Journal of Cardiology 73 (2019) 97–101

Contents lists available at ScienceDirect

Journal of Cardiology

journal homepage: www.elsevier.com/locate/jjcc

Review

Progress and Challenge of Cardiac Regeneration to Treat Heart Failure

Mari Isomi (BVSc), Taketaro Sadahiro (MD), Masaki Ieda (MD, PhD)*

Department of Cardiology, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan

ARTICLE INFO

Article history:
Received 3 October 2018
Accepted 9 October 2018
Available online 9 November 2018

Keywords:
Direct reprogramming
Regeneration
Fibroblasts

ABSTRACT

Cardiac muscle has limited proliferative capacity, and regenerative therapies are highly in demand as a new treatment strategy. Pharmacological and non-pharmacological therapies have been developed, but these medical therapies have limited effects to cure patients with severe heart failure. Moreover, heart transplantation is limited due to the low number of donor organs. Thus, heart regeneration holds great potential to offer innovative therapy to treat heart failure patients. Currently, there are several strategies for heart regeneration. Transplantation of somatic stem cells was safe and modestly improved cardiac function after myocardial infarction mainly through paracrine mechanisms. Alternatively, new cardiomyocytes could be generated from induced pluripotent stem cells (iPSCs) to transplant into injured hearts. However, several issues remain to be resolved prior to using iPSC-derived cardiomyocytes, such as a potential risk of tumorigenesis and poor survival of transplanted cells in the injured heart. More recently, direct cardiac reprogramming has emerged as a novel technology to regenerate damaged myocardium by directly converting endogenous cardiac fibroblasts into induced cardiomyocyte-like cells to restore cardiac function. Following our first report of cardiac reprogramming, an improvement in cardiac reprogramming efficiency, *in vivo* direct cardiac reprogramming, and cardiac reprogramming in human cells were reported by many investigators. While these previous studies have advanced regenerative research, many challenges remain. Here, we review the current status of cardiac regenerative technology, a great hope to treat cardiovascular diseases.

Contents

Somatic stem cell-based cardiac regeneration . . . 98
Pluripotent stem cell-derived cardiomyocytes for cardiac regeneration. . . . 98
Proliferation of endogenous adult cardiomyocytes to repair hearts . . . 98
Direct cardiac reprogramming for heart regeneration. . . . 99
Direct reprogramming of fibroblasts into cardiomyocytes by defined factors . . . 99
Modification of signaling pathways and epigenetics. . . . 100
In vivo cardiac repair and regeneration . . . 100
Conflict of interest. . . . 100
Funding . . . 100
Acknowledgements . . . 101
References . . . 101

Introduction

Cardiovascular diseases are the leading cause of death and disability worldwide. As adult cardiomyocytes have poor regenerative ability, dead cardiomyocytes are replaced by fibroblasts, leading to the formation of fibrosis and myocardial remodeling. Many therapeutic approaches have been developed, but the

* Corresponding author : Masaki Ieda, MD, PhD, Department of Cardiology, Faculty of Medicine, University of Tsukuba, 1-1-1, Tennoudai, Tsukuba City, Ibaraki 305-8575, Japan.
E-mail address: mieda@md.tsukuba.ac.jp (M. Ieda).

https://doi.org/10.1016/j.jjcc.2018.10.002
0914-5087/

mortality of patients with severe heart failure remains high. Fundamental treatment of end stage heart failure is heart transplantation, which is limited by lack of donors, criteria of selected patients, and risk of surgery. Cardiomyocytes are considered to be in a terminally differentiated state in humans. Unlike the mammalian heart, zebrafish hearts exhibit robust regenerative responses upon injury; they can completely regenerate the lost myocardium following ventricular amputation. Nevertheless, neonatal mice have the capacity to regenerate large portions of their hearts after ventricular amputation [1]. Previous studies revealed a cardiomyocyte renewal rate of 0.5–1% per year in adult humans [2]. However, the regenerative capacity of human cardiomyocytes is insufficient to regenerate the lost myocardium. Therefore, cardiac regenerative therapy has attracted attention as a novel therapeutic approach for patients with heart disease. Early attempts of cardiac regeneration involved somatic stem cells to stimulate regeneration and improve cardiac function. The first clinical trial was transplantation of bone marrow-derived mononuclear cells (BMMNCs). Although early clinical trials improved cardiac function, other studies did not replicate these findings [3]. In the next generation, researchers focused on cardiac progenitor cells (CPCs). They have the ability to proliferate and differentiate into cardiac lineage cells to replace the damaged and lost cardiomyocytes [4]. Injection of autologous cultured CPCs showed a potential positive improvement on cardiac function, and appeared to be safe in small-scale trials. Nevertheless, engraftment of transplanted cells remains low, and transplanted stem cells had a limited capacity to differentiate into cardiomyocytes. Thus, the benefits of cellular injections are probably due to paracrine effects [5]. Another promising approach to repair heart disease is using cardiomyocytes derived from allogeneic pluripotent stem cells such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). These cells differentiate into functional cardiomyocytes, which can be transplanted into the injured heart. However, pluripotent stem cell-based therapies have many obstacles before their practical use in patients, including engraftment rate and potential risk of tumorigenesis. To overcome these major issues arising from the use of pluripotent stem cells, we discovered a novel approach to repair hearts. We found direct cardiac reprogramming, in which resident cardiac fibroblasts (CFs) are converted to induced cardiomyocyte-like cells (iCMs) without reverting to pluripotent stem cells by transduction of defined cardiac-specific factors (Fig. 1). In this review, we summarize advances in cardiac regeneration for the treatment of damaged hearts, and discuss perspectives and challenges for future clinical application.

Somatic stem cell-based cardiac regeneration

In an early stage of regenerative medical research, BMMNCs have garnered considerable interest in cardiac regeneration, because they showed cardiogenic potential *in vitro*, and BMMNCs demonstrated beneficial contribution of cardiac regeneration in rodent myocardial infarction (MI) models. Despite the beneficial preclinical outcomes, there have been conflicting results in several clinical trials based on BMMNCs. Although early small randomized human clinical studies of injection of BMMNCs demonstrated a modest increase in ejection fraction, multiple well-randomized and double-blinded clinical trials did not reproduce these promising results [3]. Bone marrow-derived mesenchymal stem cells (MSCs) are another adult stem cell source thought to be suitable for cardiac regeneration; they also showed cardiogenic potential *in vitro* and improvement in cardiac function in animal MI models. However, clinical trials such as the POSEIDON trials showed modest improvements in cardiac function, and subsequent studies revealed that MSCs had no ability to differentiate into cardiomyocytes [6,7].

Next, our interests shifted to cardiac progenitor cells (CPCs), which had ability to proliferate and differentiate into three cardiac lineage cells, including cardiomyocytes, smooth muscle cells, and endothelial cells. CPCs are characterized by different cell surface markers such as hematopoietic progenitor marker c-kit. In several preclinical trials, c-kit+ CPCs demonstrated positive results in small and large animal models [8]. Subsequently, investigators initiated first clinical trials, SCIPIO, in which autologous c-kit+ CPCs isolated from cardiac tissues were injected into patients with ischemic cardiomyopathy. This study reported slight increase in ejection fraction and decrease in infarct size [9]. In the next study, CADUCEUS trial used cardiosphere-derived cells, a mixed population of CPCs, for intracoronary administration into the patients with prior MI [5]. They showed intracoronary infusion of autologous CPCs after myocardial infarction was safe, but the effects of treatment were again modest. The results of these two clinical trials have been a matter of controversy. Because of the low engraftment rate in preclinical trials, potential benefits are probably due to paracrine effects of CPCs. Recently, conflicting findings on the c-kit+ CPCs were reported by using lineage-tracing mice. The study showed that c-kit+ CPCs minimally contributed to cardiomyocytes but mainly gave rise to endothelial cells, indicating that c-kit+ CPCs are endothelial progenitors that are likely to be involved in vascular repair in injured hearts [10].

Pluripotent stem cell-derived cardiomyocytes for cardiac regeneration

Another future therapeutic approach employs cardiomyocytes derived from allogeneic ESCs or iPSCs. Both approaches tried to generate functional cardiomyocytes *in vitro*, and used differentiated cardiomyocytes as a patch or direct injection into injured hearts [11]. Although transplantation of ESC-derived cardiomyocytes into animal MI model have shown improvements in cardiac function, there is ethical opposition to use of human ESCs for clinical translation [12]. Therefore, discovery of iPSCs solved the ethical problems and have great potential for cardiac regeneration. Yamanaka et al. reported that mouse and human fibroblasts could be directly reprogrammed into cells with characteristic ESC morphology using a combination of four transcription factors, Oct4, Sox2, Klf4, and c-Myc (also known as the Yamanaka factors) [13]. Recently, numerous attempts have been made to demonstrate regenerative therapies using iPSCs, which extensively advanced this field. Shiba et al. reported that transplanted allogenic iPSC-derived cardiomyocytes improved cardiac contractile function, and notably, the grafted cardiomyocytes survived for 12 weeks in immunosuppressed macaques [14]. However, in this study significant ventricular tachycardias were observed, which could be attributed to immature cardiomyocytes. Heterogeneity of iPSC-derived cardiomyocytes may be one of the obstacles to develop this approach to clinical trials. Many studies have improved generation of more mature and pure cardiac cells from iPSCs, which may increase the engraftment rate and solve issues related to tumorigenesis and arrhythmias [15].

Proliferation of endogenous adult cardiomyocytes to repair hearts

Another approach to heart regeneration is activating endogenous regeneration potentials. As cardiomyocytes are terminally differentiated cells and mammalian cardiomyocytes already exit from the cell-cycle, the regenerative capacity of cardiomyocytes is limited. However, if we could control cardiac proliferative capacity, it would be a potent technology to repair mammalian

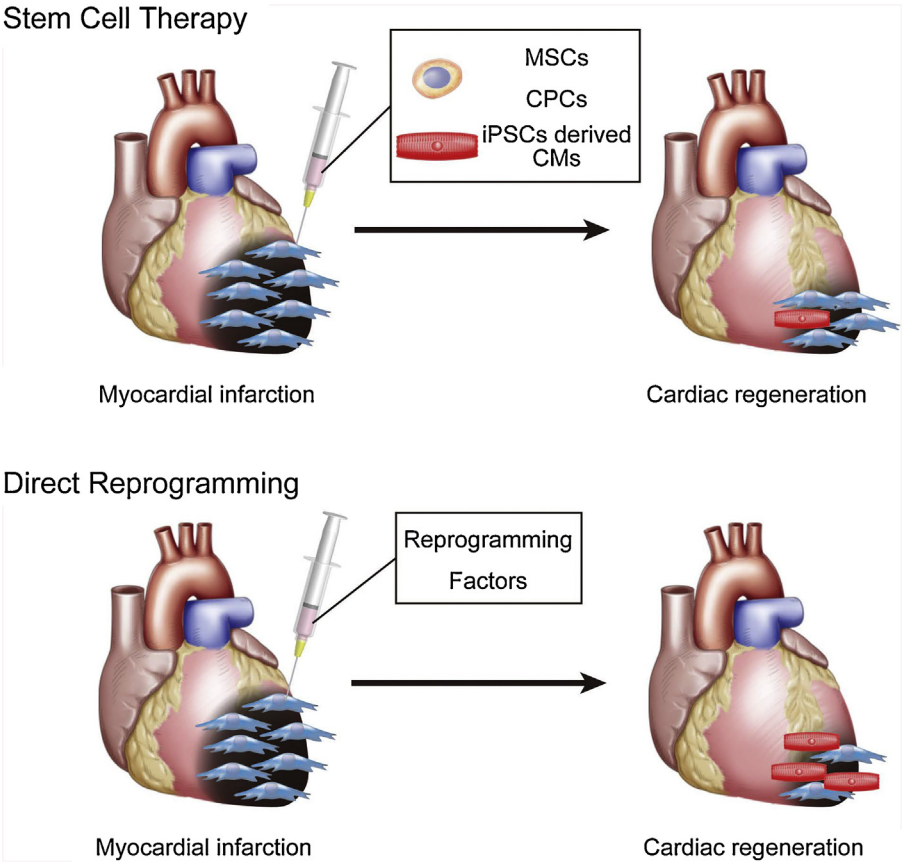

Fig. 1. Strategy for heart regeneration. In stem cell therapies, several kinds of stem cells or cardiomyocytes derived from iPSCs are transplanted into patients with heart failure. In direct cardiac reprogramming, reprogramming factors are injected into hearts and induce new cardiomyocytes *in situ*. *Abbreviations*: MSCs, mesenchymal stem cells; CPCs, cardiac progenitor cells; iPSCs, induced pluripotent stem cells; CMs, cardiomyocyte-like cells.

postnatal hearts. Mohamed et al. screened cell-cycle regulators in fetal cardiomyocytes, and found the combination of several cyclin-dependent kinases (CDKs) to promote cell division *in vitro* and *in vivo* [16]. Overexpression of CDKs not only induced cell proliferation but also reduced scar size after MI and improved heart function in mice. Another approach for cardiomyocyte proliferation is a modulation of oxidative stress. Tao et al. demonstrated that scavenging reactive oxygen species (ROS) or inhibiting DNA damage response (DDR) pathways induced cardiomyocyte proliferation and repaired adult hearts [17]. Soon after birth, energy metabolism switches from glycolytic to oxidative metabolism due to a dramatic change in oxygenation state. Increased ROS in mitochondria leads to activation of the DDR pathway, which is responsible for cell-cycle arrest of postnatal cardiomyocytes. Recently, Kimura et al. demonstrated that gradual exposure to systemic hypoxia led to decreases in ROS production and oxidative DNA damage, which reactivated cardiomyocyte mitosis in adult mice. These regenerative responses improved cardiac functions after MI [18]. Thus, stimulation of endogenous regenerative capacity of adult endogenous cardiomyocytes may be a promising approach for heart regeneration.

Direct cardiac reprogramming for heart regeneration

Direct reprogramming of fibroblasts into cardiomyocytes by defined factors

Besides cell-based transplantation therapy and induced endogenous cardiomyocyte proliferation, direct reprogramming approach, converting resident CFs to a cardiomyocyte, may be a game changer for heart regeneration. Taking the concept of innovative discovery of iPSCs reprogramming with the Yamanaka factors, we hypothesized that combinations of multiple cardiac-specific factors could directly convert resident CFs into iCMs without needing to first become a stem cell that would bypass concerns of tumor formation and the low engraftment rates of transplanted cells. We found that a combination of three transcription factors, Gata4, Mef2c, and Tbx5 (G, M, and T; GMT), could directly convert CFs into iCMs [19]. The iCMs had cardiomyocyte characteristics such as well-organized sarcomeric structures, global gene expression profiles, action potentials, and spontaneous contractions. To utilize the direct reprogramming approach in clinical situations, it would be necessary to translate the mouse system into humans. Unfortunately, GMT was not sufficient for cardiac reprograming of human CFs. Human iCMs reprogramming requires more factors with GMT [20,21]. Despite low reprogramming efficiency and absence of spontaneous beating, the cells matured to exhibit action potentials and contract synchronously in coculture with murine cardiomyocytes. Recently, Cao et al. demonstrated human fibroblasts were converted into cardiomyocyte-like cells with a combination of nine chemical compounds which were used for chemical reprogramming to iPSCs [22]. Thus, human cardiac reprogramming is more challenging than in mouse cells. The difficulty could be owing to the difference in the cellular context, such as chromatin, proteome, and metabolome, between mouse and human fibroblasts. These findings of human cardiac reprogramming represent an important step toward potential clinical applications.

Modification of signaling pathways and epigenetics

During direct cardiac reprogramming, a variety of signaling pathways, such as transforming growth factor-β (TGF-β), rho-associated kinase (ROCK), WNT, Notch and Akt, interact with each other, therefore modification of these pathways may affect reprograming efficiency. Notably, the TGF-β pathway is one of the active pathways in fibroblasts. Ifkovits et al. found that inhibition of TGF-β increased cardiac reprogramming [23]. Subsequently, Mohamed et al. confirmed TGF-β and Wnt signaling inhibition greatly enhanced cardiac reprogramming in adult human CFs [24]. Thus, profibrotic signaling acted as a barrier to cardiac reprogramming, which must be subsequently suppressed for successful conversion.

In addition to profibrotic signaling, epigenetic barriers are other hurdles to the direct reprogramming process. To achieve successful reprogramming, reprogramming factors must be able to engage genes that are developmentally silenced and inappropriate for expression in the starting cell population. Epigenetic state controls accessibility of transcription factors by marking with histone methylation, acetylation, and ubiquitination [19]. Recently, Zhou et al. identified Bmi1, polycomb complex protein, as a critical epigenetic barrier to direct cardiac reprogramming [25]. The authors demonstrated that Bmi1 regulated key cardiogenic genes through direct binding at these loci in fibroblasts, and Bmi1 inhibition promoted an open chromatin status. Thus, modification of signaling pathways and epigenetic state provably contribute to improve iCM induction, but a large part of molecular mechanisms during cardiac reprogramming is still elusive. It is intriguing to compare the complex molecular path of direct reprogramming with that of heart development.

In vivo cardiac repair and regeneration

The ultimate goal of direct reprogramming is to repair damaged heart and improve cardiac function by converting endogenous cardiac fibroblasts to CMs. Several studies reported *in vivo* direct reprogramming by delivery of reprogramming factors (GMT, GMT and Hand2, miR combo) to ischemic hearts in mice [26–28]. To demonstrate these iCMs were originated from resident cardiac fibroblasts, some groups used lineage-tracing experiments to confirm that resident CFs and non-cardiomyocytes were the origin of iCMs, which were not derived from cell fusion with resident CMs. Interestingly, these studies demonstrated that *in vivo* iCMs more closely resemble endogenous cardiomyocytes than those produced *in vitro*. This may result from factors within the native microenvironment, such as extracellular matrix, secreted proteins, and tissue stiffness. Although *in vivo* reprogramming can improve cardiac function and fibrosis after MI, the use of retroviral and lentiviral vectors leads to insertional mutagenesis. To achieve translation of direct cardiac reprogramming to clinical applications, non-integrating methods were demanded. Recently, we developed a polycistronic Sendai virus vector expressing GMT (SeV-GMT), and demonstrated direct reprogramming *in vitro* and *in vivo* [29]. SeV, non-segmented, negative-stranded RNA virus replicates only in the cytoplasm and does not integrate into the host genome. Notably, this new integration-free reprogramming vector promoted the efficiency of cardiac reprogramming *in vivo*, which resulted in improved cardiac function and reduced fibrosis of mice after MI compared to conventional retroviral injection of GMT. Mechanistically, robust transgene expression could achieve efficient cardiac reprogramming with SeV-GMT. Although further refinements are needed, cardiac reprogramming with SeV-GMT might be a potential treatment for heart diseases in future.

Conclusions

The existing therapeutic approaches for heart failure are unable to prevent remodeling process completely and to repair the injured heart. Since heart has a limited self-renewing capacity, establishment of a strategy for heart regeneration has been desired. Steady progress has been made in the heart regeneration field over the past years. The cell-based therapy has been suggested as a promising approach for heart regeneration. However, the results from clinical trials using somatic stem cells revealed modest effects on cardiac function. One of the reasons for this discouraging result may be due to low engraftment of transplanted cells. It is likely that further examination of cell dose, delivery route and timing of injection, and new technologies such as biomaterial-based delivery system and tissue-engineering techniques may overcome these problems. Notably, the progress of iPSC field has grown enormously in past years, and a first-in-man clinical trial using iPSC-derived cardiomyocytes has been planned by Dr Sawa and colleagues in 2018, in which the iPSC-derived cardiomyocyte sheets were transplanted into the patients with ischemic cardiomyopathy.

Direct cardiac reprogramming has great potential to become one of the main streams of regenerative medicine in heart failure. Cardiac fibroblasts account for a large population of cells in the heart, which become activated and turn to myofibroblasts, contributing to fibrosis after cardiac injury such as MI. After the discovery of three cardiac reprograming factors, technology of direct reprogramming in hearts made dramatic progress toward translation to clinical application. However, there are several hurdles we must overcome prior to clinical trials. First, the reprogramming efficiency remains low and the generated iCMs show heterogeneous maturity, although the reprogramming efficiency has been improved with identification of additional transcription factors, microRNAs, chemical compounds, development of techniques to modify epigenetic states, and conditioning of culture medium. Second, we need to optimize the standard protocol for generation of iCMs. The molecular mechanisms during cardiac reprogramming remain elusive. Understanding the molecular mechanisms might provide a novel technology of cardiac regeneration.

Finally, experiments in chronic heart failure models are required. Although *in vivo* cardiac reprogramming has improved cardiac function and fibrosis, all *in vivo* studies were performed in the acute stage of MI. It remains unknown whether *in vivo* reprogramming could be applied to chronic heart failure models, in which regenerative medicine is in high demand.

Despite many challenges remaining, continuation of basic research may offer a bright future for cardiac regeneration to treat heart failure.

Conflict of interest

The authors declare no conflict of interest.

Funding

Masaki Ieda received research grants from the Advanced Research & Development Programs for Medical Innovation solotype, the Research Center Network for Realization of Regenerative Medicine, and the Practical Research Project for Rare/Intractable Diseases from Japan Agency for Medical Research and Development (AMED), the Japan Society for the Promotion of Science (JSPS) (17H04179, 17K19678), and Takeda Science Foundation.

Acknowledgments

We are grateful to the members of the Ieda laboratory for valuable discussion and contributions.

References

[1] Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN, et al. Transient regenerative potential of the neonatal mouse heart. Science 2011;331:1078–80.
[2] Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabe-Heider F, Walsh S, et al. Evidence for cardiomyocyte renewal in humans. Science 2009;324:98–102.
[3] Behfar A, Crespo-Diaz R, Terzic A, Gersh BJ. Cell therapy for cardiac repair – lessons from clinical trials. Nat Rev Cardiol 2014;11:232–46.
[4] Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 2003;114:763–76.
[5] Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, et al. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. Lancet 2012;379:895–904.
[6] Hare JM, Fishman JE, Gerstenblith G, DiFede Velazquez DL, Zambrano JP, Suncion VY, et al. Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. JAMA 2012;308:2369–79.
[7] Dixon JA, Gorman RC, Stroud RE, Bouges S, Hirotsugu H, Gorman 3rd JH, et al. Mesenchymal cell transplantation and myocardial remodeling after myocardial infarction. Circulation 2009;120:S220–9.
[8] Bolli R, Tang XL, Sanganalmath SK, Rimoldi O, Mosna F, Abdel-Latif A, et al. Intracoronary delivery of autologous cardiac stem cells improves cardiac function in a porcine model of chronic ischemic cardiomyopathy. Circulation 2013;128:122–31.
[9] Bolli R, Chugh AR, D'Amario D, Loughran JH, Stoddard MF, Ikram S, et al. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. Lancet 2011;378:1847–57.
[10] van Berlo JH, Kanisicak O, Maillet M, Vagnozzi RJ, Karch J, Lin SC, et al. c-kit+ cells minimally contribute cardiomyocytes to the heart. Nature 2014;509:337–41.
[11] Kawamura M, Miyagawa S, Miki K, Saito A, Fukushima S, Higuchi T, et al. Feasibility, safety, and therapeutic efficacy of human induced pluripotent stem cell-derived cardiomyocyte sheets in a porcine ischemic cardiomyopathy model. Circulation 2012;126:S29–37.
[12] Qiao H, Zhang H, Yamanaka S, Patel VV, Petrenko NB, Huang B, et al. Long-term improvement in postinfarct left ventricular global and regional contractile function is mediated by embryonic stem cell-derived cardiomyocytes. Circ Cardiovasc Imaging 2011;4:33–41.
[13] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861–72.
[14] Shiba Y, Gomibuchi T, Seto T, Wada Y, Ichimura H, Tanaka Y, et al. Allogeneic transplantation of iPS cell-derived cardiomyocytes regenerates primate hearts. Nature 2016;538:388–91.
[15] Tohyama S, Hattori F, Sano M, Hishiki T, Nagahata Y, Matsuura T, et al. Distinct metabolic flow enables large-scale purification of mouse and human pluripotent stem cell-derived cardiomyocytes. Cell Stem Cell 2013;12:127–37.
[16] Mohamed TMA, Ang YS, Radzinsky E, Zhou P, Huang Y, Elfenbein A, et al. Regulation of cell cycle to stimulate adult cardiomyocyte proliferation and cardiac regeneration. Cell 2018;173. 104–16 e12.
[17] Tao G, Kahr PC, Morikawa Y, Zhang M, Rahmani M, Heallen TR, et al. Pitx2 promotes heart repair by activating the antioxidant response after cardiac injury. Nature 2016;534:119–23.
[18] Nakada Y, Canseco DC, Thet S, Abdisalaam S, Asaithamby A, Santos CX, et al. Hypoxia induces heart regeneration in adult mice. Nature 2017;541:222–7.
[19] Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, et al. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. Cell 2010;142:375–86.
[20] Nam YJ, Song K, Luo X, Daniel E, Lambeth K, West K, et al. Reprogramming of human fibroblasts toward a cardiac fate. Proc Natl Acad Sci U S A 2013;110:5588–93.
[21] Wada R, Muraoka N, Inagawa K, Yamakawa H, Miyamoto K, Sadahiro T, et al. Induction of human cardiomyocyte-like cells from fibroblasts by defined factors. Proc Natl Acad Sci U S A 2013;110:12667–72.
[22] Cao N, Huang Y, Zheng J, Spencer CI, Zhang Y, Fu JD, et al. Conversion of human fibroblasts into functional cardiomyocytes by small molecules. Science 2016;352:1216–20.
[23] Ifkovits JL, Addis RC, Epstein JA, Gearhart JD. Inhibition of TGFbeta signaling increases direct conversion of fibroblasts to induced cardiomyocytes. PLoS ONE 2014;9:e89678.
[24] Mohamed TM, Stone NR, Berry EC, Radzinsky E, Huang Y, Pratt K, et al. Chemical enhancement of in vitro and in vivo direct cardiac reprogramming. Circulation 2017;135:978–95.
[25] Zhou Y, Wang L, Vaseghi HR, Liu Z, Lu R, Alimohamadi S, et al. Bmi1 is a key epigenetic barrier to direct cardiac reprogramming. Cell Stem Cell 2016;18:382–95.
[26] Qian L, Huang Y, Spencer CI, Foley A, Vedantham V, Liu L, et al. In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. Nature 2012;485:593–8.
[27] Song K, Nam YJ, Luo X, Qi X, Tan W, Huang GN, et al. Heart repair by reprogramming non-myocytes with cardiac transcription factors. Nature 2012;485:599–604.
[28] Jayawardena TM, Finch EA, Zhang L, Zhang H, Hodgkinson C, Pratt RE, et al. MicroRNA induced cardiac reprogramming in vivo: evidence for mature cardiac myocytes and improved cardiac function. Circ Res 2015;116:418–24.
[29] Miyamoto K, Akiyama M, Tamura F, Isomi M, Yamakawa H, Sadahiro T, et al. Direct in vivo reprogramming with Sendai virus vectors improves cardiac function after myocardial infarction. Cell Stem Cell 2018;22. 91–103 e5.