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Heart Regeneration in Adult Mammals after Myocardial Damage

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Heart regeneration remains a critical question in current basic research and clinical practice. The adult mammalian heart exhibits a very limited regeneration capacity. In contrast, adult zebrafish and neonatal mice retain a remarkable ability of heart regeneration after damage. Understanding the mechanisms of heart regeneration would be very valuable to help design efficient treatment strategies against myocardial damage and heart failure. While inherent regeneration of the heart occurs after damage with varying efficiency among species, regeneration may also be induced exogenously. In this study, we briefly review the different approaches and current progress in improving heart regeneration.

Key Words: Heart • Heart failure • Mice • Regeneration • Zebrafish

The mammalian heart is considered to be a post-mitotic organ with a very low capacity for regeneration. When the heart undergoes damage such as myocardial infarction (MI), it usually leads to a loss of billions of cardiomyocytes as well as damage to the peripheral vessels in the ischemic myocardium. Owing to the extremely low proliferation capability of adult cardiomyocytes, heart tissue regeneration is very limited, while scar formation takes place at the injured sites, leading to cardiac remodeling and a consequent decline in cardiac contractility and function.[1] In severe conditions, these events progressively lead to heart failure which is the end stage of all cardiac diseases. One fourth of cases of heart failure have been reported to be caused by MI.[2] The reported incidence of heart failure in humans ranges from 0.3% to 1% and increases with age, with the number reaching 6% in people above 65 years and 10% in those above 70 years.[2] In addition to its high incidence, the severe impact of MI on patients' quality and length of life is one of the main causes of death among elderly people.

For years, cardiomyocytes of postnatal mammals and humans were considered to be "terminally differentiated" and to be restrained in the G0 phase of the cell cycle throughout life.[3] This assumption was changed several years ago by Bergmann, who applied ^{14}C dating and proved the occurrence of cardiomyocyte renewal in the human heart, with a yearly rate gradually decreasing with age from 1% at 20 years of age to 0.4% at 75 years of age.[4] Approximately 45% of cardiomyocytes undergo regeneration throughout life.[4] However, the limited capacity of regeneration and proliferation of adult hearts still cannot compensate for the massive loss of cardiomyocytes in a single attack of MI. With the activation of repair-associated pathways following cardiac injury, the original injured sites of cardiac tissue are gradually occupied by fibrotic scars.[5]

In contrast to humans, zebrafish and salamanders, as vertebrates, possess a robust capacity of heart regeneration. In 2002, studies showed that adult zebrafish can undergo complete heart regeneration after a resection of ~20% of the ventricular myocardium.[6] Inflammatory cells infiltrated at the injury site 1 day post-resection,

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and 9 days later, a large amount of fibrosis could be observed in the apex. Newly formed ventricular wall appeared 30 days after the operation with little fibrosis remaining, and the heart regenerated completely in 60 days post-resection.[6,7] Understanding the inherent regeneration process of the heart as well as the mechanisms of proliferation of cardiomyocytes will definitely provide important information for improving the treatment for MI and heart failure.

Currently, exogenous strategies exist to induce heart regeneration after damage. These strategies mainly include transplantation of various types of stem cells, *in vivo* reprogramming of resident cardiac cells, and activating proliferation of existing cardiomyocytes. However, clinical studies have shown that strategies targeting regeneration of the heart tissues are not satisfactory. Heart transplantation remains the only option for patients with end-stage heart failure. More than 40 million people suffer from heart failure globally.[8] In China, chronic heart failure has also long been a major public health burden. The study of heart regeneration is therefore of profound significance to be able to understand and improve heart repair.

INHERENT REGENERATION CAPACITY OF THE HEART

Current clinical treatments for myocardial damage include angiotensin converting enzyme inhibitors, β-adrenergic blockers, and anti-platelet agents, which are used to inhibit excessive activation of neurohumoral regulation and improve patients' cardiac function. However, these treatments usually have no effect on healing the injuries and cannot radically recover damaged cardiac function.[9,10] Rather than simply alleviate the symptoms, only regenerative medicine can possibly regenerate a degenerated heart and permanently cure the disease. To date, studies in the field of cardiac regeneration have mainly focused on transplantation of different types of stem cells.[11-14] However, enhancing inherent heart regenerative capacity in mammals is an other important possibility for the treatment of cardiac damage.

Cardiac regeneration in adult zebrafish and neonatal mice

Zebrafish are commonly used model organisms in cardiac regeneration studies. Compared to mammals, zebrafish exhibit a potent regenerative capacity following tissue injury. After resection of 20% of the left ventricle, the damaged site will immediately form a hematoma and stop bleeding within a few seconds in adult zebrafish. Proliferation of cardiomyocytes then occurs and the heart undergoes complete regeneration in ~1-2 months.[6] Neonatal mice have been shown to undergo regeneration after apex resection in a process similar to that observed in zebrafish. Of note, neonatal mice have a higher blood pressure than zebrafish, and hypothermia anesthesia may help mitigate postoperative hemorrhage.[15]

Although it can serve as a model of cardiac regeneration, apex resection does not represent the whole pathologic process of MI.[16,17] The process of regeneration after MI in zebrafish was observed in a frost damage model, in which a cryoprobe immersed in liquid nitrogen was placed at the apex causing cardiomyocyte death.[16,17] The injured apex then regenerated in 60-90 days leaving collagen scars at the damaged site. Dry ice damage mainly led to the formation of fibrous scars, indicating that different damage may induce different regenerative processes.[16,17]

Myocardial regeneration replenishes lost myocardium

In lineage tracing experiments, new zebrafish cardiomyocytes have been found to be derived from dedifferentiation of pre-existing cardiomyocytes to form an electrically coupled contractile syncytium.[7,18,19] After resection, the pre-existing cardiomyocytes re-entered the cell cycle, with sarcomeres disorderly arranged and exhibiting profound deoxyribonucleic acid (DNA) synthesis.[19,20] Kikuchi et al. used zebrafish with reporter genes to re-express the cardiogenic transcription factor Gata4 after heart damage and found that the expression of Gata4 could activate epicardial cells. Moreover, Gata4-positive cardiomyocytes proliferated and migrated to the site of injury, indicating that cardiomyocyte migration played an important role in cardiac regeneration.[19] Cell lineage tracing techniques have shown that cardiomyocyte migration is essential in heart regeneration. The chemical signaling pathway Cxcl12a/Cxcr4b has also been shown to be essential in this process.[19] In addition, the transcription factor hand2 has been shown to expressed in heart regeneration in zebrafish, and its overexpression has been shown to promote cardiomyocyte proliferation.[20]

The hearts of neonatal mice can regenerate after ventricular resection, however, they become unable to regenerate 7 days after birth.[15] Neuregulin 1 (Nrg1) has been shown to play an important role in the cardiac regeneration of zebrafish and neonatal mice. After cardiac injury, the perivascular cells of zebrafish have been shown to highly express Nrg1,[21] and blocking the Nrg1 receptor Erbb2 with AG1478 inhibited cardiac regeneration in zebrafish. Adult zebrafish cardiomyocytes are mainly mononuclear cells, while adult mammalian cardiomyocytes are mainly binuclear and multinuclear cells. Similar to zebrafish, neonatal mouse cardiomyocytes are mainly mononuclear. Nrg1 can stimulate the proliferation of neonatal mouse cardiomyocytes.[22]

The role of the nervous system

Cholinergic nerves have also been shown to play an important role in the regeneration of certain organisms.[22] After blocking the transportation of acetylcholine, the proliferation of myocardial cells in adult zebrafish and neonatal mice has been shown to be inhibited after cardiac apical resection.[23] When cardiac vagotomy was performed on neonatal mice, scarring was formed 21 days after the cardiac apical resection, and the mortality of the mice increased. Another study reported that when sympathetic nerves of neonatal mice were blocked by chemical reagents, significant scarring developed 21 days after the cardiac apical resection.[24] A previous study showed that cyclin P2, cyclin-dependent kinase 4, growth factor Nrg1 and nerve growth factors all decreased because of vagotomy, and that after removing the vagus nerve, supplementations of growth factor Nrg1 and other nerve growth factors improved myocardial proliferation and regeneration.[23]

In addition to cardiomyocyte proliferation, revascularization has also been recorded in adult zebrafish and neonatal mice after cardiac apical resection.[15,23,25,26] Apical resection has also been shown to lead to activation of the epicardium which then plays a vital role in promoting the activities of vascular pericytes during cardiac regeneration and revascularization, and it has also been shown to be the origin of signals in the regeneration process.[15,23,25,26]

Cardiac regeneration and the role of inflammation during fibrosis

Inflammation is an indispensable process in repair after cardiac injury.[27-29] After cardiac apical resection, platelets accumulate in the lesion to form a hematoma, providing platelet-derived growth factors and regulating epicardial function and neovascularization. During heart regeneration in a zebrafish apical resection model, the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signaling pathway was activated in the myocardium near the injury.[30] In addition, if the super-suppressor l kB was over-expressed, the number of regenerated cardiomyocytes decreased and the heart did not regenerate completely.[30] The NF-kB signaling pathway is also activated after myocardial infarction in mammals.[31]

Inflammation produces double-facet effects during heart regeneration, causing both damage and repair to tissues. Macrophages of 1-day-old mice have been shown to promote regeneration, while macrophages of adult mice only have the ability to remove cell debris and secrete dissolution factors to promote scar formation.[28] Macrophages of 1-day-old mice have also been shown to regulate angiogenesis rather than the proliferation of cardiomyocytes.[28]

In summary, inherent regeneration is critical to determine the final repair effects in heart regeneration. Different species possess various heart regeneration capacities, and understanding why animals such as adult zebrafish and salamanders have potent heart regeneration abilities is important to help us design better treatments for patients with MI and heart failure (Table 1 and Figure 1).

INDUCING HEART REGENERATION BY EXOGENOUS METHODS

Inducing heart regeneration by stem cell transplantation

Stem cells are known for their ability of self-renewal and multipotency, and they possess multi-lineage differentiation potential and thus can develop into diverse cells.[32] A number of preclinical studies have used stem cell therapy for myocardial damage, and many have shown significant positive effects in animal MI models, including restoration of cardiac function and reduction of scar size.[33] Different types of stem cells have been transplanted and tested for their treatment effects on myocardial damage, including mesenchymal stem cells,

Table 1. Landmark studies of mechanisms regulating inherent myocardial regeneration in zebrafish and mice

Name	Animal	Materials/design	Main results	Refs
Ángel Raya et al., 2003	Zebrafish	Myosin light-chain2a(mlc2a)-enhanced GFP and CARP-EGFP transgenic zebrafish	Adult zebrafish have a remarkable capacity to regenerate the heart in a process that involves up-regulation of msxB and msxC genes.	16
Kazu Kikuchi et al., 2010	Zebrafish	Genetic fate-mapping approaches	Identify a population of cardiomyocytes that become activated after resection of the ventricular apex and contribute prominently to cardiac muscle regeneration.	40
Itou J et al., 2012	Zebrafish	Pharmacological blocking of Cxcr4 function and genetic loss of cxcr4b function	Migration of cardiomyocytes to the injury site is essential for heart regeneration and Cxcl12/Cxcr4b pathway plays an important role.	21
Mahmoud Al et al., 2015	Zebrafish and mouse	Tg(cmlc2:sema3aa)^{pd106}	Nerves are required for cardiomyocyte proliferation during both zebrafish and neonatal mouse heart regeneration.	24
Gemberling M et al., 2015	Zebrafish	βactin2: loxp-mCherry-STOP-loxp-DTA, cmlc2: CreER, NF-kB: eGFP, gata4: eGFP, gata5: eGFP and tcf21: nuceGFP transgenic zebrafish	NF-kB signaling acts as a key node between cardiac injury and tissue regeneration.	22
Porrello ER et al., 2011	Mouse	N/A	The hearts of 1-day-old neonatal mice can regenerate after partial apex resection, but this capacity is lost by 7 days of age.	15
White IA et al., 2015	Mouse	Cre/+; tdTomato/+	The profound regenerative capacity of the neonatal mammalian heart requires sympathetic innervation.	25
Auraro Ab Fau et al., 2014	Mouse	N/A	Macrophages provide necessary signals to drive angiogenesis and regeneration of the neonatal mouse heart.	28

CARP, CARP is a direct target of Nkx2.5 whose expression is limited to heart structures during cardiac development; DTA, diphtheria fragment A; EGFP (eGFP), enhanced green fluorescent protein; GFP, green fluorescent protein.

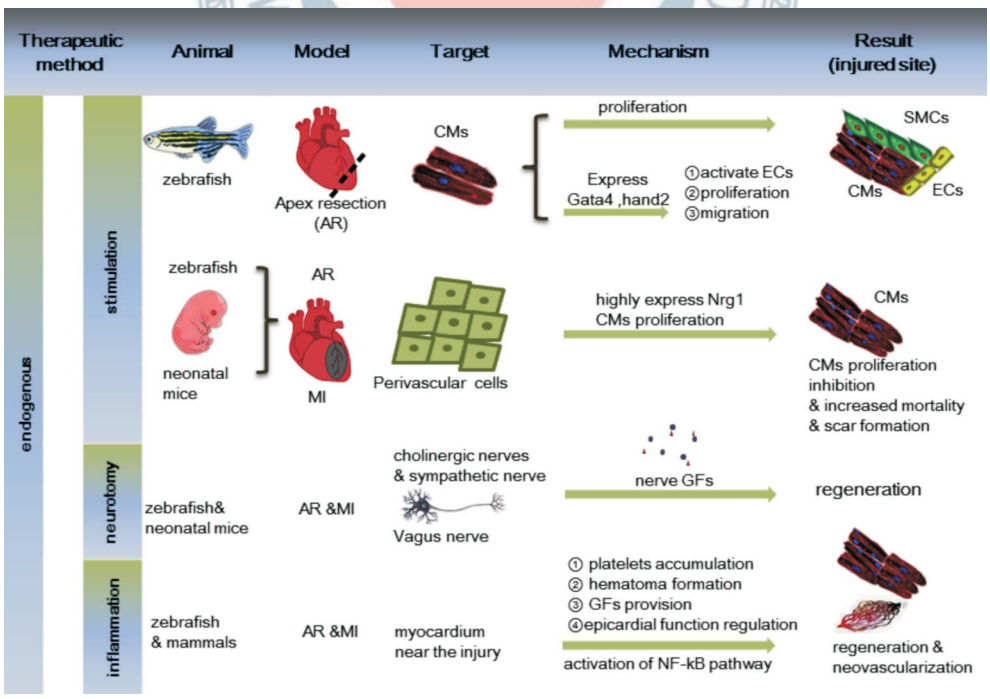

Figure 1. *Various mechanisms regulating inherent myocardial regeneration in zebrafish and mice. AR, apical resection; ECs, endothelial cells; GFs, growth factors; MI, myocardial infarction; SMCs, smooth muscle cells.*

cardiac stem cells (CSCs), endothelial progenitor cells, adipose stem cells, and blood-derived mononuclear cells.[34-37] These cells have either been administered into veins or coronary arteries, or directly injected into cardiac tissues. Studies of stem cell therapy for myocardial damage have been extensively reviewed elsewhere.[38,39] However, even though stem cell transplantation has been shown to play a positive role in cardiomyocyte regeneration and vascularization and thereby improve cardiac function,[33,36] few become cardiomyocytes and integrate into host heart tissues.[36,40] Autocrine and paracrine (mediated by exosomes) mechanisms may contribute to the beneficial effects and induction of myocardial regeneration.[40] The detailed mechanisms of how exogenous transplanted stem cells induce heart regeneration are still largely unknown and deserve further investigations.

Recent studies on induced pluripotent stem cells (iPS cells) derived from personalized somatic cells have opened a new era of stem cell research.[41] iPS cells, obtained through cellular reprogramming by the exogenous overexpression of transcription factors Oct3/4, Sox2, c-Myc and Klf4, can be induced from autologous somatic cells and thereby may avoid immunorejection after transplantation. iPS-derived cardiomyocytes have been transplanted into primate hearts but resulted in severe ventricular arrhythmia.[42,43] Further studies are necessary to investigate whether iPS-derived cardiomyocytes are beneficial in heart regeneration and whether they can successfully be integrated into the host myocardium.

Inducing heart regeneration by *in vivo* reprogramming

In recent years, the induction of resident cardiac cells such as cardiac fibroblasts to trans-differentiate into cardiomyocytes has become another important method to induce regeneration in the heart. This was first reported by Leda and coworkers in 2010, who showed that new cardiomyocytes could be reprogrammed from adult murine cardiac and skin fibroblasts through co-transduction of typical cardiomyogenic factors including Gata4, Mef2c and Tbx5 (GMT).[44] In addition, *in vitro* reprogramming has produced immature induced cardiomyocytes, whereas mature cardiomyocytes can only be obtained through *in vivo* reprogramming.[45] New cardiomyocytes have been shown to form after 2 weeks of *in vivo* reprogramming.[44] Qian et al. demonstrated that non-myocyte cells in murine hearts can be converted into cardiomyocyte-like cells through reprogramming after the intracoronary administration of transcription factors GMT.[46] However, the process of *in vivo* reprogramming is still relatively ineffective, and the epigenetic mechanisms regulating trans-differentiation need to be further elucidated.

Inducing heart regeneration by hypoxia

Mammals retain the capacity for complete cardiac regeneration for a short time after birth, while the capacity diminishes in adulthood. It would thus be ideal to stimulate cardiac regeneration potential in the adult heart to the same degree as in the neonatal stage to improve regeneration after damage.

In 2014, Puente et al. reported that a postnatal environment with rich oxygen can cause DNA damage, leading to the arrest/termination of the cell cycle in cardiomyocytes.[47] In 2015, Kimura et al. discovered that in the adult heart, cell cycle re-entry was induced by hypoxia.[48] In 2016, Nakada et al. found that gradually dropping the fraction of inspired oxygen from room air oxygen to 7% helped with the inhibition of aerobic metabolism as well as decreasing the production of reactive oxygen species (ROS), which enabled cardiac regeneration.[49] In their study, transgenic mice were marked with αMHC-MerCreMer-R26/tdTomato for lineage tracing, in which cardiomyocytes were reversibly marked by tamoxifen-induced tdTomato. The induced MI model was established 10 days after tamoxifen injection, and the fraction of inspired oxygen was gradually dropped from room air oxygen to 7% by 1% per day for 2 weeks. Regeneration of cardiomyocytes and blood vessels were subsequently observed. Furthermore, most of the newly formed cardiomyocytes in the regenerated and border zone were tdTomato-positive, which indicated that most of the newly formed myocardium was derived from pre-existing cardiomyocytes. This specific study showed that hypoxia can stimulate the potential of heart regeneration capacity in adult mice. However, this gradual hypoxia procedure is not clinically practical, since more than half of the mice underwent MI died during the hypoxia process.

Inducing heart regeneration by neonatal-specific extracellular matrix protein agrin

A very recent study by Bassat et al. showed that a major component of the neonatal extracellular matrix

protein agrin can affect cardiomyocyte growth and differentiation in mice.[50] The authors found that agrin was required for the full regenerative capacity of neonatal mouse hearts, and that recombinant agrin not only promoted the division of cardiomyocytes derived from mice- and human-induced pluripotent stem cells, but also promoted cardiac regeneration in adult mice after myocardial infarction. This enhancement in regeneration was primarily through a mechanism involving the disassembly of the dystrophin-glycoprotein complex, and Yap- and ERK-mediated signaling. This study suggested that, in addition to hypoxia, there are additional mechanisms of mammalian heart regeneration, and that further new inducers of adult mammalian heart regeneration could be identified in the future (Table 2 and Figure 2).

FUTURE PROSPECTS

Unlike inferior vertebrates, mammalian endogenous cardiac regeneration is very limited and is unable to spontaneously regenerate cardiomyocytes to repair a damaged heart. It is believed that the development and improvement in studies of heart regeneration, other than hypoxia and

Table 2. Landmark studies of exogenous methods inducing cardiac regeneration in mice

Name	Animal	Materials/design	Important factors/pathway	Main results	Refs
Leda M et al., 2010	N/A	Induced pluripotent stem cells (iPSCs)	Gata4, Mef2c and Tbx5 (GMT)	· Gata4, Mef2c, and Tbx5 are sufficient cardiomyocyte-inducing factors for cardiomyocyte induction · Infuced cardiomyocytes originate from differentiated fibroblasts and are directly reprogrammed	44
Shen YH et al., 2012	N/A	Stem cells in thoracic aortic aneurysms and dissections	N/A	Stem cells might be involved in reparative and destructive remodeling processes in thoracic aortic aneurysms and dissections	38
Puente BN et al., 2014	· Adult zebrafish. · Neonatal mice	Be exposured to hypoxic environment	Activation of DNA damage response pathway	· Observe the activation of DNA damage response pathway in postnatal mouse heart · Reactive oxygen species induce postnatal cardiomyocyte cell-cycle arrest · Prolonging postnatal cardiomyocyte proliferation after: · Scavenging reactive oxygen species · Mitochondrial-specific scavenging of ROS · Pharmacological inhibition of DNA damage response pathway	47
Kimura W et al., 2015	C57-BL6J mice	Mice transgened with fusion protein of CAG-promoter-driven CreERT2-ODD	· Hif-1a · Hypoxic stress response · Ubiquitin-proteasome-mediated degradation of Hif-1a	The newly formed myocardium is derived from pre-existing cardiomyocytes	40
Shiba Y et al., 2016	Female swines	MI mice induced by occlusion of the mid-left anterior	Oct3/4, Sox2, c-Myc and Klf4	Observe cardiac differentiation, vasculogenesis and infarct evolution	48
Nakada Y et al., 2017	C57BL/6J mice	· R26R-tdTomato · αMHC-MerCreMer mice	N/A	Exposure to hypoxaemia 1 week after induction of myocardial infarction can induce: · A robust regenerative response · Decreased myocardial fibrosis · Improvement of left ventricular systolic function	49

DNA, deoxyribonucleic acid; GMT, Gata4, Mef2c and Tbx5; ROS, reactive oxygen.

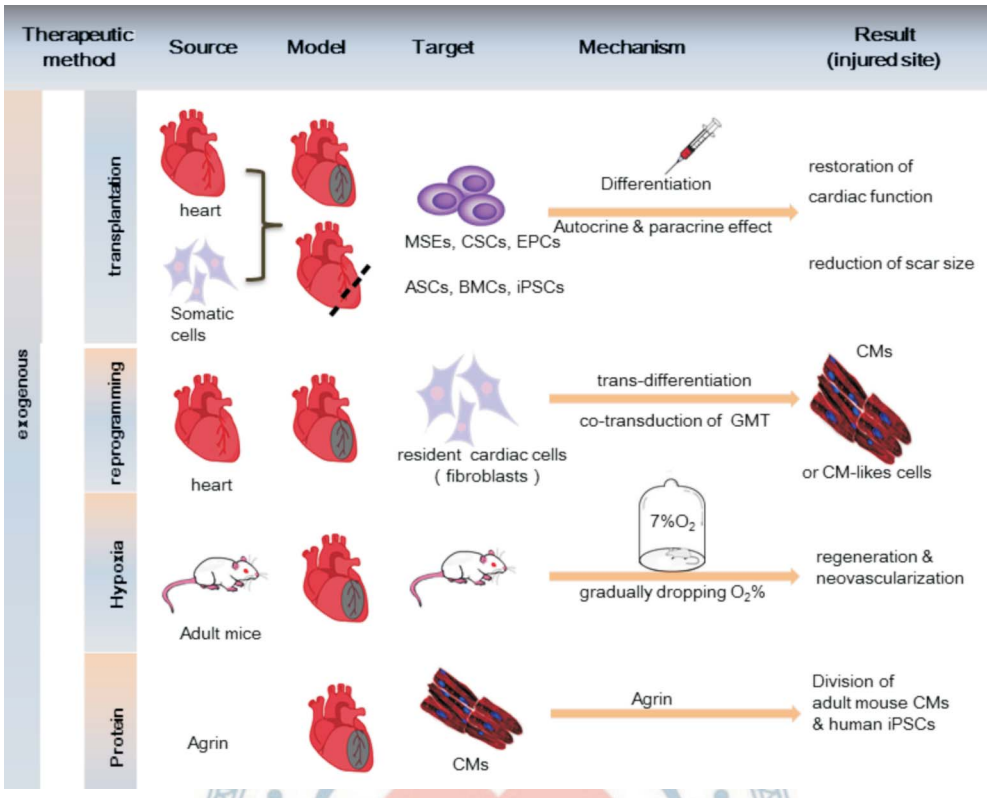

Figure 2. *Exogenous methods inducing cardiac regeneration in mice. ASCs, adipose stem cells; BMCs, blood-derived mononuclear cells; CSCs, cardiac stem cells; EPCs, endothelial progenitor cells; iPSCs, induced pluripotent stem cells; MSEs, mesenchymal stem cells.*

agrin, will lead to more factors being discovered for cell cycle re-entry, cell proliferation acceleration, cardiac regeneration stimulation, and finally cardiac function recovery.

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