Reprogramming Mechanisms and Application Prospects of Induced Pluripotent Stem Cells

Zijia Liu
Soochow University, Suzhou, Jiangsu, China

Abstracts: The technology of induced pluripotent stem cells (iPSCs) is of great significance.The reprogramming mechanism is to transform somatic cells from epigenetic modification to pluripotent state through transcription factors (such as OKSM), which involves a complex process such as chromatin remodeling, and the methods include transcription factor induction, vector introduction and small molecule compound assistance. However, this technology faces challenges such as low reprogramming efficiency, high safety risks and ethical controversies.In terms of applications, iPSCs can be used to construct disease models and drug screening , helping regenerative medicine, tissue engineering and personalized treatment. However, there are problems such as cellular heterogeneity and immunogenicity. In the future, it is necessary to optimize the reprogramming efficiency and stability, reduce the risk of carcinogenesis, unify the quality control standards, strengthen the ethical regulations, so as to promote the in-depth application of iPSCs technology in various fields and safeguard the health and well-being of human beings.

***Keywords*:** induced pluripotent stem cells, cell reprogramming, regenerative medicine, transcription factors, disease modeling

1. Reprogramming Mechanism of Induced Pluripotent Stem Cells

1.1 Concept and Principle of Induced Pluripotent Stem Cell Reprogramming

Induced pluripotent stem cell reprogramming is the process of "rejuvenating" a cell, that is, cells retrograde along the maturation path without changing the gene sequence, and returns to a more immature state through epigenetic modification, and ultimately transforms into a pluripotent state. Cell reprogramming technology has broken the traditional perception of cell differentiation, making differentiated somatic cells converse into pluripotent cells, such as the creation of induced pluripotent stem cells (iPSCs). The role of transcription factors is at the core of this technology. Among them, the OSKM (OCT3/4, SOX2, KLF4, c-MYC) combination is the most critical, and introducing them into somatic cells can transform them into iPSCs[1]. This process involves complex molecular mechanisms such as chromatin remodeling and altered gene expression patterns. Regarding reprogramming, the stochastic model suggests that all cells have the potential, only the complexity of the steps leads to fewer successful cells; the elite model suggests that only specific cells such as progenitors and stem cells can be reprogrammed into iPSCs, but terminally differentiated cells can be reprogrammed as well amends this model.

1.2 Methods of Inducing Reprogramming of Pluripotent Stem Cells

1.2.1 Transcription Factor Induction

Takahashi and Yamanaka demonstrated in 2006 that the introduction of OCT4, SOX2, KLF4 and c-MYC (OSKM) into mouse fibroblasts resulted in the reprogramming of the cells into iPSCs, and this method is also applicable to human cells. In addition, the combination of OCT3/4, SOX2, NANOG and LIN28, as well as the substitution of c-MYC by GLIS1 and OCT3/4 by NR5A2 can also realize reprogramming[2].

1.2.2 Vector Import Technology

Reprogramming is dependent on vector transporter transcription factors. Although early retroviruses and lentiviruses can efficiently integrate reprogramming genes, stably express transcription factors, and improve reprogramming efficiency, the risk of reactivation of the transgene limits clinical application[3]. Adenoviral vectors are safe but inefficient. Sendai virus replicates outside the nucleus, which is safe and can efficiently induce transgene-free iPSCs.

The efficiency of plasmid transfection in DNA vector is low. If add-on plasmids contain special sequences, they can be amplified inside the cell after transfection. The transposon PiggyBac, which integrates reprogramming factors, is a non-integrating method that removes iPSCs from the genome once they are established. In addition, synthetic RNAs encoding reprogramming factors can induce pluripotency in combination with measures to reduce immune response. The recombinant

protein and cell penetrating peptide are fused into cells, but the technology is difficult.

1.2.3 Small Molecule Compounds Assist

Small molecule compounds promote reprogramming by modulating intracellular signaling pathways and epigenetic modifications. For example, 5-azacytidine and RG108 can enhance the efficiency of DNA methylation modification; histone deacetylase inhibitors such as sodium butyrate, and histone methylation modifiers such as Neplanocin A can regulate histone modification to promote iPSC generation; and small molecules such as 8-bromocyclic adenosine cyclophosphate can activate specific protein kinases to promote the reprogramming process.

1.3 Highlights of the Induced Pluripotent Stem Cell Reprogramming Process

Reprogramming of iPSCs has been a shining light in the field of regenerative medicine.

(1) Transcription factors: Yamanaka and Takahashi demonstrated that OSKM is the core transcription factor, which reprograms cells by activating pluripotent genes, repressing somatic genes, and having various alternative combinations.

(2) Molecular processes and epigenetic inheritance: Reprogramming begins with the down-regulation of somatic markers and mesenchymal-to-epithelial transition, followed by the activation of early pluripotency markers, and ultimately the activation of key pluripotency genes and telomerase to bring the cell to a mature pluripotent state. Epigenetic modification of UTX, Wdr5 regulate histones can help reprogramming by reducing DNA methylation level.

(3) Mechanisms and enhancement strategies: There are models of reprogramming such as stochastic and stoichiometric seesaw, which are divided into two stages: early repression of somatic genes and late activation of pluripotent genes[4]. A variety of somatic cells can initiate reprogramming, and iPSCs have been realized in multiple species.Modulation of signaling pathways and epigenetics with small molecule compounds and optimization of culture conditions can enhance reprogramming efficiency and quality.

1.4 Challenges in Induced Pluripotent Stem Cell Reprogramming

Although induced pluripotent stem cells (iPSCs) reprogramming technology has a wide range of applications, it still faces challenges.

(1) Technical challenges: the complexity of the molecular mechanism of reprogramming and the high demand for synergy among the various parts of the reprogramming process have led to extremely low efficiency. Large-scale acquisition of iPSCs is costly and difficult.Different iPSC clones are affected by the heterogeneity of starting cells, reprogramming randomness and culture environment, which will interfere with subsequent applications. Moreover, the lack of maturity of functional cells differentiated from iPSCs may affect drug evaluation and disease mimicry [5].

(2) Safety risks: viral vector reprogramming is prone to insertion mutations that increase the risk of cancer; non-integrative methods may destabilize the genome.

(3) Ethical and social issues: The high cost of iPSCs technology will lead to unequal distribution of medical resources. If it is used for reproduction, it will lead to changes in the human gene pool and raise ethical issues[6].

2. Prospects for the application of induced pluripotent stem cells

2.1 Disease Modeling and Drug Screening

The technology of iPSCs has made great contributions to the construction of disease models and drug screening in biomedicine.

(1) Disease model construction: Patient skin fibroblasts and other somatic cells carry genetic information and can be reprogrammed to obtain iPSCs. iPSCs can differentiate into disease-related cells such as dopaminergic neurons in Parkinson's disease under specific induction. iPSCs can also be used as a model for the development of disease-related cells such as dopaminergic neurons[7]. Compared with traditional models, iPSCs disease models can overcome the problems of animal species differences and genetic alteration of cell lines, which is more conducive to in vitro research and personalized medicine. The construction process is as follows: obtain somatic cells reprogrammed into iPSCs, identify pluripotency and genetic stability of qualified induced differentiation into target cells, and verify the validity of the model in terms of cell morphology, genetics, and function.

(2) Drug screening: After the iPSCs disease model cells receive drugs, we use testing technology to monitor cell morphology, gene expression and signaling pathway changes to assess drug efficacy and safety. For example, in Parkinson's disease, the corresponding neuronal model is used to screen for drugs that improve neuronal function and prevent neuronal death.This method avoids generic differences, improves the success rate of R&D and reduces the cost cycle. However, the accuracy of the model needs to be improved, and the differentiation of iPSCs will affect the results and there is a gap between

in vivo and ex vivo results.

2.2 Regenerative Medicine and Tissue Engineering

Regenerative medicine and tissue engineering are promising fields in modern medicine, and iPSCs also play a role in them. iPSCs can be differentiated into functional cells for regenerative medicine. For example, in the treatment of Parkinson's disease, the patient's somatic cells can be reprogrammed into iPSCs and wait for them to differentiate into dopaminergic neurons for transplantation. The shortage of organ transplants can be solved by combining iPSCs technology with 3D printing to build small functional organs. The technology can also build disease models to aid pathogenesis research and new drug development.

With their unique differentiation potential, iPSCs are ideal seed cells for tissue engineering. It can be combined with biomaterials to construct tissue substitutes for skin wound repair and so on. Its differentiated vascular endothelial cells participate in angiogenesis to promote the survival and functional recovery of tissue constructs.

Even though iPSCs are promising, scientists still need to address the issues of immunogenicity, tumorigenic risk, differentiation regulation and clinical translational assessment.[8].

2.3 Personalized Therapy

Personalized medicine has become a cutting-edge field in the process of medical precision, in which induced pluripotent stem cells (iPSCs) technology plays an indispensable role. iPSCs technology focuses on somatic cell reprogramming. The focus of iPSCs technology is on somatic cell reprogramming. Researchers obtain somatic cells from patients' skin fibroblasts, and introduce key transcription factors such as Oct4, Sox2, Klf4 and c-Myc into the cells to reprogram them into iPSCs carrying all the patient's genetic information.

By virtue of their pluripotency, iPSCs can differentiate into disease-associated cells under specific induction. For example, when treating Parkinson's disease, iPSCs can differentiate into dopaminergic neurons to replenish the damaged cells of the patient, which can effectively improve motor function and circumvent the problem of immune rejection[9].By constructing disease models with the help of patient-derived iPSCs, drug screening can monitor the effects of drugs on cell morphology and gene expression levels to accurately screen suitable drugs, thus promoting the development of personalized medicine.

3. Challenges and Solutions to Induced Pluripotent Stem Cell Applications

3.1 Cell Reprogramming Efficiency and Stability

Induced pluripotent stem cells (iPSCs) have been widely used, but their reprogramming efficiency and stability still need to be refined. In terms of reprogramming efficiency, due to the transcriptional and epigenetic differences between somatic cells and pluripotent stem cells, resulting in complex and difficult to regulate reprogramming molecular events, and reprogramming factor introduction and other factors affecting the transformation, the efficiency of iPSCs induction was only 0.01% - 0.1% at the early stage, and the efficiency is still very low after improvement.

In terms of stability, some iPSCs are incompletely reprogrammed, and their differentiation is easily controlled and affected by epigenetic memory and fluctuating culture conditions.

We can screen the combination of reprogramming factors to solve these problems, such as the use of small molecules, such as synergistic or alternative safe introduction; add growth factors to optimize the culture conditions; and combine with gene editing to select high-quality clones.We will work together to study the molecular mechanism and help iPSCs technology break through the bottleneck of application.

3.2 Carcinogenicity and Safety

Carcinogenicity and safety are still a major obstacle to the clinical application of iPSCs. The value-added differentiation of undifferentiated iPSCs is easily out of control, and teratomas are easily generated when injected into immunodeficient animals. During reprogramming, the introduction of retroviral vectors can cause insertion mutations, and after silencing, there is still residual cellular activity that interferes with growth. In addition, due to the unstable genome of iPSCs, the risk of cancer is higher in reprogramming and long-term culture[10].

Reprogramming, culture and differentiation can easily alter cell surface antigens or introduce animal components that can trigger receptor immune responses, and the risk of incomplete differentiation leads to a low safety profile. Experimenters can use cell surface markers to remove undifferentiated cells; or develop reprogramming techniques without integration risk; build animal-free culture systems and set strict quality control standards. Correct abnormal genes with the help of gene editing technology .

3.3 Cell Line Differences and Quality Control

In addition to the above problems, iPSCs technology has a major flaw waiting to be perfected. Different iPSCs cell lines differ significantly in differentiation potential, gene expression and epigenetic modification. Due to the differences in donor cell genetic background, reprogramming methods, culture conditions and number of passages, and the influence of gene expression and epigenetic modification patterns of each cell line, they have different different differentiation efficiencies and functional performances when differentiated into neural cells.

The current quality control of iPSCs suffers from the shortcomings of no unified standard, complicated detection methods, difficult to unify pluripotency assessment, difficult to detect subtle changes, and insufficient means of detecting cells after differentiation. Detectors need to set up uniform standards and testing procedures, develop advanced technologies, strengthen the whole process of monitoring and control key conditions to establish high-quality cell banks and strictly screen the cells to be banked.

4. Future Rrospects

Induced Pluripotent Stem Cells (iPSC) can be applied in many fields.Through in-depth study of their reprogramming and differentiation processes, we can unravel the mechanisms that control cell fate regulation and thus promote innovation in biological theories. iPSCs can be transplanted into differentiated cells to avoid immune rejection, and when combined with gene editing technology, they can be used to conquer hereditary diseases. Enhanced differentiation efficiency can lead to safer and more effective treatments. iPSC-based disease models have the potential to accelerate drug screening and shorten drug development cycles.However, iPSCs technology still faces challenges, including technical safety issues as well as optimization methods. Guidelines at the ethical and societal levels should be developed to enhance scientific popularization and public acceptance.

5. Conclusion

The potential of iPSCs technology is not to be underestimated, and it has shown remarkable promise in many cutting-edge fields, such as constructing disease models, screening drugs, promoting the development of regenerative medicine, and realizing personalized treatment. However, iPSCs are currently facing multiple challenges and constraints, including technical barriers, safety considerations, and ethical issues. Researchers have the important task of complying with the norms and exploring the unknown. We believe that iPSCs will play an increasingly important role in ensuring human health and well-being.

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