies for converting cells. We know that the cells in the tissue are susceptible to conversion, but conditions such as cell death and the conversion to the unwanted types of cells are factors that decrease the efficacy of this method.[74,75] Therefore, we should look for ways to increase the efficiency of cell reprogramming.

According to the above, it is clear that with the advancement of stem cell science and technology we can gain a clearer understanding of these cells and the processes occurring during cell reprogramming, and develop the technology of internal re-programming with a brighter and more open view.

Another major obstacle to the development of in-vivo reprogramming is delivering pathways of compounds and factors. Reprogramming compounds must be delivered

safely, effectively, and optimized. Viral and plasmid vectors are the usual methods, but due to their disadvantages, they are not still ready human treatment applications.[76,77] Although it is possible to deliver the reprogramming factors to the actual locations of damage, but their risk is still very high. On the other hand, transcription factors should be expressed to a large extent, whose effects and consequences are still unclear. Given the limitations of genetic reprogramming, today, chemical reprogramming using small molecule compounds has received attention. In this method, although the need for high expression of transcription factors has been almost eliminated, still some obstacles remained. Delivering the compounds to the site of lesion, and maintaining their therapeutic concentrations over a long period of time are the major problems. It's worth noting, however, that nanotechnology has opened up new door to overcome these problems. Another hope is also injecting high concentrations of mRNAs to the damaged tissue to transiently increase the expression of desired genes.

After all, the safety and effectiveness of this method should be tested in animal models evolutionary close to humans including monkeys. These tests help us to test the quality of this method in human-size animals.

With the advancements of in-vivo programming technology, gradually more efficient methods of reprogramming and targeting will be developed. These methods should all receive the necessary approvals from the relevant organizations, but nevertheless the need for this technology will make the verification processes move faster. Of course, for patients who are disappointed with other therapies, it is possible to use in vivo reprogramming more quickly.

Conclusion

At the end, in short, in vivo reprogramming technology is a regenerative medicine therapeutic method based on developmental biology and cell reprogramming. Recently, many studies have been published on chronic diseases associated with pancreatic, cardiac, and neural tissues of small laboratory animals that have reported promising results. Despite all the positive aspects of this method, there are still challenges facing this new technology, which we hope they would be solved gradually. With the gradual removal of obstacles and challenges, the use of this method for medical purposes is not far from mind. We hope that this technology will progress as quickly as possible, and we will soon see chronic and nonchronic diseases patients treated with this approach.

Conflict of Interests

Authors have no conflict of interests.

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