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Research Progress and Prospects of Direct Cardiac Reprogramming Technology

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Abstract: ***As adult cardiomyocytes are in a state of terminal differentiation, they have no regenerative capacity. Once the heart is subjected to ischemia and hypoxia, cardiomyocytes suffer irreversible damage, and damaged and necrotic cardiomyocytes are replaced by myocardial fibroblasts, which in turn form scar tissue leading to cardiac remodeling and ultimately to heart failure. The discovery of direct cardiac reprogramming, in which myocardial fibroblasts are induced to become cardiomyocyte-like cells by specific means, holds new promise for the treatment of cardiac diseases and the regeneration of the heart. In this paper, we will provide an overview of the development of direct cardiac reprogramming, summarize the transcription factor-mediated reprogramming approach, and discuss its molecular mechanisms, including key transcription factors, signaling pathways, immune response and autophagy. At the same time, we will analyze the current challenges faced by the field and the future direction of development.***

Keywords: Direct reprogramming, Fibroblasts, Challenges, Perspectives.

1. Introduction

According to relevant statistics, Cardiovascular Disease (CVD) has become one of the leading fatal diseases in the world. Moreover, the prevalence and mortality rate of CVD continue to rise [1]. Studies have shown that the number of CVD deaths globally will be in the tens of millions in 2022, and the annual number of CVD deaths globally is expected to reach 23 million in 2030 due to population growth and aging, which highlights the serious public health challenges posed by the disease, and the burden on society will continue to increase [2]. Adult cardiomyocytes are in a state of terminal differentiation and therefore have no regenerative capacity. When the heart is subjected to ischemia and hypoxia, cardiomyocytes suffer irreversible damage, and damaged and necrotic cardiomyocytes are replaced by myocardial fibroblasts, which form scar tissue leading to cardiac remodeling, ultimately leading to heart failure. Currently, pharmacological treatments can only alleviate the patient's symptoms, but cannot address the underlying problem of cardiomyocyte damage and necrosis, and invasive cardiac transplantation or surgical treatments have a high risk as well as an economic burden. To address this problem, several strategies have been developed to repair damaged myocardial tissues, including the stimulation of endogenous cardiomyocyte proliferation, and the transplantation of cardiac progenitor cells or the use of induced pluripotent stem cells (IPSCs) to treat myocardial failure, including the use of cardiac fibroblasts. induced pluripotent stem cells (iPSCs) to regenerate the heart. In recent years, significant progress has been made regarding the direct reprogramming of induced fibroblasts into cardiomyocyte-like cells. Compared with the previous two methods, direct cardiac reprogramming, can directly induce fibroblasts into cardiomyocyte-like cells without the need for in vitro cell transplantation, and avoids the risks of delivering potential oncogenic factors as well as immune rejection [3].

2. Historical Origin and Development of Direct Heart Reprogramming

Direct reprogramming, also known as trans differentiation, refers to the transformation of cells by directly inducing a certain type of cell into another mature cell under specific conditions, without going through intermediate stages such as multifunctional stem cells, or progenitor cells. In 1987, Davis' team reported for the first time that fibroblasts were transformed into myofibroblasts through the expression of the transcription factor MyoD, which provided feasibility for the realization of transformation of cells [4]. However, it was not until 2007 that the team of Japanese scientist Shinya Yamanaka made a breakthrough in the study of induced pluripotent stem cells [5], an achievement that greatly facilitated the direct reprogramming of one type of somatic cell to another and provided a key theoretical support and technological impetus to the induction of multifunctional stem cells. In recent years, researchers have successfully realized direct cardiac reprogramming by using overexpression of transcription factors, miRNAs or small molecule treatments. In this paper, we focus on direct cardiac reprogramming regulated by transcription factors.

In 2010, Ieda's team [6] screened fourteen transcription factors and finally demonstrated, for the first time, that direct cardiac reprogramming was able to induce fibroblasts into cardiomyocyte-like cells by overexpressing three transcription factors, Gata4, Mef2c, and Tbx5 (GMT) and found that a combination of GMTs was sufficient to transform murine cardiac fibroblasts into iCMs with cardiomyocyte-like functional characteristics that including calcium oscillations, contraction, and other electrophysiological properties. In addition, it was found that direct cardiac reprogramming is influenced by a variety of factors, including cell-intrinsic and cell-extrinsic factors, in which the expression level and combination of specific transcription factors are critical for

the efficiency of direct cardiac reprogramming. Based on this, several subsequent studies have attempted to further optimize the efficiency of direct reprogramming by screening for the best transcription factor combinations, modulating epigenetics, application of small molecule compounds, and microenvironmental regulation, etc. For example, researchers have found [7] that the addition of the transcription factors Hand2 and Nkx2.5 to GMT increased the efficiency of direct cardiac reprogramming by more than 50-fold and achieved a higher transformation rate. It has also been found that fusion manipulation of four transcription factors (GMTH), Gata4, Mef2c, Tbx5 and Hand2, with the MyoD trans-activating structural domains significantly enhances the beat-to-beat function of reprogrammed cardiomyocytes. Experimental data showed that the pacing capacity of cardiomyocytes treated with this technique was 15-fold higher than that of the traditional method [8].

3. Molecular Mechanisms of Direct Cardiac Reprogramming

3.1 Regulation of Transcription Factors

Core transcription factors (Gata4, Mef2c and Tbx5 (GMT)) play a dominant role in direct cardiac reprogramming. It has been found that Gata4, as an initiator of reprogramming, not only participates in several key physiological processes of cardiac development, such as cardiomyocyte division [9], but also activates genes related to cardiac development by binding to and facilitating the modification of H3k27ac [10].Mef2c plays an important role in muscle cell development and function, and it can regulate cardiomyocyte Mef2c plays an important role in muscle cell development and function by regulating cardiomyocyte contraction and metabolism-related gene expression [11], while Tbx5 dominates cardiac morphogenesis and atrial septum formation [12], and synergistically activates cardiac-specific gene programs with GMT in reprogramming to ensure cellular transformation into the cardiac lineage. In addition to the core transcription factors, the synergistic enhancer Tbx20 as well as the co-regulator Hand2/Nkx2.5 (see Table 1), which interact with each other, activate the expression of cardiac genes as well as repress non-cardiomyocyte-associated genes through the formation of a dynamic regulatory network, and finally, achieve highly efficient and precise cardiac regeneration.

Table 1: Regulatory network diagram of cardiac direct reprogramming transcription factors

Classification	Factor	Functional Effects
core transcription factor	Gata4, Mef2c and Tbx5	Basic combination of reprogramming to induce fibroblast transformation into cardiomyocytes [9]
synergistic enhancer	Tbx20	There is a synergistic regulatory effect of Tbx20 in combination with GMT transcription factors in the enhancer regions of cardiac contraction-related genes. This combined effect significantly enhanced the transcriptional activity of target genes by enhancing the binding efficiency of the transcription factor complex to chromatin [11]
co-regulator	Hand2/Nkx 2.5	Enhancement of the number and functional maturity of iCMs [7]

3.2 Modulation of Signaling Channels

The regulation of major signaling pathways plays an important role in the process of direct cardiac reprogramming. Mohamed's team [13], after a series of studies, finally determined that inhibition of the TGF-β and Wnt signaling pathways can effectively improve the efficiency of direct reprogramming. The TGF-β signaling pathway is a central pathway in the regulation of the fibrotic process. Once activated, pro-fibrotic signaling impairs the effect of cardiac reprogramming. It was found [14] that the activation of pro-fibrotic signaling could be reduced by inhibiting the expression of TGF-β and Rho-related kinases, thus promoting the direct reprogramming of embryonic fibroblasts into functional induced cardiomyocytes (iCMs). Wnt signaling pathway is also one of the key pathways in cardiac regeneration. Activation of the Wnt signaling pathway promotes the transformation of fibroblasts into cardiomyocyte-like cells by regulating the activity and expression of relevant transcription factors that affect cell proliferation and differentiation. In addition, Zhou et al [15] found that activation of protein kinase B (Akt) pathway promotes direct cardiac reprogramming, and Abad et al [16] found that the efficiency of GHMT-mediated reprogramming could be improved by inhibition of Notch signaling pathway. Thus, the regulation of visible signaling has an important role in both achieving direct cardiac reprogramming and improving the efficiency of direct reprogramming.

3.3 Immune Response and Autophagy

Previous studies have demonstrated that the innate immune response plays an extremely critical role in reprogramming of other systems [17], and autophagy response should not be ignored in cardiac reprogramming. It has been found that efficient direct cardiac reprogramming can be achieved by appropriate immune signaling stimulation [18]. Muraoka et al. found [19] that diclofenac sodium (a nonsteroidal anti-inflammatory drug), when applied in combination with GMT or GHMT, reduced the expression of fibroblasts, inflammation-related genes, and thus significantly enhanced the efficiency of cardiac reprogramming. However, the relationship between direct cardiac reprogramming and immune response still needs further investigation. And autophagy, as an intracellular self-degradation mechanism, also has an important role in cardiac reprogramming. Autophagy can inhibit the activation of the TGF-β signaling pathway, thereby reducing the generation of pro-fibrotic signals, which in turn promotes the occurrence of direct cardiac reprogramming and improves the efficiency of direct cardiac reprogramming. At the same time, TGF-β signaling pathway also has a certain regulatory effect on autophagy, which can regulate the expression of autophagy-related proteins and activate autophagy, and moderate autophagy can remove damaged organelles in the cell, which can improve the internal environment for cell reprogramming. Studies have shown that appropriate activation of cellular autophagy can improve the efficiency of direct cardiac reprogramming [20]. The above indicates that autophagy plays an important role in cardiac reprogramming.

4. Challenges and Prospects

In recent years, cardiac regenerative therapy has become a promising therapeutic strategy due to the continuous development of direct cardiac reprogramming technology, but

the molecular mechanism of direct cardiac reprogramming is not clear enough and needs to be studied in depth, in addition, the reprogramming technology also faces many challenges. First, the efficiency of reprogramming technology needs to be further improved. Although the efficiency of direct reprogramming has been further optimized by screening the best combination of transcription factors, epigenetic regulation, application of small molecule compounds, and microenvironmental regulation, the efficiency is still relatively low, limiting its clinical application. Secondly, studies have shown that there are differences in the direct cardiac reprogramming process between humans and mice [21,22], for example, it was found that GHMT, a combination of transcription factors in mice, could not activate the expression of human fibroblast-related genes [23]. In addition to this, cardiomyocyte-like cells induced by direct reprogramming techniques have some deficiencies in the maturation of their cellular functions. There is still a gap between these cells and natural cardiomyocytes in terms of electrophysiological properties, contractile function, etc., so the reprogramming method needs to be further optimized to obtain iCMs with functions closer to those of natural cardiomyocytes. lastly, the safety aspect is also a key concern. The reprogramming process may involve risks such as genovirus overexpression and increased tumorigenesis [24].

Cardiac direct reprogramming technology has shown remarkable potential in the field of cardiac regeneration by inducing myocardial fibroblasts to transform into cardiomyocyte-like cells. And in recent years, with the continuous development of cardiac direct reprogramming technology, the technology has made great progress, but still faces challenges such as insufficient analysis of molecular mechanisms, programming efficiency and maturity. Looking ahead, we still need to make breakthroughs in the following aspects: firstly, in-depth study of the molecular mechanism of direct cardiac reprogramming and elucidation of its specific mechanism; secondly, optimization of the existing strategies of reprogramming, such as through small molecule interventions and other means, to enhance the efficiency of reprogramming and the maturity of cellular function; and thirdly, improvement of the safety of reprogramming to avoid the risk of tumor formation. Finally, as basic research and clinical exploration go hand in hand, it is expected to promote the direct cardiac reprogramming technology from the laboratory to clinical application, opening up new paths and bringing new vitality to the treatment of heart diseases such as heart failure.

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