

# Recent advances in myocardial regeneration strategy

Journal of International Medical Research


2019, Vol. 47(11) 5453–5464

© The Author(s) 2019

Article reuse guidelines:

[sagepub.com/journals-permissions](http://sagepub.com/journals-permissions)

DOI: 10.1177/0300060519862663

[journals.sagepub.com/home/imr](http://journals.sagepub.com/home/imr)**Kai Sheng<sup>1</sup>, Yu Nie<sup>1,2</sup> and Bingren Gao<sup>3</sup> **

## Abstract

This review described the current status of research into the regeneration potential of myocardial cells after myocardial injury, focussing on possible mechanisms of regeneration and the application of animal models to human biology, all with the aim of evaluating any novel approaches to the regeneration of human cardiomyocytes. A literature review was undertaken of the PubMed<sup>®</sup> and The Cochrane Library databases using the search terms ‘regeneration’, ‘heart regeneration’, ‘cardiac regeneration’, ‘proliferation’, ‘animal model’, ‘repair’ and ‘myocardial cell injury’ in English language publications only. The search covered publications between 1 January 2002 to 31 December 2017. The cardiac regeneration capability significantly differed among different species. In lower vertebrates, such as zebrafish, cardiomyocytes possess a sustained regeneration capacity under specific conditions. In mammalian animals, such as mice, the cardiomyocytes retain a regeneration capability under specific conditions, which gradually declines. Inflammation, non-coding RNA, gene regulatory elements, signal transduction and cell phenotype transformation play pivotal roles in cardiomyocyte regeneration. Myocardial regeneration appears to be a viable repair strategy for cardiomyocyte loss, which deserves further research in order to validate its clinical applicability in humans.

## Keywords

**Heart regeneration, proliferation, animal model, repair, cardiomyocyte injury**

Date received: 8 April 2019; accepted: 19 June 2019

<sup>1</sup>Department of Cardiac Intensive Care Unit, Lanzhou University Second Hospital, Lanzhou, Gansu Province, China

<sup>2</sup>State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Centre for Cardiovascular Disease, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

<sup>3</sup>Department of Cardiac Surgery, Lanzhou University Second Hospital, Lanzhou, Gansu Province, China

### Corresponding author:

Bingren Gao, Department of Cardiac Surgery, Lanzhou University Second Hospital, 82 Cuiyingmen Road, Lanzhou 730000, Gansu Province, China.

Email: [gaobingren@yandex.com](mailto:gaobingren@yandex.com)



## Introduction

Heart failure is a serious, advanced-stage manifestation of heart disease and has become a major global public health problem.<sup>1</sup> The primary cause of the onset and progression of heart failure is the loss of myocardial cells and their regeneration capability.<sup>2</sup> The human heart has almost no ability to replenish the myocardial cells, which can subsequently be replaced by fibrous tissue, resulting in the heart gradually losing function.<sup>3</sup> Drugs, cardiac resynchronization therapy, intra-aortic balloon pumping, extracorporeal membrane oxygenation and Impella devices have been widely recognized and applied in clinical practice.<sup>4</sup> However, none of these solutions can resolve the problems of heart repair and regeneration. Although heart transplantation fundamentally resolves these issues, the serious shortage of donor hearts severely limits its widespread application by surgeons. Therefore, it is necessary to investigate the clinical efficacy of cardiac regeneration in the management of heart failure. In this review article, the relevant studies were reviewed and summarized to deepen the understanding of the clinical significance of myocardial regeneration strategies.

## Search strategy

The literature search involved a MeSH word search combined with a free search. The retrieval strategy included the following search terms: 'regeneration' or 'heart regeneration' or 'cardiac regeneration'; 'proliferation'; 'animal model'; 'repair'; 'myocardial cell injury'. The search covered publications published between 1 January 2002 to 31 December 2017. Irrelevant documents and some documents with low impact factors were eliminated. The search was limited to publications published in English. The PubMed<sup>®</sup> and The Cochrane

Library databases were searched. Relevant documents on cardiac regeneration were searched. Documents were manually removed if the Journal Citation Reports impact factor was  $\leq 4.0$  or if the report was unrelated to the theme of cardiac regeneration.

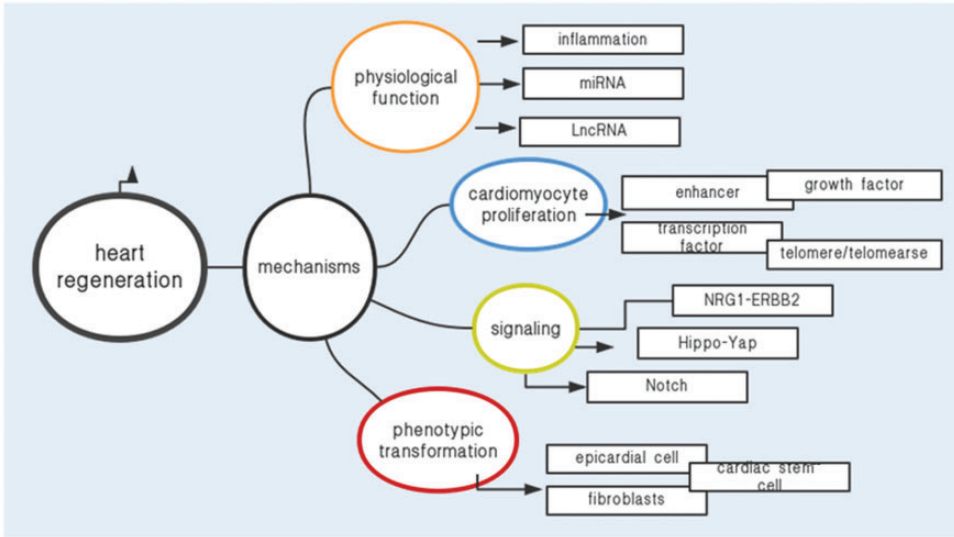
## Cardiac regeneration

Cardiomyocytes were previously considered to be irreplaceable terminally-differentiated cells. Nevertheless, in 2002, researchers observed that cardiomyocytes could be completely restored within 2 months after the removal of the apex of adult zebrafish, accounting for approximately 20% of the ventricular area.<sup>1</sup> Since then, research into the mechanisms of cardiac regeneration has attracted widespread attention from scientists and physicians. In 2014, researchers from Australia reported that the mammalian heart still has a temporary regenerative capacity during the embryonic and early neonatal stage.<sup>2</sup> In addition, cardiomyocytes can regenerate after surgical resection of the left ventricular apex in 1-day-old newborn mice, whereas this regenerative capacity had spontaneously diminished in 7-day-old mice.<sup>3</sup> The mechanisms underlying the process of heart regeneration are illustrated in Figure 1.

## Effect on promoting the proliferation of cardiomyocytes

### Inflammation

An acute inflammatory reaction occurs after myocardial cells are damaged, and neutrophils and macrophages are rapidly recruited, which can release various cytokines to regulate the microenvironment of the damaged myocardium.<sup>5</sup> It is generally believed that acute inflammation exerts a negative effect upon cellular regeneration by promoting scar formation, whereas



**Figure 1.** Flowchart of potential mechanisms underlying the process of cardiac regeneration. miRNA, microRNA; LncRNA, long non-coding RNA; NRG1, neuregulin-1; ERBB2, erythroblastic leukaemia viral oncogene homolog 2.

related animal experiments have reached the opposite conclusion.<sup>6</sup> In 2015, yeast glycan A was microinjected into the myocardium of newborn mice receiving apical resection and demonstrated that acute inflammation mediated by interleukin-6-signal transducer and activator of transcription 3 plays a role in promoting cardiac regeneration in newborn mice.<sup>4</sup> In addition, another study reported that embryo-derived macrophages can promote regeneration through myocardial cell proliferation and angiogenesis.<sup>5</sup> Macrophages have also been shown to promote cardiac angiogenesis in newborn mice.<sup>6</sup> The above research collectively demonstrates that acute inflammation and inflammatory cells can stimulate cardiac regeneration in newborn mice, but this effect yields completely different results between 1-day-old and 14-day-old mice, indicating that age may be the key determinant in myocardial regeneration or fibrous scar repair. In addition, alternative factors that may affect the standardization of surgery, such as resection scope, injury

degree and surgeon proficiency, will also lead to different research results, which remains to be elucidated by subsequent investigations.

### microRNAs

MicroRNAs (miRNAs) are short non-coding regulatory RNA molecules with a molecular length of approximately 22 nucleotides, which regulate gene expression by binding to complementary sequences on their target mRNAs.<sup>7</sup> According to the different effects of miRNA upon cardiac regeneration, these molecules have been divided into regenerative, inhibitory and autophagy types of miRNAs.<sup>8</sup>

With regard to regenerative miRNA, a function screening study that examined a whole genome library found that miR-590 and miR-199a could significantly promote cardiac regeneration through the proliferation of cardiomyocytes, preventing the deterioration of cardiac function after acute myocardial infarction (AMI).<sup>7</sup>

With regard to inhibitory miRNA, a study found that the knockdown of miR-15 can prolong the time window for the proliferation of cardiomyocytes after AMI in 1-day-old mice.<sup>8</sup> Inhibition of miR-15 can promote the proliferation of cardiomyocytes and improve ejection fraction.<sup>8</sup> Similarly, another study demonstrated that the downregulation of miR-26a can upregulate the high expression of *EZH2* gene in the downstream signalling pathway in the injured myocardium of zebrafish and mice, thereby stimulating the proliferation of cardiomyocytes.<sup>9</sup> In addition, *EZH2* is conducive to the proliferation of cardiomyocytes and the effect is gradually strengthened along with the differentiation and expression of cardiomyocytes.<sup>9</sup> A previous study demonstrated that the levels of Sirt1, cell cycle protein D1 and Bcl2 proteins are associated with cell proliferation and survival *in vivo* and *in vitro* in newborn and adult mouse myocardial infarction (MI) models with coronary ligation,<sup>10</sup> indicating that miR-15, miR-26a and miR-34a can be regarded as inhibitory miRNA, and long-term suppression of miR through anti-miR can promote the cardiac regeneration instead.

Autophagy is a pathophysiological process by which cardiomyocytes maintain intracellular homeostasis and activation of autophagy can promote cardiac regeneration.<sup>11</sup> As mentioned previously, the ability of cardiomyocytes to regenerate gradually disappears with prolonged injury time. Therefore, it has been speculated that the specific deletion of the autophagic *Atg5* gene in the mouse heart leads to the inability of myocardial regeneration, which is characterized by decreased cardiac function, cardiac hypertrophy and fibrosis.<sup>11</sup> With regard to autophagic miRNA, miRNAs have been identified as a key regulatory factor for cardiac pathophysiology,<sup>11</sup> but little research has been performed on the role of miRNA in regulating cardiac autophagy.<sup>11</sup> Recent research has

demonstrated that inhibition of miR-22 can activate cardiac autophagy and improve cardiac function after AMI, especially in the aging myocardium.<sup>11</sup> Research using fluorescence-activated cell sorting to measure the autophagic flux in cardiomyocytes following the transfection of a precursor miRNA library of 380 miRNAs found that miR-22 is highly expressed in elderly mice with myocardial infarction.<sup>11</sup> Pharmacological inhibition of miR-22 in the infarction area could activate autophagy and the heart function was significantly improved compared with that in the control group.<sup>11</sup> However, this effect was not evident in young mice with low levels of miR-22.<sup>11</sup> In a human cohort study, the serum level of miR-22 was negatively correlated with an early mortality rate in 198 patients with systolic heart failure.<sup>11</sup>

The above-mentioned experiments collectively demonstrate that although miRNA can regulate autophagy and improve the function of aging myocardium, the limitations have to be acknowledged. miRNAs appear to exert their effects upon multiple targets probably through the cumulative effects of different miRNAs rather than a single miRNA. All the above studies were conducted based on the regulation of a single miRNA, so they cannot be directly applied to clinical practice. How to establish a one-to-one drug target or regulate multiple targets simultaneously is the direction of future research.

### **LncRNAs**

Long non-coding RNAs (lncRNA) are another category of non-coding RNA that are distinct from miRNAs, which are defined as being >200 base pairs in length and highly structurally conserved.<sup>12</sup> LncRNAs play a pivotal role in post-transcriptional regulation, whereas the role of lncRNAs in angiogenesis and remodeling remains poorly understood.<sup>13</sup>

Moreover, research related to the origin of coronary artery development has primarily focused on the initial stage of angiogenesis in the embryonic stage.<sup>13</sup> The relationship between lncRNAs and the development of coronary arteries after birth has been seldom studied.<sup>13</sup> A previous study found that heart regeneration damage is correlated with the decrease in the quantity of coronary vessels.<sup>12</sup> A study using genetic lineage tracing demonstrated that the majority of the coronary vessels in newborn mice appear after birth rather than expanding from the pre-existing embryonic vasculature.<sup>13</sup> Silencing of the expression of metastasis-related lung adenocarcinoma transcript (MALAT1, an lncRNA) in the vascular endothelial cells through small interfering RNA and pharmacological inhibition induced endothelial cells to change to a migratory but nonproliferative phenotype, resulting in impaired angiogenesis.<sup>12</sup> The technical strategies employed to specifically inhibit target RNA expression *in vivo* remain in their infancy and more research is required to determine how best to accurately and effectively target the post-transcriptional effects of lncRNAs. However, these preliminary findings are undoubtedly promising and collectively demonstrate that upregulating the expression of lncRNAs can promote the proliferation and regeneration of vascular endothelial cells.<sup>12</sup> However, more evidence-based studies are required before firm conclusions can be drawn. In addition, these preliminary findings were made under anoxic conditions, so whether these findings apply to a wider range of situations remains to be elucidated.

Taken together, these preliminary findings suggest that lncRNAs play an increasingly important role in coronary heart disease, heart failure and hypertension.<sup>12</sup> We speculate that certain lncRNAs might be used as diagnostic markers and novel therapeutic targets for cardiovascular

diseases in the future. However, we acknowledge that the quantity of detected circulating lncRNA is extremely small, the cell source is unclear and the relationship between these lncRNAs and cardiac regeneration does not properly correspond, which significantly decreases the sensitivity and specificity. In addition, we speculate that lncRNAs probably coordinate with miRNAs, but the relationship between them remains largely unknown. It is of necessity to improve RNA sequencing technology and explore the significance of lncRNAs in cardiac regeneration in clinical practice.

## Regulatory factors for promoting myocardial cell proliferation

### Enhancers

Enhancers can upregulate the expression levels of the target genes and can be utilized to regulate tissue specificity during embryo development.<sup>13</sup> A previous study revealed that leptin b is highly expressed in the myocardium of zebrafish and newborn mice after injury through transcriptomic analysis, suggesting that enhancers probably have the potential to promote myocardial cell proliferation and regulate cardiac regeneration.<sup>14</sup>

### Transcription factors

The GATA-4 zinc-finger transcription factor plays a crucial role in embryonic and cardiac development and angiogenesis.<sup>15</sup> The expression of the *GATA4* gene was upregulated in the injured myocardium of mice after cardiac apex resection before that of the surrounding intact cardiomyocytes with proliferative capacity.<sup>16</sup> The proliferation of cardiomyocytes was enhanced and the cardiac function was significantly improved at 4 weeks after cardiac infarction in MI mouse models that over-expressed Tbx20.<sup>17</sup> The proliferation rate of

cardiomyocytes was increased by nearly three times by the knockdown of the transcript factor *Meis1* in myocardial cells after MI in newborn mice because overexpression of *Meis1* mediates the withdrawal of the cardiomyocytes from the cell cycle by activating cyclin-dependent kinase (CDK) inhibitors, such as P15, P16 and P21, resulting in decreased myocardial cell proliferation and impaired ejection fraction.<sup>18</sup>

### **Growth factors**

The interaction between growth factors and transcription factors can regulate cell proliferation and is a potential approach to develop candidate drugs for cardiac regeneration. For example, a study that specifically induced the deletion of *GATA4* gene expression using a newborn mouse model of heart freezing and apical resection found that the cardiac function was gradually decreased along with the downregulation of fibroblast growth factor-16.<sup>19</sup> Another study established a neonatal mouse model with frozen myocardial cells to induce cardiac dysfunction.<sup>20</sup> The recombinant growth factor neuromodulator protein-1 (rNRG1) was administered to mice from birth to 34 days, which improved cardiac function and reduced the incidence of transmural myocardial scarring.<sup>20</sup> In the biological experiment using human cardiomyocytes, the application of rNRG1 improved cardiac function and promoted the development of cardiomyocytes.<sup>20</sup> Although NRG1 can promote cardiac regeneration, it may exert nerve or carcinogenic effects, which limits its wider clinical application.<sup>20</sup>

### **Telomeres and telomerase**

Researchers have adopted adenovirus to express cardiac specific telomerase (Tert).<sup>21</sup> Compared with the control

group, the expression of Tert in a mouse model of MI was up-regulated and the degree of cardiac enlargement, ejection fraction and infarction after the scar were also improved, and the survival rate was increased by 17%.<sup>21</sup> In addition, the elongation of the telomeres after Tert treatment yields a significant increase in the quantity of cardiomyocytes expressing Ki67 and pH3, suggesting that telomerase activation may be a potential strategy for myocardial cell regeneration.<sup>21</sup>

We think that unlike non-coding RNA sequences, the regulatory components represented by enhancers, transcription factors, growth factors and promoters play an important role in gene expression and function of cardiac regeneration. Researchers think that cardiac regeneration can be promoted by producing new cardiomyocytes by multiplication from the perspectives of inflammation, non-coding RNA sequences and gene transcription components. However, it is unconvincing to regard it as a unique strategy for myocardial regeneration because signalling pathway conduction also plays an important role in myocardial regeneration.<sup>21</sup> Which of these two mechanisms of proliferation and dedifferentiation of myocardial cells is the key process remain to be elucidated.

### **Signal transduction**

In recent years, many studies have shown that cardiac regeneration involves multiple signalling pathways and these may play a role in promoting cell proliferation by working on combination with the above-mentioned regulatory factors. A review of the three main signalling pathways is presented below.

#### ***NRG1-ERBB2* signalling pathway**

Neuregulin-1 (NRG1) activates the erythroblastic leukaemia viral oncogene homolog

(ERBB) kinase receptor family consisting of ERBB2, ERBB3 and ERBB4, which promote cardiac development and regenerate cardiomyocytes.<sup>21</sup> A previous study demonstrated that the levels of ERBB2 in the cardiomyocytes of neonatal, young and adult mice were regulated by an exogenous injection of doxycycline.<sup>21</sup> A functional study has further shown that the expression of ERBB2 can effectively alleviate the damage caused by the ligation of the left anterior descending branch in mouse models, characterized by regenerative manifestations, especially in the neonatal mice.<sup>21</sup>

### *Hippo-Yap signalling pathway*

The Hippo-Yap signalling pathway can activate insulin-like growth factor and Wnt to promote the proliferation and development of embryonic myocardial cells.<sup>22</sup> The specific knockout of Yap can hinder cardiac regeneration in newborn mice, leading to the repair of the myocardial scar.<sup>22</sup> In contrast, the persistent high expression of Yap in adult mouse hearts can promote cardiac regeneration and improve cardiac function.<sup>22</sup>

### *Notch signalling pathway*

The Notch signalling pathway exerts slight effects on the promotion of cardiac regeneration in adult mice, which contradicts the notion that the activation of the Notch signalling pathway serves as a means of inducing myocardial regeneration after MI.<sup>23</sup> Activation of the Notch signalling pathway plays an essential role in mediating the proliferation of cardiomyocytes after birth.<sup>23</sup> Whether the Notch signalling pathway can induce cell regeneration after MI in adult mice remains to be elucidated by subsequent investigations.

## **Transformation of cell phenotype**

The survival of cardiomyocytes depends upon both the promotion of their proliferation and signalling transduction, but the proliferation rate is extremely slow and limited.<sup>3</sup> Recent studies have reported that non-myocardial cells can be transformed into cardiomyocytes by phenotypic transformation, thereby playing a role in promoting cardiac regeneration.<sup>3</sup> This notion is completely different from the traditional thinking of accelerating cardiac regeneration by promoting the proliferation of cardiomyocytes. In the field of cardiac regeneration medicine, the exploration of a novel approach has attracted widespread attention and achieved rapid progress.<sup>4,5</sup> The key research in this field is summarized below.

### *Epicardial cells*

The epicardial membrane plays an important role in cardiac regeneration.<sup>24</sup> The reprogramming of epicardial cells into cardiomyocytes can be promoted by treating 7-day-old newborn mice that have received apical excision with thymosin (TB4).<sup>24</sup> TB4 may promote the reprogramming of Wilm's tumour 1-labeled embryonic epicardial cells into cardiomyocytes in 7-day-old mice, suggesting that TB4 treatment could restore the potential of cardiac regeneration in 7-day-old mice.<sup>24</sup> However, whether other circulating stem cells are involved in this process remains to be elucidated. It has been proposed that the epicardium is essential for muscle regeneration in the zebrafish model with innate heart regeneration, and the epicardium also participates in fibrotic responses in mammalian hearts.<sup>25</sup> This structure acts as a source of crucial cells including vascular smooth muscle cells, pericytes and fibroblasts.<sup>25</sup> The epicardium also secretes factors that are essential for

the proliferation and survival of cardiomyocytes.<sup>25</sup>

### Fibroblasts

Skeletal muscle fibroblasts can also be transformed into cardiomyocytes, which have been evaluated in animal experiments in the United States.<sup>26</sup> Nevertheless, this mode of transformation is inefficient and likely to lead to arrhythmia and heart failure.<sup>26</sup> A previous study investigated the role of epicardium-derived fibroblasts in myocardial infarction.<sup>27</sup> The authors concluded that epicardium-derived fibroblasts are the main origin of cardiac fibroblasts in the ischaemic heart.<sup>27</sup> Adult resident epicardium-derived fibroblasts contribute to the cardiac fibroblast compartment in a time- and disease-dependent manner, which may contribute to the development of new therapies to treat myocardial infarction.<sup>27</sup>

### Stem cells

A distinctive functional significance of Cdx2 cells beyond their established role in embryonic patterning has been demonstrated.<sup>28</sup> The therapeutic use of Cdx2 cells may represent a vital advance, as these cells are multipotent and immunologically naive, with a unique proteome, compared with embryonic stem cells.<sup>28</sup> Moreover, they exhibit the ability to selectively home to sites of injury.<sup>28</sup> These characteristics pave the way for novel allogeneic stem cell therapy for cardiac diseases. Stem cells have unlimited and long-term capabilities of self-renewal and multi-directional differentiation, which are characterized by unlimited proliferative potential and lack of cell line markers. All these features can make one daughter cell differentiate into a cell with a specific function, whereas the other daughter cell retains the characteristics of a stem cell.<sup>29</sup> At present, stem cell-based promotion of cardiac regeneration mainly

focuses on embryonic stem cells (ESC), bone marrow mesenchymal stem cells (BMSC), endogenous cardiac stem cells/progenitor cells (CS/PC) and induced pluripotent stem cells (iPSC).<sup>30</sup> We suggest that the commonly-used 'drug delivery' routes are peripheral intravenous injection, intracoronary injection via catheter, intracardiac injection and epicardial attachment. The effect of stem cells upon promoting cardiac regeneration is a hot topic in the field of cardiac regeneration. A previous study has shown that ESCs have the capability of self-renewal, clone formation and are multi-potent, which is the target of myocardial regeneration *in vivo*.<sup>30</sup> Compared with adult-derived cardiac stem cells (CSCs), CSCs obtained from neonatal counterparts have a higher ability to preserve myocardial function and enhance vascular function.<sup>30</sup> Nevertheless, neither adult nor neonatal cardiomyocytes can offer a suitable microenvironment for the differentiation from ESC into cardiomyocytes, which probably has multiple disadvantages, such as tumorigenicity, low graft survival rate, lack of sources and certain ethical issues.<sup>30</sup> Consequently, the role of ESC in accelerating cardiac regeneration has been evaluated in animal studies rather than clinical trials.<sup>30</sup>

In addition, a study has proposed that novel paracrine cytokines secreted by mesenchymal stem cells can promote heart repair through the PI3K–AKT–CDK7 bypass signalling pathway, suggesting that BMSC can differentiate into mature and functional cardiomyocytes.<sup>31</sup> Nevertheless, the ethics, tumorigenicity and safety issues constrain their widespread application in cardiac regeneration and repair. How to choose the best transportation mode for transplanted cells, how to improve the transplantation effect and enhance the survival rate of BMSC transplantation severely limits BMSC from becoming an effective approach to promote cardiac regeneration.



## CS/PC

Cardiac stem cells/progenitor cells include c-kit<sup>+</sup> cells, epicardial-derived cells, cardiosphere-derived cells and sca-1<sup>+</sup> cells.<sup>29</sup> All these cells can be differentiated into cardiomyocytes, smooth muscle cells or endothelial cells.<sup>30</sup> Therefore, CS/PC can be isolated from the autologous body and differentiated into cardiomyocytes by directional induction *in vitro* to maintain the proliferative ability, and then these cells can be transplanted back into the host body, which might promote cardiac regeneration.<sup>32</sup> Some researchers have successfully injected c-kit<sup>+</sup> into the diseased heart, which yielded diverse effects.<sup>29</sup> Taken together, the application of CS/PC to promoting cardiac regeneration requires further investigations.

## iPSC

Induced pluripotent stem cells can differentiate into cardiomyocytes and they share the same characteristics as ESC-derived cells.<sup>29</sup> Nevertheless, the efficiency of iPSC differentiation into cardiomyocytes is extremely low.<sup>33</sup> In addition, iPSC-differentiated cardiomyocytes are immature and not suitable for human cardiac regeneration models, which have multiple disadvantages, such as teratogenicity and arrhythmias.<sup>33</sup> Therefore, the potential use of iPSC for cardiac regeneration is not promising.

## Research and review limitations

In terms of the critical work that is being undertaken in this field, cardiac regeneration is at the cutting edge and the potential to transfer the findings into the clinic are great. The regeneration of myocardium by stem cells is currently the most controversial topic in this field. Although the use of stem cells with the same properties as their own host cells to regenerate new myocardial cells would be the ideal situation and this

research goal is being actively pursued, there is still a long way to go before stem cells can be used to regenerate the myocardium. With the improvement in technology, in-depth research will be able to explore other mechanisms of myocardial regeneration to find possible solutions to regenerate the heart.

In terms of limitations with the previous research in this field, there were some methodological problems in the original studies. First, the time-point used for the animal models of myocardial cell injury is extremely important and there is no clear standardization of surgery-induced myocardial cell injury in these models. There is no fixed and quantitative standard for the scope, degree and time of myocardial cell injury. This may be one reason why different experiments have drawn different conclusions at the time-point of myocardial regeneration. Secondly, extracellular matrix (ECM) proteins may also play a role in promoting cardiac regeneration. In this review, different viewpoints are simply presented rather than being summarized or analysed, which is a limitation of the scope of the review.

## Conclusion

This review summarizes the recent progress made in research into cardiac regeneration strategies based on the three major mechanisms of action: (i) promoting myocardial cell proliferation; (ii) signal transduction; and (iii) cell phenotype transformation. However, several limitations have to be acknowledged. First, previous research demonstrated that unlike the cardiomyocyte regeneration that occurs after apical resection in 1-day-old mice, cardiomyocyte regeneration is inhibited after permanent ligation of the left anterior descending branch, indicating that different injury models appear to result in different mechanisms and outcomes of myocardial

repair.<sup>32,33</sup> Secondly, the standards of cardiac surgery have not consistent between studies and it is difficult to establish a fixed quantitative standard for the extent and degree of myocardial cell injury and the time of injury, which may explain the contradictory findings demonstrated by different studies.<sup>33</sup> This review also has some limitations. Limb regeneration requires the control of the peripheral nervous system, but whether myocardial regeneration requires neuronal control remains poorly understood. Although 2-day-old neonatal mouse models have been established and have demonstrated that cardiomyocyte regeneration requires the control of autonomic nerves after resection of the left ventricular apex,<sup>34</sup> there has been no further explanation of the different roles of different branches of the sympathetic and parasympathetic nervous systems during the process of cardiac regeneration. It should also be noted that ECM proteins also play a role in promoting cardiac regeneration.<sup>35,36</sup> This review has not summarized and analysed other important mechanisms that might affect cardiac regeneration, which need to be validated by additional animal research and clinical trials. Undoubtedly, cardiac regeneration will be a feasible strategy for the treatment of myocardial cell loss. In our opinion, cardiac regeneration can be expected to be used to treat cardiac cell loss in clinical practice in the future, which should bring clinical benefits to patients with cardiovascular diseases.

#### Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

#### Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

#### ORCID iD

Bingren Gao  <https://orcid.org/0000-0003-2617-8301>

#### References

1. Ezekowitz JA, O'Meara E, McDonald MA, et al. 2017 Comprehensive Update of the Canadian Cardiovascular Society Guidelines for the Management of Heart Failure. *Can J Cardiol* 2017; 33: 1342–1433.
2. Bui AL, Horwich TB and Fonarow GC. Epidemiology and risk profile of heart failure. *Nat Rev Cardiol* 2011; 8: 30–41.
3. Bergmann O, Bhardwaj RD, Bernard S, et al. Evidence for cardiomyocyte renewal in humans. *Science* 2009; 324: 98–102.
4. Brogan TV, Lequier L, Lorusso R, et al (eds). *Extracorporeal Life Support: The ELSO Red Book*. 5th ed. Ann Arbo: ELSO, pp.488–489.
5. Lavine KJ, Epelman S, Uchida K, et al. Distinct macrophage lineages contribute to disparate patterns of cardiac recovery and remodeling in the neonatal and adult heart. *Proc Natl Acad Sci USA* 2014; 111: 16029–16034.
6. Aurora AB, Porrello ER, Tan W, et al. Macrophages are required for neonatal heart regeneration. *J Clin Invest* 2014; 124: 1382–1392.
7. Eulalio A, Mano M, Dal Ferro M, et al. Functional screening identifies miRNAs inducing cardiac regeneration. *Nature* 2012; 492: 376–381.
8. Porrello ER, Mahmoud AI, Simpson E, et al. Regulation of neonatal and adult mammalian heart regeneration by the miR-15 family. *Proc Natl Acad Sci U S A* 2013; 110: 187–192.
9. Crippa S, Nemir M, Ounzain S, et al. Comparative transcriptome profiling of the injured zebrafish and mouse hearts identifies miRNA-dependent repair pathways. *Cardiovasc Res* 2016; 110: 73–84.
10. Yang Y, Cheng HW, Qiu Y, et al. MicroRNA-34a plays a key role in cardiac repair and regeneration following myocardial infarction. *Circ Res* 2015; 117: 450–459.

11. Gupta SK, Foinquinos A, Thum S, et al. Preclinical development of a microRNA-based therapy for elderly patients with myocardial infarction. *J Am Coll Cardiol* 2016; 68: 1557–1571.
12. Michalik KM, You X, Manavski Y, et al. Long noncoding RNA MALAT1 regulates endothelial cell function and vessel growth. *Circ Res* 2014; 114: 1389–1397.
13. Tian X, Hu T, Zhang H, et al. Vessel formation. De novo formation of a distinct coronary vascular population in neonatal heart. *Science* 2014; 345: 90–94.
14. Kang J, Hu J, Karra R, et al. Modulation of tissue repair by regeneration enhancer elements. *Nature* 2016; 532: 201–206.
15. Hecklen-Klein A and Evans T. T-box binding sites are required for activity of a cardiac GATA-4 enhancer. *Dev Biol* 2004; 267: 490–504.
16. Kikuchi K, Holdway JE, Werdich AA, et al. Primary contribution to zebrafish heart regeneration by gata4(+) cardiomyocytes. *Nature* 2010; 464: 601–605.
17. Xiang FL, Guo M and Yutzey KE. Overexpression of Tbx20 in adult cardiomyocytes promotes proliferation and improves cardiac function after myocardial infarction. *Circulation* 2016; 133: 1081–1092.
18. Mahmoud AI, Kocabas F, Muralidhar SA, et al. Meis1 regulates postnatal cardiomyocyte cell cycle arrest. *Nature* 2013; 497: 249–253.
19. Yu W, Huang X, Tian X, et al. GATA4 regulates Fgf16 to promote heart repair after injury. *Development* 2016; 143: 936–949.
20. Polizzotti BD, Ganapathy B, Walsh S, et al. Neuregulin stimulation of cardiomyocyte regeneration in mice and human myocardium reveals a therapeutic window. *Sci Transl Med* 2015; 7: 281ra45.
21. D’Uva G, Aharonov A, Lauriola M, et al. ERBB2 triggers mammalian heart regeneration by promoting cardiomyocyte dedifferentiation and proliferation. *Nat Cell Biol* 2015; 17: 627–638.
22. Xin M, Kim Y, Sutherland LB, et al. Hippo pathway effector Yap promotes cardiac regeneration. *Proc Natl Acad Sci U S A* 2013; 110: 13839–13844.
23. Felician G, Collesi C, Lusic M, et al. Epigenetic modification at Notch responsive promoters blunts efficacy of inducing notch pathway reactivation after myocardial infarction. *Circ Res* 2014; 115: 636–649.
24. Rui L, Yu N, Hong L, et al. Extending the time window of mammalian heart regeneration by thymosin beta 4. *J Cell Mol Med* 2014; 18: 2417–2124.
25. Cao J and Poss KD. The epicardium as a hub for heart regeneration. *Nat Rev Cardiol* 2018; 15: 631–647.
26. Sanganalmath SK and Bolli R. Cell therapy for heart future: a comprehensive overview of experimental and clinical studies, current challenges, and future directions. *Circ Res* 2013; 113: 810–834.
27. Ruiz-Villalba A, Simón AM, Pogontke C, et al. Interacting resident epicardium-derived fibroblasts and recruited bone marrow cells form myocardial infarction scar. *J Am Coll Cardiol* 2015; 65: 2057–2066.
28. Vadakke-Madathil S, LaRocca G, Raedschelders K, et al. Multipotent fetal-derived Cdx2 cells from placenta regenerate the heart. *Proc Natl Acad Sci U S A* 2019; 116: 11786–11795.
29. Jesty SA, Steffey MA, Lee FK, et al. c-kit+ precursors support postinfarction myogenesis in the neonatal, but not adult, heart. *Proc Natl Acad Sci U S A* 2012; 109: 13380–13385.
30. Simpson DL, Mishra R, Sharma S, et al. A strong regenerative ability of cardiac stem cells derived from neonatal hearts. *Circulation* 2012; 126: S46–S53.
31. Beigi F, Schmeckpeper J, Pow-Anpongkul P, et al. C3orf58, a novel paracrine protein, stimulates cardiomyocyte cell-cycle progression through the PI3K-AKT-CDK7 pathway. *Circ Res* 2013; 113: 372–380.
32. Konfino T, Landa N, Ben-Mordechai T, et al. The type of injury dictates the mode of repair in neonatal and adult heart. *J Am Heart Assoc* 2015; 4: e001320.
33. Mahmoud AI, Porrello ER, Kimura W, et al. Surgical models for cardiac regeneration in neonatal mice. *Nat Protoc* 2014; 9: 305–311.

34. White IA, Gordon J, Balkan W, et al. Sympathetic reinnervation is required for mammalian cardiac regeneration. *Circ Res* 2015; 117: 990–994.
35. Bassat E, Mutlak YE, Genzelinakh A, et al. The extracellular matrix protein agrin promotes heart regeneration in mice. *Nature* 2017; 547: 179–184.
36. Li J, Gao E, Vite A, et al. Alpha-catenins control cardiomyocyte proliferation by regulating Yap activity. *Circ Res* 2015; 116: 70–79.