Research progress on myocardial regeneration: what is new?

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Abstract

The regeneration capacity of cardiomyocytes (CMs) is retained in neonatal mouse hearts but is limited in adult mouse hearts. Myocardial infarction (MI) in adult hearts usually leads to the loss of large amounts of cardiac tissue, and then accelerates the process of cardiac remodeling and heart failure. Therefore, it is necessary to explore the potential mechanisms of CM regeneration in the neonates and develop potential therapies aimed at promoting CM regeneration and cardiac repair in adults. Currently, studies indicate that a number of mechanisms are involved in neonatal endogenous myocardial regeneration, including cell cycle regulators, transcription factors, non-coding RNA, signaling pathways, acute inflammation, hypoxia, protein kinases, and others. Understanding the mechanisms of regeneration in neonatal CMs after MI provides theoretical support for the studies related to the promotion of heart repair after MI in adult mammals. However, several difficulties in the study of CM regeneration still need to be overcome. This article reviews the potential mechanisms of endogenous CM regeneration in neonatal mouse hearts and discusses possible therapeutic targets and future research directions.

Keywords: Myocardial infarction; Cardiomyocyte; Endogenous regeneration; Mechanism

Introduction

Ischemic heart disease (IHD) remains a leading cause of morbidity and mortality worldwide, killing about 7.4 million people every year.[1] Myocardial infarction (MI), as the most serious manifestation of IHD, usually leads to the loss of large amounts of cardiac tissue, which will then be replaced by fibrous scar tissue. Consequently, the formation of fibrous scar in patients with MI will inevitably lead to myocardial remodeling, cardiac dysfunction, and eventual heart failure.[2] Therefore, reducing fibrous scar area and promoting myocardial regeneration is of great significance to reverse or delay the disease course after MI. To date, there are three main potential strategies of cardiomyocyte (CM) replacement in mammals, including transplantation of stem and progenitor cells, transdifferentiation of resident cardiac fibroblasts, and reactivation of endogenous regeneration mechanisms of CMs.[3] The first two approaches are characterized by significant limitations and significant defects. This reality tells us that it is wrong to achieve cardiac regeneration through the transdifferentiation of bone marrow cells or putative adult resident cardiac progenitors.[4] In terms of fibroblasts, although direct reprogramming of cardiac fibroblasts into CMs might be a new method of cardiac regeneration,[5] the current reprogramming efficiency is relatively low and the mechanism is not completely clear.[6] On the contrary, a large number of studies have shown that CM regeneration after MI really occurred in neonatal mice,[7-9] so proliferation of pre-existing CMs remains a hopeful strategy of myocardial regeneration after MI.

Senyo *et al*[10] found that adult mammalian CMs did not actively join the mitotic events, and CMs maintained a very low capacity to self-divide and proliferate. The annual self-renewal rate of human CMs was about 1% in adolescence to 0.45% in old age,[11] which is hardly enough to replenish for the loss of CMs after MI in adult mammals. In contrast, neonatal mice within 7 days after birth have the ability of complete cardiac regeneration, hence myocardial injury including MI can induce CM proliferation and complete recovery of cardiac function and structure within one month. The regeneration ability, however, is rapidly lost on the postpartum on the 7th day.[12] Like neonatal mice, neonatal humans may also have this intrinsic ability to replenish damaged myocardium and fully recover cardiac function after myocardial injury.[13] With the establishment of the MI model of neonatal mice through apical resection, and ligating left anterior descending coronary artery,[14] further studies can explore the genetic and cytological mechanisms of cardiac regeneration. Thus, this article reviews the research progress of endogenous

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regeneration, and discusses future research directions as well as some potential therapeutic targets on promoting CM regeneration after MI.

Cell cycle regulators and cardiac regeneration

Cell cycle activity plays an important role in all cell types, including DNA synthesis, G1/S phase, and effective progression of G2/M phase.[15] Cell cycle in mammals is accurately regulated by a complex set of proteins, including cyclins, cyclin dependent kinases (CDKs), CDK inhibitors (CDKIs), CDK activated kinases (CAK), and retinoblastoma (Rb).[16] It has been reported that cell cycle regulators can regulate CM proliferation [Table 1].[17]

Initially, Poss *et al*[18] found that zebrafish heart can completely regenerate after removing 20% of the ventricle, and the expression of some cell cycle regulators, such as polo-like kinase 1 (Plk1) and monopolar spindle 1 (Mps1), are essential in the process. With further research, other regulators have been found: cyclin A2, a cyclin that promotes the G1/S and G2/M phase, which participates in the CM proliferation.[19] Overexpression of cyclin A2 in α-myosin heavy chain (α-MHC)-cyclin A2 transgenic mice can improve the cardiac function, and induce DNA synthesis and CM Mitosis after MI.[20] Furthermore, it has been found that overexpression of cyclin D2 and cyclin B,[21] positive regulators that respectively drive G1/S and G2/M transition, can induce DNA synthesis and the proliferation of mammalian CMs.[22] In addition, CKIs influence the cell cycle by regulating cyclin-CDK complexes. For example, knockout of *p21*, *p27*, and *p57* genes may induce active proliferation in resting adult CM after MI.[23]

Recently, Mohamed *et al*[17] screened cell cycle regulators expressed in fetal CMs by using the methods of intramyocardial injection and controlling adenoviruses to the peri-infarct site of mice. They found that overexpression of CDK1, CDK4, cyclin B1, and cyclin D1 could effectively induce CM proliferation in adult mouse, rat, and human. In conclusion, cell cycle may be the final process of CM proliferation,[24] and some other mechanisms may highly promote endogenous regeneration of CMs by regulating cell cycle regulators, which play an important role in cardiac regeneration. Therefore, cell cycle regulation may serve as a therapeutic target after MI.

Transcription factors and myocardial regeneration

In recent years, it has been discovered that transcription factors (TFs) may also play important roles in CM proliferation after injury. Myeloid ecotropic viral integration site 1 (Meis1), a member of three-amino-acid loop extension (TALE) homeodomain TFs, has been proven to be associated with hemopoiesis and cardiac development at the embryonic stage, and it may also play a negative role in postpartum CM cell cycle arrest.[16] With the application of cardiomyocyte-specific *Meis1* knockout mice, which were generated by crossing α-MHC-Cre mice with *Meis1*$^{f/f}$ mice, Mahmoud *et al*[25] showed that the deletion of *Meis1* in CMs of neonatal mice was sufficient to extend the CM proliferation window after heart injury. On the contrary, overexpression of *Meis1* would inhibit the CM proliferation in neonatal heart after MI.[25] It has been demonstrated that Meis1 could promote transcription of Ink4b-Arf-Ink4a and CDK interacting protein (CIP)/kinase inhibitor protein (KIP) family (p21 and p57) by interacting with the promoters of the two kinds of synergistic CDK inhibitors.[26] In addition, the absence of Meis1 may induce the up-regulation of some positive cell cycle regulators such as CDKs, minichromosome maintenance 3 (MCM3)[16] and the down-regulation of negative regulators including CDKIs.[26]

In addition to Meis1, other TFs might also be associated with the CM proliferation after MI in mice. As a transcription factor, E2F could regulate the proliferation and differentiation of CMs by forming a complex network with pocket proteins in the cell cycle by injecting adenovirus carrying E2F transcription factor 2 (E2F2) into the left ventricular free wall and the interventricular septum.[27] Ebelt *et al*[28] demonstrated that overexpression of *E2F2* could induce robust expressions of cyclins A/E and proliferation of CMs in adult mice. It has been found that transcription factor T-box 20 (Tbx20)[29] may also promote CM proliferation after MI in adult mice by binding to genes of *p21/Meis1* and then inhibiting its expression. In summary, TFs affect the state of the CM cell

Table 1: Cell cycle regulators associated with cardiomyocyte regeneration.

Cell cycle regulators	Research objects	Observed effects	References
Plk1/Mps1	Zebrafish	The essential cell cycle regulators in cardiac regeneration	[18]
Cyclin A2	Mouse/ swine	A positive cyclin that promotes the G1/S and G2/M phase, and induces CM mitosis after MI	[19,20]
Cyclin D2	Mouse	A positive regulator that drives G1/S transition, induces DNA synthesis and CM proliferation in adulthood	[21]
Cyclin B	Rat	A positive regulator that drives G2/M transition, and induces the proliferation of differentiated CMs	[21]
CKIs	Mouse	Regulators that influence cell cycle of adult mammal by regulating cyclin-CDK complexes	[23]
Cyclin B1/ D1	Mouse/rat/human	Positive regulators that can effectively induce cell division in post-mitotic mouse, rat, and human CMs	[17]

Plk1: Polo-like kinase 1; Mps1: Monopolar spindle 1; CM: Cardiomyocyte; MI: Myocardial infarction; CKIs: Cyclin dependent kinase inhibitors.

cycle by regulating the transcription of target genes, which may serve as a potential strategy to promote myocardial regeneration after MI in adults.

Non-coding RNA (ncRNA) and cardiac regeneration

A large number of studies have shown that non-coding RNA (ncRNA) may play an important role in the process of cell proliferation, differentiation, apoptosis, and inflammation.[30,31] The function of ncRNA including microRNA (miRNAs), long noncoding RNA (lncRNA), and circular RNA (circRNA) in the cardiovascular field is emerging [Table 2].[30]

MicroRNA and cardiac regeneration

MiRNAs were first reported in the 1990s, and the research on it has rapidly developed into a mature field. There are increasing studies confirming that miRNAs are associated with heart disease and cardiac functions including cardiac proliferation.[32] However, its inhibitory regulation of gene expression contributes to degradation or inhibition of target mRNA by binding to it.[33]

Initially, with the application of miRNA expression profile analysis, Porrello *et al*[34] showed that in comparison with 1-day-old mice, 7-day-old mice had 71 miRNAs which were significantly up-regulated or down-regulated in CMs. Among them, several miRNA families (such as the miRNA-15, miRNA-30, and let-7 families) were up-regulated and miRNA-195 (a member of the miRNA-15 family) was confirmed to be the most up-regulated. Obviously, the results showed that the loss of CM regeneration potential in adulthood maybe because of the up-regulation of these miRNAs, which may induce postpartum cell cycle arrest. Thereafter, by injecting anti-miRNAs modified by locked nucleic acid (LNA) to postnatal mice, Porrello *et al*[35] further showed that the inhibition of miRNA-15 family does increase adult CM proliferation and promote recovery of cardiac function after MI. Similarly, it has been demonstrated that the inhibition of miRNA-34a would bring benefit to adult heart post-MI via CM proliferation.[36] Recently, Huang *et al*[37] generated the cardiac-specific miRNA-128 knock-out mice and found that the deletion of miRNA-128 could also promote endogenous regeneration of adult CM by suppressing p27 (a negative cell cycle regulator) expression and promote the activation of cyclin E/CDK2 (positive cell cycle regulators).

Meanwhile, miRNA can also promote CM endogenous regeneration. Eulalio *et al*[38] found that miRNA-590 and miRNA-199 can induce the cell cycle re-entry of adult CMs in vitro, and the introduction of has-miRNA-590 and has-miRNA-199a in the heart of adult mice with MI can contribute to CM regeneration. Therefore, with the application of cardiac-specific gene knock-in mice or other

Table 2: Non-coding RNAs associated with cardiomyocyte regeneration.

Non-coding RNAs	Research objects	Observed effects	References
miRNA-195	Mouse	Inhibition of the miRNA-15 family does increase adult CM proliferation and promote cardiac function after MI	[34]
miRNA-34a	Mouse	Inhibition of miRNA-34a may bring benefit to adult heart post-MI through CM proliferation	[36]
miRNA-128	Mouse	Deletion of miRNA-128 can promote CM endogenous regeneration in adult mice	[37]
miRNA-590/199	Mouse	Introduction of it can contribute to the cell cycle re-entry of adult CMs as well as CM endogenous regeneration	[38]
miRNA-17-92	Mouse	Expression of it in adult mice with MI can promote CM proliferation	[39]
miRNA-204	Mouse/rat	Overexpression of it may promote CM proliferation of adult and neonates in vitro and vivo	[40]
miRNA-302-367	Mouse	A miRNA that promote CM proliferation by inhibiting Hippo signaling pathway	[32]
miRNA-19a/19b	Mouse	Overexpression of it promotes CM proliferation and improve cardiac function after MI	[41]
miRNA-294	Mouse	Transient introduction of miRNA-294 in adult mice can promote CM cell cycle reentry and cardiac repair after MI	[42]
lncRNA ECRAR	Rat	A novel lncRNA that can promote DNA synthesis and cell mitosis in adult rat CMs	[30]
lncRNA CPR	Mouse	A lncRNA that obstructs CM proliferation by inhibiting the transcription of MCM3	[43]
lncRNA uc.457	Mouse	Expression of it may inhibit the differentiation and proliferation of CMs	[44]
circRNA Nfix	Mouse/rat	Inactivation or silencing of circRNA Nfix may promote adult CM proliferation and functional recovery after MI	[47]

miRNA: microRNA; lncRNA: Long non-coding RNA; circRNA: circular RNA; CM: Cardiomyocyte; MI: Myocardial infarction; ECRAR: Endogenous cardiac regeneration-associated regulator; CPR: Cardiomyocyte proliferation regulator; MCM3: Minichromosome maintenance 3; Nfix: Nuclear factor I-X.

methods, miRNA-17–92 cluster,[39] miRNA-204,[40] and miRNA-302-367[32] have also been reported to potentially promote CM proliferation of adult mammalian heart.

In 2019, Gao *et al*[41] found that overexpression of miRNA-19a/19b in mouse hearts by intramyocardial injection of miRNA-19a/19b mimics or adeno-associated virus 9 (AAV9) carrying miRNA-19a/19b could promote CM proliferation and improve cardiac function after MI. Coincidentally, Borden *et al*[42] found that transient reintroduction of miRNA-294 (which was found in embryonic stem cell exosomes) by delivering AAV-9-miRNA-294 vectors to adult mice heart could promote CM cell cycle reentry and cardiac repair after MI.

Thus, current studies indicate that miRNA plays an important role in cardiac repair. However, to make the miRNA a potential strategy for treating MI in adults, some substantial difficulties need to be resolved, such as how to effectively deliver miRNAs to the target site as well as whether there are other side effects with adenovirus as the carrier.

Long non-coding RNA (lncRNA) and cardiac regeneration

Many studies have found that lncRNA, a kind of non-coding RNA composed of more than 200 nucleotides, may play a significant role in cardiac hypertrophy, inherited cardiomyopathy, and other cardiovascular diseases. Meanwhile, the roles of lncRNA in CM proliferation and regeneration are explored systematically.

Chen *et al*[30] found that 3092 lncRNAs were differentially expressed in human hearts during the fetal-to-adult transition by analyzing publicly available RNA-seq data of cardiac tissues. Then, they identified endogenous cardiac regeneration-associated regulator (ECRAR), a novel upregulated fetal lncRNA that could promote DNA synthesis and cell mitosis in 7-day-old and adult rat CMs. The ECRAR functions by activating extracellular signal-regulated kinases 1 and 2 (ERK1/2) signaling. By generating cardiomyocyte proliferation regulator (CPR) knockout mice, Ponnusamy *et al*[43] found that lncRNA CPR could inhibit the transcription of MCM3 (an initiator of eukaryotic genome replication), and proved that deletion of CPR could significantly promote CM proliferation and improve cardiac function in postnatal and adult hearts after MI. The expression of lncRNA uc.457[44] also inhibited the differentiation and proliferation of CMs, which is similar to the function of lncRNA CPR. In summary, lncRNAs take part in the regulation of CM proliferation and cardiac repair, which may provide a new therapeutic strategy for effective cardiac regeneration after MI.

CircRNAs and myocardial regeneration

As a novel class of ncRNAs that are circularized by joining the 3' end to the 5' end of the RNA,[45] circRNA may play a fundamental role in the regulation of CM proliferation and cardiac regeneration.[46] For example, in 2019, Huang *et al*[47] found that inactivation or silencing of the nuclear factor I-X (Nfix), a circRNA enriched in adult mice CMs, could promote CM proliferation and functional recovery after MI by inhibiting Y box binding protein 1 (Ybx1) ubiquitin-dependent degradation and increasing activity of miR-214.

Signaling pathway and cardiac regeneration

The Hippo signaling pathway is evolutionarily conserved and was first identified in Drosophila melanogaster.[48] The Hippo signaling pathway in mammals consists of a series of kinase cascades, which mainly includes the mammalian sterile 20-like kinases 1/2 (MST1/2), Salvador homolog 1 (SAV1), large tumor suppressor 1/2 (LATS1/2), Mps one binder kinase activator-like 1 (MOB1), and Yes-associated Protein (YAP)/ transcriptional coactivator with PDZ-binding motif (TAZ).[49] The terminal effector of Hippo pathway is YAP/TAZ, a transcription coactivator regulating gene expression that promotes cell proliferation, survival, and metabolic function.[50] Many groups have shown that YAP is essential for CM proliferation and regeneration, and Lin *et al*[51] found that the damaged heart can no longer regenerate when the *YAP* gene is specifically knocked out in embryonic CMs. However, YAP/TAZ only works when it has entered the nucleus, and the activation of Hippo signaling pathway can finally obstruct the expression of proliferation-related genes by phosphorylating YAP/TAZ and then preventing it from entering into the nucleus.[52] In contrast, Heallen *et al*[53] demonstrated that CMs specific knockout of the gene of *SAV1* or *LATS1/2* (components of Hippo signaling pathway) may contribute to cell cycle re-entry and proliferation of CMs. It was shown that Hippo signaling pathway may limit CMs proliferation and control heart size by negatively regulating Wnt signaling.[54] Therefore, the activation of YAP may promote CM proliferation and cardiac repair after MI in adulthood, while inhibition of Hippo pathway may also result in this outcome [Figure 1].

Likewise, there are other signaling pathways playing potential roles in CM endogenous proliferation and regeneration after MI. Inhibition of the phosphatidylinositol 3 kinase (PI3K)/protein kinase B (AKT), an intracellular signaling pathway, can accelerate the degradation of cyclin D1 and then negatively regulate CM cell cycle,[55] which means that PI3K-AKT signaling pathway may be a positive regulator of CM proliferation.

Also, in mouse myocarditis models, the recovery of cardiac structure and function after myocardial injury was caused by the re-entry of the cell cycle, in which Janus kinases (JAK)/ signal transducers and activators of transcription (STAT) signaling pathway may play an important role.[56] STAT3 may also participate in CM proliferation induced by acute inflammation. The Wnt/β-catenin and p38 signaling pathways[24] may also participate in the mechanism of cardiac regeneration.

Acute inflammation and myocardial regeneration

Acute inflammation is known to occur immediately after myocardial injury, this triggers a significant fibrotic response in adult hearts followed by deterioration of cardiac function.[57] However, acute inflammation may be

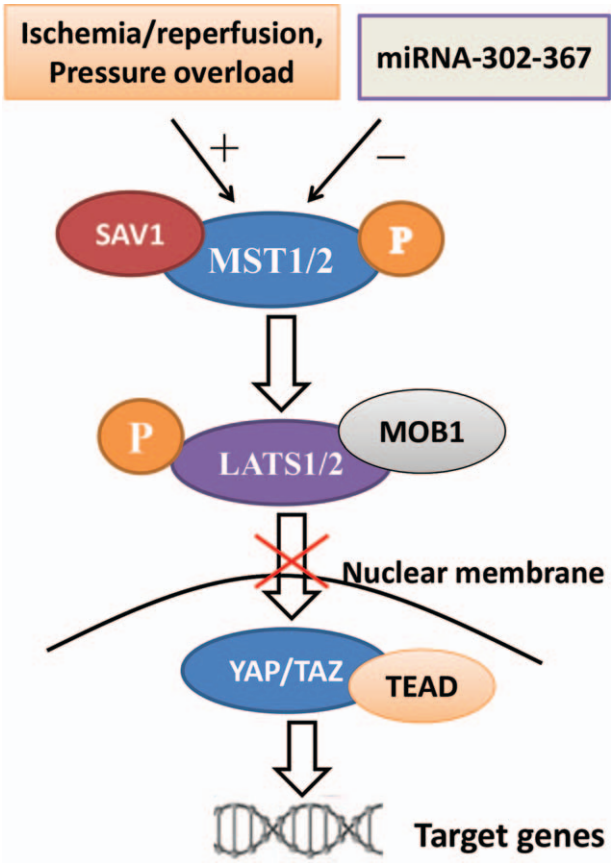

Figure 1: The components of canonical hippo signaling pathway. YAP/TAZ, the terminal effectors of hippo pathway, enter the nucleus and promote gene expression by binding to transcription factors such as TEAD. Activation of hippo pathway prevents YAP from entering nucleus by phosphorylation (P) and inhibits cardiomyocyte proliferation. Ischemia/reperfusion and pressure load have been known to activate the pathway, while miRNA-302-367 inhibit it. miRNA: microRNA; MST1/2: Mammalian sterile 20-like kinases 1/2; SAV1: Salvador homolog 1; LATS1/2: Large tumor suppressor kinase 1/2; MOB1: Mps one binder kinase activator-like 1; YAP: Yes-associated protein; TAZ: Transcriptional coactivator with PDZ-binding motif; TEAD: Transcriptional enhanced associate domain.

closely related to the proliferation of CMs in neonatal hearts. Han *et al*[58] found that by injecting the immunogenic zymosan A (ZA) into the myocardium, the induction of acute aseptic inflammation in the hearts of neonatal mice could induce endogenous CM proliferation, which, however, could be suspended after suppressing the immune response of neonatal mice. Moreover, they found that damaged hearts in neonatal mice could not live up to regeneration without interleukin-6 (IL-6). A similar phenomenon could be observed with the absence of STAT3, a major downstream effector activated by tyrosine phosphorylation of IL-6.[59]

Interestingly, the different outcomes of result from acute inflammation between neonatal and adult hearts might be due to the different sources of macrophages.[60] It has been found that some embryonic-derived cardiac resident macrophages could be immediately mobilized in heart of neonatal mice after myocardial injury, while the preferential increase of bone-marrow-derived macrophages was observed in the heart of adult mice.[61] Compared with the latter, embryo-derived macrophages have more significant angiogenesis ability *in vitro*.[61] Unfortunately, although acute inflammation may play an important role in CM regeneration in mammals, there will still be a long way from achieving the goal to promote cardiac regeneration in adults by targeting acute inflammation after MI.

Hypoxia and myocardial regeneration

In recent years, the adverse effects of oxidative stress on CM proliferation have become a focus. Induction of systemic hypoxia in patients with MI may be a future research direction and a potential therapeutic strategy.

The function of reactive oxygen species

Mitochondrial oxidative phosphorylation produces adenosine triphosphate (ATP) for the organism more than anaerobic metabolism, which, in turn, contributes to more generation of mitochondrial reactive oxygen species (ROS) that can lead to cellular dysfunction and senescence.[62] It is known that mitochondrion-derived ROS can induce CM cell cycle arrest by stimulating DNA damage responses.[63] Kimura *et al*[64] found that inhibiting ROS activity with N-acetyl-L-cysteine could promote CM regeneration after ischemia/reperfusion injury in mice, while scavenging ROS could also achieve the goal. Further, the toxic or physiological effects that ROS generated are actually influenced by the level, source, type of ROS, and other factors. For example, in contrary to mitochondrion-derived ROS, the production of cytoplasmic H_2O_2 induced by NADPH oxidase 4 (Nox4) can extend the time window for postpartum CM proliferation.[64]

The role of hypoxia in myocardial regeneration

The presence of oxygen in the environment will influence the production and clearance of ROS through the oxygen content in the circulatory system, and hence influence the proliferation of CMs. It has been found that the transition from intrauterine hypoxic environments to postpartum hyperoxic environments can lead to cell cycle arrest of CMs in neonatal mammals through oxidative DNA damage induced by mitochondrial ROS.[64] More significantly, by exposing mice to low oxygen tension (7% O_2) for 2 weeks, Nakada *et al*[65] found that induction of systemic hypoxia could effectively reduce the production of mitochondrion-derived ROS, attenuate DNA damage, and finally activate CM mitosis. In addition, CM proliferation induced by systemic hypoxia may also be related to hypoxia-inducible factor (HIFs) and HIF-regulating prolyl-hydroxylase domain enzymes (PHDs).[66] It has been shown that inhibition of PHDs activity can reduce myocardial infarction area and improve cardiac function.[67]

Protein kinase and myocardial regeneration

Protein kinase, a kind of enzyme that can phosphorylate substrate proteins and regulate cell growth, differentiation, proliferation, and apoptosis has become a focus of research in recent years and may become a potential therapy for CHD.

Wang *et al*[68] found that CHIR-99021, an inhibitor of glycogen synthase kinase 3-beta (GSK-3β) kinase, could

induce human atrial CM proliferation by increasing the level of β-catenin in the cell nucleus. However, it remains indefinite that the observation in atrial CMs would also be directly applicable to ventricular CMs. In contrast, Yester *et al*[22] found that induction of active erb-b2 receptor tyrosine kinase 2 (ERBB2) in neonatal and adult CMs may lead to CM proliferation and cardiac regeneration after MI, a process including sequential CM dedifferentiation, proliferation, and finally redifferentiation. Further studies found that this process might be mediated by extracellular signal-regulated kinases (ERK), AKT, and GSK 3β/β-catenin signaling pathways.[69] In addition, p38 mitogen-activated protein kinase (MAPK), a member of the serine/threonine protein kinase family, may play a negative role in CM proliferation post-MI both *in vitro* and *in vivo*.[70] This has been verified in a series of mice[71] and even human randomized controlled trials.[72] Inhibition of p38 MAPK through inhibitors may increase the expression of cyclin A2 and cyclin B, and then promote the expression of genes responsible for CM proliferation and regeneration.[70]

Moreover, it has been found that growth and differentiation factor 11 (GDF11)[73] and Pim1 kinase[74] may also play underlying roles in CM proliferation and reduction of infarction area in neonatal mice after MI. With the application of quantitative phosphorylation proteomics method, more kinases, which may be involved in the endogenous CM proliferation, can be discovered. This may provide new conceptions for further studies on cardiac regeneration in mammals.

Epigenetics and other factors in CM regeneration

Epigenetic regulation of CM proliferation is a new research direction. It is known that post-translational modification of histones such as DNA methylation, deacetylation and phosphorylation can affect the expression of adult cardiac cyclin.[75] In mammal heart, the combination of epigenetic modifying proteins with cardiac-specific transcriptional factors (such as GATA binding protein 4, Tbx5) and cell cycle regulators (such as Rb/p130) contributes to modifications of histones at promoter regions, which may determine the phenotype of adult CM.[75] Additionally, studies showed that the recombinant growth factor neuregulin-1 (rNRG1)[76] and the activation of complement receptor C5aR1[77] may also participate in the process of CM regeneration in neonatal mice. In 2019, Hirose *et al*[78] found that increased levels of circulating thyroxine could deprive the heart of the ability to regenerate in adult CMs.

Conclusion

For decades, stem cells derived from the bone marrow and other tissues had been infused into the coronary arteries or injected directly into the myocardium,[79] which has been thought to be a method to promote CM regeneration and recovery of cardiac function after MI. However, subsequent studies have shown that stem cells may not be able to differentiate into cardiomyocytes, and the benefits generated from stem cell therapy for patients may originate from the subsequent paracrine effects.[80] Given the generally unsatisfactory and uncertain results from stem cell method, the European Society of Cardiology concluded that the promise of cell therapy has not been realized. Conversely, the endogenous proliferation of adult CMs may be a promising method to promote the recovery of cardiac function after MI, as shown in animal models. However, some challenges are yet to be addressed before the realization of clinical applications. First, we need to understand the interaction between reported mechanisms involved in cardiac regeneration. Hashmi *et al*[24] proposed a "molecular switch model" and advocated the "locked" cell cycle of adult CMs could be changed through cell cycle checkpoints. In the model, some mechanisms, including cyclins and CDKs, signaling pathways, transcription factors and others, can modulate the transition at cell cycle checkpoints. Nevertheless, how to turn off the "switch" of CM proliferation once it is turned on remains elusive. Moreover, many other mechanisms, such as ncRNA, hypoxia, and kinase are not mentioned in the suggested "molecular switch model." Second, some methodological limitations must be overcome before clinical application, such as whether the application of adenovirus will have other side effects. It is believed that the multi-step preclinical and clinical trials can deal with the limitations and generate benefits for human beings.

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Conflicts of interest

None.

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