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Research progress on reprogramming induction of small molecules

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Abstract. In the fields of medicine and biology, tissue and organ transplantation is a key area of research for scientists. In order to meet the increasing demand for tissue and organ transplantation, the artificial induction of various types of tissue and organs has gradually become a research hotspot today. The gradual reprogramming of cells can generate transplanted organs without rejection reactions and enable low-cost testing of drug effects on the human body, which has broad prospects in medicine. At present, a large amount of research has been conducted on inducing organ transplantation through recoding, and a large amount of literature has been accumulated in preclinical studies, including the mechanisms and principles of its occurrence. There have been many attempts in inducing organ development in vitro, but there is no systematic summary and analysis of the relevant content. This article reviews the research progress of reprogramming to generate iPS, from the transfer of Yamanaka factors to the induction of small molecule reprogramming. Then, considering the current difficulty in screening new highly efficient inducible small molecules, this article used a reprogramming related protein.

Keywords: iPS, Cell Reprogramming, Stem Cells

1. Introduction

Cell reprogramming can dedifferentiate mature cells into stem cells. Organs re-induced from stem cells will not produce rejection reactions during transplantation, which has broad prospects in medicine. It was first discovered by Japanese scientist Yamanaka in 2006.(1) Yamanaka noticed that transplanting nuclei from somatic cells into nucleus-less ES could continue to develop as ES,(2) Therefore, it is speculated that factors related to maintaining ES stemness in ES can also induce the stemness recovery of somatic cells. Finally, the minimum four combinations of transcription factor genes required by somatic cells were screened out, which can induce somatic cells to dedifferentiate into stem cells, that is, the genes that introduce the four transcription factors OCT3/4, Sox2, c-Myc and Klf4 will Induces reprogramming of somatic cells into stem cells.

This discovery has great application prospects, but it still faces several problems that need to be solved.The success rate of reprogramming is extremely low,and the method of gene transfer puts cells at risk of becoming cancerous. Therefore, we should consider minimizing the transfer of Yamanaka factors and reducing the impact of gene transfer on the recipient cell genome. We should use other methods to induce reprogramming, reduce the risks caused by gene transfer, and improve the success rate of reprogramming. As a result, reprogramming of cells was induced by small molecule induction to

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replace the transferred genes. Small molecules often induce reprogramming by regulating epigenetic modifications or signaling pathways, because small molecules are mostly regulators of various epigenetic modification-related proteins or ligands (agonists or inhibitors) of protein receptors. For small molecules, Screening also often targets these related proteins. With the development of computer technology in recent years, virtual screening methods for drugs have made great progress, which can greatly improve the efficiency of screening. For example, for Wnt, an important protein receptor in reprogramming, there is still a lack of cheap small molecule agonists.

For this protein, virtual screening methods can be used to find more suitable small molecule agonists to promote reprogramming induction. The same can be used Similar approaches have led to the development of more small molecule ligands targeting protein receptors.

2. Inducible reprogramming through gene transfer

After Yamanaka Shinya, other teams also discovered factor combination schemes and different factor introduction methods that can achieve similar effects.

Table 1. Different induction cocktails

Reprogramming cocktail	Transfer method	Year
OKSM	Retroviral	2006
OSM+Esrrb	Retroviral	2009
KSM+Nr5a1/Nr5a2	Retroviral	2009
KSM+Tet1	lentiviral vector	2013
OS+Nanog+LIN28	entiviral vector	2007
OKSM	Cre recombinase	2009
OKSM	Adenovirus vector	2007

In 2007, researchers found a new factor combination solution for situations where the c-Myc gene would cause ES death or differentiation.(3)Reprogramming can also be induced using OCT4, SOX2,NANOG and LIN28 (it can also be induced in the absence LIN28,). Other researchers later found that pore-related genes can also play a role in reprogramming, such as two studies in 2009, the combination of OCT4, SOX2, c-Myc and Esrr b, and the combination of Klf4, SOX2, c-Myc and Nr5a1 or Nr5a2, respectively, were found to also induce reprogramming. Research in 2013 found that the transfer of the Tet1 gene can also induce reprogramming.(4)This shows that the factors that induce reprogramming are not fixed, and reprogramming should be achieved through some factor-related pathway. Therefore, some researchers have introduced other factors to activate reprogramming-related factors through signaling pathways. These retroviral transfer methods still have the risk of damaging the genome due to genome integration. Therefore, in 2008(3) and 2009(5), two new methods were adopted to remove the influence of external factors. In a 2009 study, researchers tried to use Cre recombinase to remove gene fragments of four factors in iPS and found that the iPS after removal could still maintain stemness and could avoid subsequent canceration caused by these factors to a certain extent. wait. Research in 2008 used adenovirus methods to construct adenovirus plasmids to avoid damage to the genome by foreign factors as much as possible. However, the induction efficiency of adenovirus vector is significantly lower than that of traditional methods. The common problem between these two studies is that they cannot completely avoid the damage to the genome by the transfer factor, or they cannot ensure that the excision is complete, or they cannot ensure that the adenoviral vector does not integrate into the chromosome.

In addition to the method of gene transfer, small molecule induction methods have also been found to be used to regulate gene expression and signaling pathways to achieve induced reprogramming.(6) The study has identified two parameters, the differentiation status and methylation state of the donor

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cell, that strongly influence the efficiency of ES cell derivation following NT. Because nuclear transfer-derived ES cells can be used to dissect mechanisms of disease as well as a potential therapy for disease, it is of practical significance to identify means of improving reprogramming efficiency.

3. Method of Induction by Small Molecules

The use of small molecules to regulate genome modifications at the epigenetic level can also induce cell reprogramming. In Yamanaka's previous research, he found that stemness is related to the methylation level of the genome in experiments on nuclear transfer.

Nuclear transfer of methyltransferase mutants has a higher success rate in restoring stemness. Applying exogenous inhibitors to regulate epigenetic modifications can also partially restore the stemness of cells. Another study in 2006(7) used the method of exogenous molecule regulation, using exogenous drugs to modulate the genome appearance of cells. genetic characteristics. Using TSA, a histone deacetylase (HDAC) inhibitor, to treat cells after nuclear transplantation, it was found that the success rate was greatly improved, and another HDAC inhibitor, Apicidin, also had a similar effect, further indicating that the inhibition of HDAC played a role. This follows Yamanaka's groundbreaking discovery of epigenetic regulation related to SCNT and stemness, and it is known that inhibiting methylation and inhibiting deacetylation are beneficial to the restoration of cell stemness. In 2008, after the results of Shinya Yamanaka were released, researchers combined the method of dsing small molecules to improve the success rate of nuclear transplantation with the use of Yamanaka factors, and tried to treat iPS with HDAC inhibitors to see whether the efficiency could be improved. Therefore, three methods were used. There were three types of HDAC inhibitors, SAHA, TSA and VPA, and finally it was found that the reprogramming efficiency increased the most after VPA treatment. In addition, it was also observed that reprogramming can be achieved without transferring oncogenic c-Myc factors after chemical treatment, and changes in different genes after VPA application were detected. It is speculated that genes specifically highly expressed in ES are up-regulated. Genes characterized by high expression in MEFs were downregulated. This is an early discovery of the use of small molecules to replace certain factors to directly reduce the number of transferred genes, which initially demonstrates the feasibility of chemical reprogramming. This study was only conducted in mouse cells. In view of the conservation of reprogramming factors, this experiment on mice may be successful in human cells in the future. In the future, it is hoped that high-throughput screening of small molecule libraries can achieve pure Chemical reprogramming. The specific regulatory sites and regulatory mechanisms of epigenetic modification regulation were partially explained in a 2009 study,(8) it is the methylation modification on the K4 site of histone H3 that affects the binding of OSK expression products in Yamanaka factors as transcription factors. In addition to inhibiting methylation, a 2009 study found that inhibiting demethylation to a certain extent can also promote reprogramming.(9) This study selected tranylcypromine to inhibit LSD1 (lysine-specific demethylase 1, also known as KDM1A) and inhibit demethylation, thereby maintaining the methylation of some sites and facilitating the binding of factors.

4. Further Screening of Small Molecule Inducers

In addition to direct epigenetic regulation, other pathways of genes related to Yamanaka factors can also help to improve the understanding of reprogramming and develop new methods to induce reprogramming. The 2009 study introduced the method of bioinformatics analysis and provided an enriched network of genes related to Yamanaka factors.(10) In subsequent studies, this group of researchers paid attention to the signal transduction of TGF-β involved in the network, so they chose an inhibitor of the TGF-β receptor Alk5 and found that it could replace c-myc or sox2. Then they tested activators and found that Reprogramming was inhibited, indicating that it was indeed the action of the Alk5 inhibitor that promoted reprogramming and replaced the factor. The mechanism of this pathway was partially revealed in the same year. (11)The tgf-β and BMP pathways have antagonistic effects on nanog. The types of smad proteins of the two are different. Therefore, inhibiting the tgf-β pathway can achieve the function of increasing nanog expression, thereby increasing nanog expression. amount to achieve the purpose of bypassing sox2. This shows that the target of small molecules can be found

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through pathway analysis, making it possible to bypass factors. In 2010, the new TGF-β receptor inhibitor 616452 (repox) was also used for the induction of reprogramming, replacing the sox2 factor by promoting the expression of nanog.

C-myc is a relatively familiar cancer-related gene, so the signaling pathways related to this factor are relatively clear. It is known that the activation and inactivation of c-myc protein can be regulated by phosphorylation and dephosphorylation. The specific mechanism was revealed in a 2012 study.(12) The sequence of c-myc contains a phosphodegron sequence. The phosphorylation of serine at position 62 can enhance the stability of myc, but it can also initiate the second step of myc protein. Hypophosphorylation, the second phosphorylation is catalyzed by GSK-3β. GSK phosphorylates threonine at position 58, giving the F-box protein Fbw7 binding site, which ultimately leads to ubiquitination and de gradation of the myc protein. Therefore, the activity of GSK-3β will reduce the stability of myc protein and promote its degradation. Inhibition of GSK-3β is an important process in reprogramming. Among the many GSK-3β inhibitors, researchers found that CHIR99021 is the most suitable GSK-3β inhibitor.(13)

As a GSK inhibitor, CHIR99021 works intracellularly. However, with the gradual development of iPS induction technology, direct GSK inhibitors also have some shortcomings:

In the latest research, staged pure chemical reprogramming has been achieved. The reprogramming uses the Waddington-OT algorithm to determine the different stages of reprogramming, and cells are induced in different culture media at different stages, inhibitors of the CHIR99021 intracellular receptor may remain in the cell and are difficult to regulate.

2) CHIR99021 can only work in a range of about nanomolar, and there is a possibility of off-target. If the concentration is too high, it will have other effects, and staged culture may be difficult to control its concentration, and it is easy to exceed the range.(13)

We therefore considered whether surface receptor sites on the same pathway could be used to replace CHIR99021 as a new GSK inhibitor. It is known that GSK-3β protein is an important intracellular protein in the Wnt signal transduction pathway. After the Wnt surface receptor Fzd protein accepts the regulation of Wnt signal, dvl is the bridge that mediates Frizzled and subsequent downstream pathways. The traditional view is that dvl is in Frizzled activation by the received Wnt signal near the membrane destroys the DVL complex, thereby inhibiting GSK, new research believes that it is through other methods.(14) In short, the activity of GSK can be inhibited when the Wnt pathway is activated. Therefore, it is considered whether it is possible to activate the membrane receptor of the Wnt pathway. Also inhibits GSK.

Traditional Wnt receptor agonists use recombinant proteins or purified proteins, but the cost of protein ligands is obviously high. If small molecule drugs can be used as FZD activators, the cost can be greatly reduced. However, the development of small molecule ligands for Fzd agonists has always been difficult, and effective small molecule agonists have not been found.(15) A 2018 study analyzed found that the crystal of FZD4 exhibits a ligand-free structure.(16) This may indicate that the core of FZD is a hydrophobic region unlike other receptor proteins, which means that it is almost impossible to find small molecule agonists of FZD. Some researchers also believe that this is related to FZD forming heterodimers to achieve signal transduction. The development of technologies related to virtual screening and molecular dynamics simulation may indicate new hope for the development of Fzd agonists. A 2018 study found that an inhibitory small molecule ligand of FZD4 has the following properties after changing part of its structure to form FZM1.8 through computational structure-related methods. The ability to make FZD allosteric and activate downstream pathways to a certain extent. However, this pathway is biased toward P13K rather than the classic pathway. Perhaps different allosteries of FZD will lead to different pathway activations. This document also shows that it is feasible to use small molecules to allosterize FZD to activate downstream pathways. A 2020 study(17) mentioned that based on the homologous similarities between SMO and FZD, an attempt was made to use SMO's small molecule agonist SAG1.3 to treat FZD It achieves partial activation of the Wnt classical pathway, and SAG1.3 can act as a partial agonist. Therefore, unlike previous studies, this study found the core of FZD and the action site of SAG, indicating that it is possible to design small molecule ligands

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of FZD as agonists. The next question is, there are many downstream pathways of the Wnt pathway, how to find and activate the one we need. In the 2023 study(18), conserved micro-switches in FZD were mentioned, that is, conformational changes in some specific amino acid sites will change the selection of pathways.

5. Conclusion

We can use virtual computing methods to explore the impact of unreasonable interference changes on protein conformation based on the conformational changes of different microswitches in FZD, and design new small molecule drugs. The FAD agonist designed in this way can have higher specificity, and the residual problem will be smaller than CHIR99021 when changing stages of culture. Similar methods can also be used to activate and inhibit other pathways involving protein conformational changes.

In addition, CiPS small molecule inducers also have many small molecule activation or inhibition sites that affect a variety of aging and cancer-related pathways, and of these related pathways, only one or two specific pathways are often related to reprogramming. Choosing to use this method to redesign or improve small molecules can also enhance pathway specificity and try to avoid problems such as cancer.

Since the discovery of Yamanaka factors, more and more inducible reprogramming methods have been discovered, including different factor combinations and small molecule induction. With the development of computer science and technology and structural biology, in the future, we can consider the structural characteristics of receptor proteins and use newly developed virtual screening technology to more efficiently find more and better small molecules to induce cell reorganization. programming.

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