Review Article

Advancements in Induced Pluripotent Stem Cell Reprogramming Methods

Hannah Zhong

College of Letters and Science, University of California, Los Angeles, California, USA

ABSTRACT

Induced pluripotent stem cells (iPSCs) hold remarkable potential for regenerative medicine, disease modeling, and drug discovery. The reprogramming of somatic cells into iPSCs has opened new avenues for personalized therapies and understanding human diseases. This review provides a comprehensive overview of iPSC reprogramming methods, highlighting recent advancements, challenges, and future prospects. Various techniques, including viral approaches, episomal vectors, protein transduction, small molecules, and microRNAs, are discussed in detail. Furthermore, strategies to enhance reprogramming efficiency, minimize genomic instability, and improve the quality of iPSCs are explored. The integration of emerging technologies would revolutionize iPSC research and accelerate clinical translation. Overall, this review aims to provide insights into the evolving landscape of iPSC reprogramming methods and their implications for biomedical applications.

Keywords: induced pluripotent stem cells, reprogramming methods, viral vectors, non-viral methods, small molecules

INTRODUCTION

Induced pluripotent stem cells (iPSCs) have emerged as a groundbreaking technology in regenerative medicine and biomedical research [1-5]. They represent a significant scientific advancement, offering tremendous potential for disease modeling, drug discovery, and personalized therapies. The reprogramming of somatic cells into iPSCs involves the activation of endogenous pluripotency genes and the establishment of a pluripotent state resembling embryonic stem cells (ESCs) [1]. The discovery of iPSCs in 2006 by Shinya Yamanaka and his team marked a paradigm shift in stem cell biology [5]. Like ESCs, iPSCs are pluripotent and can differentiate into virtually any cell type in the body, including neurons, cardiomyocytes, hepatocytes, and pancreatic beta cells. However, iPSCs circumvent the ethical issue associated with ESCs by allowing the generation of pluripotent stem cells from somatic cells without the need for embryo destruction, opening new avenues for personalized medicine and regenerative therapies [1].

One of the most significant applications of iPSCs is

Corresponding author: Hannah Zhong, E-mail: hzhong12@g.ucla.edu.

Received March 2,2024**; Accepted** March 31, 2024
https://doi.org/10.70251/HYJR2348.211115

their potential for disease modeling. By reprogramming patient-derived somatic cells into iPSCs, researchers can generate cell lines that recapitulate the genetic background of individuals affected by various diseases. These iPSC-derived disease models offer a platform for studying disease mechanisms, identifying novel therapeutic targets, and screening drug candidates in a patient-specific context. Moreover, iPSCs hold promise for cell replacement therapies, where differentiated cells derived from iPSCs could be used to replace damaged or dysfunctional tissues in patients with degenerative diseases [1]. Additionally, iPSCs have implications for drug discovery and toxicity testing. Traditional drug development processes often rely on animal models or immortalized cell lines that may not accurately recapitulate human physiology. iPSC-based platforms offer a more physiologically relevant alternative, allowing researchers to assess drug efficacy and toxicity in human cells derived from diverse genetic backgrounds (Takahashi et al., 2007). This approach has the potential to enhance the efficiency of drug development pipelines and reduce the attrition rates of candidate compounds during clinical trials [2].

The reprogramming of iPSCs involves the activation of key pluripotency genes, such as Oct4, Sox2, Klf4, and c-Myc. These transcription factors act in concert to remodel the cellular epigenome, resetting the somatic cell's identity and inducing a pluripotent state [5]. Over the past decade, significant progress has been made in developing reprogramming methods that are efficient, safe, and amenable to clinical translation. Despite these advances, several challenges remain to be addressed including improving efficiency and safety and addressing issues of genomic stability and epigenetic memory. This review provides a comprehensive overview of the diverse methodologies employed for iPSC generation, highlighting recent advancements and future directions.

VIRAL-BASED REPROGRAMMING METHODS

Viral-based reprogramming methods have been extensively used in the generation of iPSCs since the pioneering work of Shinya Yamanaka and his team in 2006 [2-8]. These methods rely on the use of viral vectors to deliver reprogramming factors into somatic cells, enabling the activation of pluripotency-related genes and the induction of a pluripotent state.

Retroviral Vectors

Retroviruses are RNA viruses capable of integrating their genetic material into the host genome, making them well-suited for stable gene transfer [1, 5]. Retroviral vectors, derived from replication-defective retroviruses, have been widely used for iPSC reprogramming due to their high transduction efficiency and ability to mediate long-term transgene expression. These vectors typically carry the reprogramming factors OCT4, SOX2, KLF4, and c-MYC, collectively known as the Yamanaka factors, which are essential for inducing pluripotency in somatic cells [1, 5].

Lentiviral Vectors

Lentiviruses are a subclass of retroviruses capable of infecting both dividing and non-dividing cells, making them versatile tools for gene delivery [2, 6]. Lentiviral vectors have been employed for iPSC reprogramming, offering higher transduction efficiency and broader tropism compared to traditional retroviral vectors [2, 6]. Lentiviral vectors can efficiently deliver reprogramming factors into a wide range of somatic cell types, including non-dividing cells such as neurons and hepatocytes.

Adenoviral Vectors

Adenoviruses are non-enveloped DNA viruses capable of infecting a broad range of mammalian cells [8, 9]. Adenoviral vectors have been explored for iPSC reprogramming, offering transient gene expression without genomic integration. Unlike retroviruses and lentiviruses, adenoviral vectors do not integrate into the host genome but instead remain episomal, leading to transient expression of the reprogramming factors [8, 9].

Adeno-Associated Viral Vectors (AAVs)

Adeno-associated viruses are small, non-pathogenic viruses that have gained attention as gene delivery vectors due to their ability to mediate long-term transgene expression without causing significant immune responses [10, 11]. AAV vectors have been investigated for iPSC reprogramming, offering the advantages of low immunogenicity, minimal cytotoxicity, and low genotoxicity.

In summary, viral-based reprogramming methods have played a crucial role in the generation of iPSCs, offering robust and efficient approaches for inducing pluripotency in somatic cells. While retroviral and lentiviral vectors provide high transduction efficiency, concerns regarding genomic integration and insertional mutagenesis necessitate careful consideration of safety issues, particularly for clinical applications. Adenoviral and adeno-associated viral vectors offer transient gene expression without genomic integration, making them attractive options for generating integration-free iPSCs

with improved safety profiles. Nonetheless, ongoing research efforts are focused on developing safer and more efficient reprogramming methods, including non-viral approaches and synthetic biology tools, to overcome existing limitations and accelerate the clinical translation of iPSC-based therapies.

EPISOMAL VECTORS-BASED REPROGRAMMING METHODS

Episomal vectors represent a promising approach for iPSC reprogramming, offering the advantage of generating integration-free iPSCs without the risk of genomic modification or insertional mutagenesis [12] (Jia et al., 2010; Okita et al., 2011; Kaji et al., 2009; Yu et al., 2009; Mali et al., 2010). These vectors are derived from plasmids or viral elements capable of replicating autonomously in the host cell, allowing transient expression of reprogramming factors without genomic integration. Episomal vectors have gained attention as a safer alternative to viral-based methods for iPSC generation, particularly for clinical applications where genomic stability and safety are paramount concerns.

Episomal vectors are typically designed to contain reprogramming factors, such as OCT4, SOX2, KLF4, and c-MYC, along with additional elements necessary for replication and maintenance in the host cell [12, 13]. These vectors may incorporate oriP/EBNA1 elements derived from Epstein-Barr virus (EBV) or other viral elements capable of facilitating episomal replication and retention in dividing cells [2]. In transfection into somatic cells, episomal vectors are maintained as extrachromosomal DNA entities, allowing transient expression of reprogramming factors and induction of pluripotency. The absence of genomic integration ensures that the host cell's genome remains unaltered, reducing the risk of insertional mutagenesis and preserving the genomic integrity of iPSCs.

In conclusion, episomal vectors represent a promising approach for iPSC reprogramming, offering integration-free generation of pluripotent stem cells with improved safety and scalability [14]. These vectors hold great potential for advancing iPSC-based therapies and personalized medicine, providing a versatile platform for disease modeling, drug discovery, and regenerative medicine. Continued research and development efforts are needed to optimize episomal vector design, enhance reprogramming efficiency, and overcome existing challenges to facilitate the widespread adoption of episomal-based iPSC reprogramming methods in clinical settings.

PROTEIN TRANSDUCTION-BASED IPSCS REPROGRAMMING METHODS

Protein transduction offers a novel avenue for induced pluripotent stem cell (iPSC) reprogramming, presenting a non-genetic method for delivering reprogramming factors directly into somatic cells. Unlike traditional approaches relying on viral-based or episomal vectors, protein transduction obviates the need for DNA transfection or viral vectors, thereby enhancing safety and minimizing the risk of genomic integration [15]. This method provides precise control over reprogramming factor expression, promising the generation of integration-free iPSCs with improved safety profiles and broader applicability in regenerative medicine, disease modeling, and drug discovery.

The mechanism of protein transduction hinges on the cellular uptake of exogenous proteins or peptides through diverse mechanisms including endocytosis, direct membrane penetration, or receptor-mediated internalization [16]. Reprogramming factors such as OCT4, SOX2, KLF4, and c-MYC are typically fused with cell-penetrating peptides (CPPs) or protein transduction domains (PTDs) to facilitate their entry into target cells. These CPPs or PTDs harbor sequences that enable efficient cellular uptake and intracellular delivery of cargo proteins, overcoming barriers imposed by the cell membrane and endosomal compartmentalization [17]. Once internalized, the transduced proteins or peptides exert their biological activity, initiating the reprogramming process and inducing pluripotency in somatic cells [18].

Despite its potential, protein transduction faces several challenges and considerations in iPSC reprogramming, including issues related to protein stability, low delivery efficiency, cell-type specificity, and potential toxicity. Addressing these challenges will be crucial for optimizing protein transduction-based approaches and unlocking their full potential in iPSC research and therapeutic applications. Continued research efforts aimed at refining protein transduction methodologies and enhancing their efficiency and safety profiles will be instrumental in advancing this promising technology towards clinical translation [19].

SMALL MOLECULE-MEDIATED REPROGRAMMING

Small molecule-mediated reprogramming has emerged as a promising strategy for generating induced pluripotent stem cells (iPSCs) without the need for

exogenous gene expression or viral vectors [20]. This approach leverages the use of small molecules to modulate key signaling pathways and epigenetic modifications involved in pluripotency regulation, thereby facilitating the transition of somatic cells into a pluripotent state [21]. Small molecules offer several advantages, including their non-genetic nature, precise control over reprogramming factors, scalability, and versatility [22]. Moreover, small molecule-mediated reprogramming eliminates the risks associated with genomic integration, insertional mutagenesis, and off-target effects, enhancing the safety profile of iPSCs generated using this method [23].

Histone deacetylase (HDAC) inhibitors, DNA methyltransferase (DNMT) inhibitors, signaling pathway modulators, and metabolic modulators are among the key classes of small molecules employed in iPSC reprogramming [24]. These molecules target specific cellular processes to promote chromatin remodeling, epigenetic modifications, and metabolic reprogramming, leading to the activation of pluripotency genes and the acquisition of pluripotency. While small molecule-mediated reprogramming offers significant advantages, optimization of protocols, enhancement of reprogramming efficiency, and assurance of long-term safety and stability remain challenges to be addressed [21]. Regulatory considerations also need to be addressed to facilitate the translation of small molecule-based iPSC reprogramming methods into clinical applications.

MICRORNAS-BASED APPROACHES

MicroRNAs (miRNAs) play crucial roles in induced pluripotent stem cell (iPSC) reprogramming by regulating gene expression at the post-transcriptional level. Several miRNAs have been identified as key players in promoting reprogramming efficiency and stabilizing the pluripotent state of iPSCs [25]. For example, the miR-302/367 cluster, miR-200 family, miR-369-3p, and Let-7 family are among the miRNAs implicated in iPSC reprogramming, with diverse roles in modulating signaling pathways, promoting epithelial-to-mesenchymal transition (EMT) or mesenchymal-to-epithelial transition (MET), and targeting pluripotency-associated genes [26] [27, 28].

MiRNA-mediated iPSC reprogramming offers advantages such as non-genetic regulation, precise control over gene expression, and potential for combinatorial approaches with other reprogramming factors or small molecules [27]. However, challenges including off-target effects, delivery methods optimization, and ensuring long-term safety and stability of iPSCs need to be addressed for clinical translation [29]. Overall, miRNAs represent promising candidates for enhancing iPSC reprogramming efficiency and quality, with ongoing research efforts focused on elucidating their mechanisms of action, optimizing delivery strategies, and evaluating their potential for clinical applications in regenerative medicine and disease modeling.

CHALLENGES AND FUTURE DIRECTIONS

Despite the significant advancements in iPSC reprogramming methods, several challenges remain to be addressed for their widespread clinical application. Genomic instability, off-target effects, and variability in reprogramming efficiency pose hurdles that need to be overcome through rigorous optimization and quality control measures. Moreover, the integration of emerging technologies such as single-cell analysis, organoid culture systems, and microfluidic platforms holds promise for dissecting the dynamics of reprogramming and improving the fidelity of iPSC generation. Future research efforts are focused on refining reprogramming protocols, enhancing the safety and scalability of iPSC production, and translating iPSC-based therapies into clinical practice.

CONCLUSION

In conclusion, iPSC reprogramming methods have witnessed remarkable progress in recent years, driven by advances in viral and non-viral delivery systems. These methodologies offer diverse approaches for generating iPSCs with high efficiency, minimal genomic modification, and improved quality. By overcoming existing challenges and integrating emerging technologies, iPSC research is poised to revolutionize regenerative medicine, disease modeling, and drug discovery in the coming years. Continued interdisciplinary collaboration and translational efforts will be essential for harnessing the full potential of iPSCs and realizing their promise for personalized medicine.

REFERENCES

1. Takahashi, K., et al., Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell, 2007. 131(5): p. 861-72.
2. Yu, J., et al., Induced pluripotent stem cell lines derived from human somatic cells. Science, 2007. 318(5858): p. 1917-20.

3. Park, I.H., et al., Reprogramming of human somatic cells to pluripotency with defined factors. Nature, 2008. 451(7175): p. 141-6.
4. Aoi, T., et al., Generation of pluripotent stem cells from adult mouse liver and stomach cells. Science, 2008. 321(5889): p. 699-702.
5. Takahashi, K. and S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell, 2006. 126(4): p. 663-76.
6. Okita, K., T. Ichisaka, and S. Yamanaka, Generation of germline-competent induced pluripotent stem cells. Nature, 2007. 448(7151): p. 313-7.
7. Wernig, M., et al., In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature, 2007. 448(7151): p. 318-24.
8. Hanna, J., et al., Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. Science, 2007. 318(5858): p. 1920-3.
9. Eminli, S., et al., Reprogramming of neural progenitor cells into induced pluripotent stem cells in the absence of exogenous Sox2 expression. Stem Cells, 2008. 26(10): p. 2467-74.
10. Maherali, N., et al., Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. Cell Stem Cell, 2007. 1(1): p. 55-70.
11. Nakagawa, M., et al., Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nat Biotechnol, 2008. 26(1): p. 101-6.
12. Jia, F., et al., A nonviral minicircle vector for deriving human iPS cells. Nat Methods, 2010. 7(3): p. 197-9.
13. Okita, K., et al., A more efficient method to generate integration-free human iPS cells. Nat Methods, 2011. 8(5): p. 409-12.
14. Mali, P., et al., Butyrate greatly enhances derivation of human induced pluripotent stem cells by promoting epigenetic remodeling and the expression of pluripotency-associated genes. Stem Cells, 2010. 28(4): p. 713-20.
15. Kim, D., et al., Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell, 2009. 4(6): p. 472-6.
16. Joliot, A. and A. Prochiantz, Transduction peptides: from technology to physiology. Nat Cell Biol, 2004. 6(3): p. 189-96.
17. Derossi, D., et al., The third helix of the Antennapedia homeodomain translocates through biological membranes. J Biol Chem, 1994. 269(14): p. 10444-50.
18. Kim, J.B., et al., Direct reprogramming of human neural stem cells by OCT4. Nature, 2009. 461(7264): p. 649-3.
19. Yamanaka, S., Induced pluripotent stem cells: past, present, and future. Cell Stem Cell, 2012. 10(6): p. 678-684.
20. Zhu, S., et al., Reprogramming of human primary somatic cells by OCT4 and chemical compounds. Cell Stem Cell, 2010. 7(6): p. 651-5.
21. Hou, P., et al., Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. Science, 2013. 341(6146): p. 651-4.
22. Li, W. and S. Ding, Small molecules that modulate embryonic stem cell fate and somatic cell reprogramming. Trends Pharmacol Sci, 2010. 31(1): p. 36-45.
23. Zhu, S., et al., Small molecules enable OCT4-mediated direct reprogramming into expandable human neural stem cells. Cell Res, 2014. 24(1): p. 126-9.
24. Huangfu, D., et al, Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. Nat Biotechnol, 2008. 26(7): p. 795-7.
25. Samavarchi-Tehrani, P., et al., Functional genomics reveals a BMP-driven mesenchymal-to-epithelial transition in the initiation of somatic cell reprogramming. Cell Stem Cell, 2010. 7(1): p. 64-77.
26. Subramanyam, D., et al., Multiple targets of miR-302 and miR-372 promote reprogramming of human fibroblasts to induced pluripotent stem cells. Nat Biotechnol, 2011. 29(5): p. 443-8.
27. Anokye-Danso, F., et al., Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell, 2011. 8(4): p. 376-88.
28. Li, Z., et al., Small RNA-mediated regulation of iPS cell generation. EMBO J, 2011. 30(5): p. 823-34.
29. Kasinski, A.L. and F.J. Slack, Epigenetics and genetics. MicroRNAs en route to the clinic: progress in validating and targeting microRNAs for cancer therapy. Nat Rev Cancer, 2011. 11(12): p. 849-64.