Review

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Regenerative Medicine

How can the adult zebrafish and neonatal mice teach us about stimulating cardiac regeneration in the human heart?

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The proliferative capacity of mammalian cardiomyocytes diminishes shortly after birth. In contrast, adult zebrafish and neonatal mice can regenerate cardiac tissues, highlighting new potential therapeutic avenues. Different factors have been found to promote cardiomyocyte proliferation in zebrafish and neonatal mice; these include maintenance of mononuclear and diploid cardiomyocytes and upregulation of the proto-oncogene c-Myc. The growth factor NRG-1 controls cell proliferation and interacts with the Hippo–Yap pathway to modulate regeneration. Key components of the extracellular matrix such as Agrin are also crucial for cardiac regeneration. Novel therapies explored in this review, include intramyocardial injection of Agrin or zebrafish-ECM and NRG-1 administration. These therapies may induce regeneration in patients and should be further explored.

Plain language summary: The heart pumps blood across the body carrying nutrients and oxygen where they are needed. If the heart is damaged (e.g., after a heart attack), it may lose its ability to pump blood, and this can lead to heart failure, where the heart cannot meet the body's needs, leaving the affected person tired and breathless. This occurs because the human heart unfortunately has a limited ability to heal and regain function. Current therapies for heart injuries focus on minimizing the problems resulting from the injury but cannot recover damaged heart tissue. Scientists have found that in contrast to adult human hearts, the hearts of baby mice and zebrafish can repair themselves after injuries and recover normal function. This review highlights some important mechanisms that occur in the hearts of baby mice and zebrafish, which may help contribute to their regenerative abilities. These mechanisms involve small messenger chemicals that stimulate heart cells to replicate and reform normal heart tissues. Further research into these pathways may help develop new therapies for damaged human hearts and help them regain function.

Tweetable abstract: Unlike humans, adult zebrafish and neonatal mice can regenerate cardiac tissues. Key regenerative processes that occur in zebrafish and neonatal mice may help the development of future therapeutic avenues for many cardiovascular diseases.

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During myocardial infarction (MI), blocked coronary arteries cause individual cells to become deprived of oxygenated blood, which could lead to death of up to one billion cardiomyocytes [1]. In the adult human heart, instead of regeneration of damaged myocardium, a fibrotic scar forms after injury, impairing cardiac function and thereby predisposing to incurable heart failure (HF) [2]. Most treatments for HF aim to either ameliorate symptoms or slow the pathological remodelling which underlies the progressive nature of the disease [3,4]. Although some HF patients undergo transplantation, the number of available donors is limited while demand continues to rise due to

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an increase in the mean age of the population and increasing survival following MI [4]. Additionally, although allogeneic transplantation theoretically improves outcomes, prognosis remains poor due to chronic rejection and the detrimental effects of long-term immunosuppression [4]. In contrast, treatment strategies that aim to replace lost cardiomyocytes via endogenous regeneration may prevent HF post-MI.

There is a divergence between species in the response to myocardial injury [4,5]. In stark contrast to the adult mammalian heart, as outlined in Figure 1, both the adult zebrafish and neonatal mouse have been shown to regenerate healthy new cardiomyocytes to replace damaged myocardium [5,6]. Indeed, following a diverse range of injury protocols, the adult zebrafish and neonatal mouse employ endogenous regenerative pathways that involve the proliferation of preexisting cardiomyocytes without the use of a progenitor cell population, ultimately facilitating scarless regeneration [5–9]. However, recent studies have illustrated that the role of different regeneration pathways is mode-of-injury dependent [10] and, as summarized in Table 1, different methods of cardiomyocyte injury have unique advantages and disadvantages that merit discussion [5,7,9,11–20]. One of the major differences between the cryoinjury and resection protocols is the amount of fibrotic tissue produced as a by-product. After resection injury, a relatively small amount of collagen localizes superficially on the cut surface of the myocardium [5], whereas in cryo-injured hearts, a significant amount of collagen transiently accumulates in place of the dead tissue mass [7]. Nevertheless, it has been demonstrated that zebrafish are capable of efficiently regenerating cardiomyocytes removed by either genetic ablation [9], where up to 60% of cardiac muscle cells are removed, or by severe cryoinjury [13]. In murine cardiomyocytes, there is a transition from hyperplastic to hypertrophic growth at postnatal day 7 (P7) that correlates with cell cycle arrest and loss of regenerative capacity [21]. In contrast, the adult human heart was long considered to be a postmitotic organ [22]. However, a recent pulse-chase experiment by Bergmann *et al.* showed the integration of ^{14}C into the DNA of cardiac cells from the atmosphere, proving they turnover at a rate of approximately 1% annually, with turnover gradually decreasing with age [23]. Although the meager rate of renewal remains insufficient for generating a meaningful response to injury, this finding nevertheless led to a paradigm shift, reinvigorating interest in regenerative strategies for treatment of cardiac disease. Despite this, the precise proliferative capacity, and the programmed timing of cell cycle arrest of cardiomyocytes in humans remains unknown. Here, mechanisms underlying the regenerative capacity of neonatal mice and adult zebrafish are reviewed and the potential for their translation to humans is discussed.

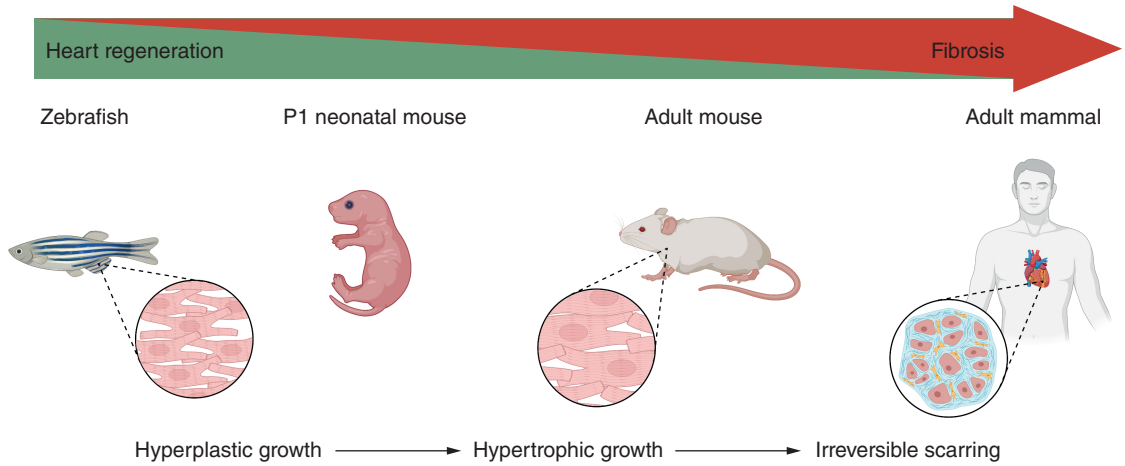
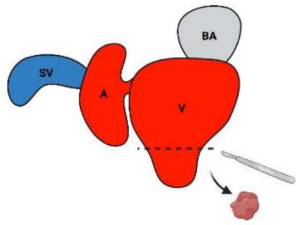
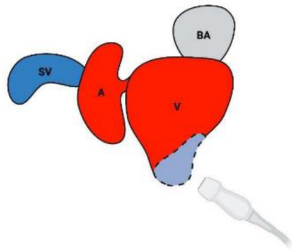
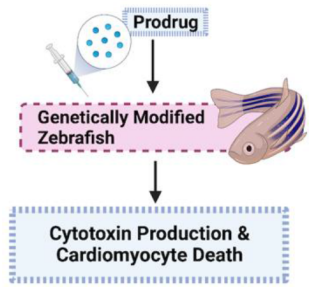
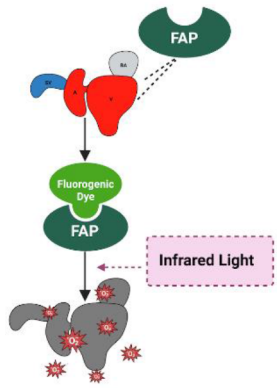
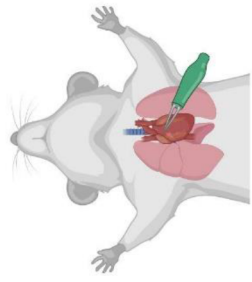


Figure 1. Illustration outlining the cardiac regenerative potential of neonatal mice and adult zebrafish. In contrast to the adult mammalian heart (on the right), both the adult zebrafish and neonatal mouse (on the left) have been shown to regenerate healthy new cardiomyocytes to replace damaged myocardium. Following a diverse range of injury protocols, the adult zebrafish and neonatal mouse employ endogenous regenerative pathways that involve the proliferation of preexisting cardiomyocytes facilitating scarless regeneration. In murine cardiomyocytes, there exists a transition from hyperplastic to hypertrophic growth at postnatal day 7 (in the middle of the figure), which correlates with the timing of cell cycle arrest and loss of regenerative capacity.
P1: Postnatal day 1.
Image created with BioRender.com.

Table 1. The different cardiac injury methods used in zebrafish and neonatal mice.

Model	Diagram	Protocol	Relation to mammalian physiology	Advantages	Limitations	Ref.
Resection		~20% ventricular myocardium surgically removed Tissue recovers within ~60 days	Thrombosis and collagen deposition superficially on wound	Fast and complete recovery of cardiac function	Time-consuming Imprecise/tedious	[5,11]
Cryoablation		Extensive cell death over ~20% of ventricular wall Apex of ventricle is exposed, and a precooled cryoprobe and dry ice is applied to freeze the ventricle Scar tissue replaced with new myocardium within ~2 months	More similar to localized damage seen after MI in mammals Inflammatory response	Extensive cell death Well tolerated	Injury observed in all cell types Morphological alterations postrecovery	[7,12,13]
Genetic ablation		~60% ventricular cardiomyocytes depleted Cardiomyocytes undergo genetic manipulation and express toxins → production of cytotoxic metabolites Tissue recovers within ~30 days	Signs of heart failure	Reversible Target specific cell lineage Reproducible Practical for large-scale analyses Noninvasive Temporal control	Complete ablation not always achieved Non-specific 'bystander effect' on neighboring cell types Does not reproduce pathological changes seen following MI	[9,14,15]
Chemoptogenetic ablation		Zebrafish express FAP in cardiomyocytes A fluorogenic dye administered binding FAP Localized near-infrared exposure results in excited photosensitizer-mediated ROS generation, which damages 30% of cardiac tissues	In humans, post-MI and particularly postreperfusion, there is an increase in ROS production, which further damages the myocardium; similarly, this chemoptogenic ablation model generates ROS that damage cardiomyocytes	This injury method did not activate the epicardium and endocardium May help identify specific cardiomyocyte responses to injury without whole organ activation	This model produces milder injury and only affects cardiomyocytes Post-MI, other cells in addition to cardiomyocytes are involved, which this model does not address	[16]
LAD ligation		LAD is permanently ligated with a single suture Infarct size ~10–15% of the left ventricle Complete cardiac regeneration within ~7 days	Mimics pathophysiological changes seen after MI	Reproducible Possibility for minimally invasive methods that minimize postsurgical mortality	Does not always stimulate complete regeneration Only feasible in mice Invasive, time-consuming Microsurgery – extensive training required	[17–20]

Different methods of cardiomyocyte injury (resection, cryoablation, genetic ablation, chemoptogenetic ablation and LAD ligation) are used in regenerative studies in zebrafish and neonatal mice. This table summarizes the advantages and limitations of each methodology, as well as their relevance to human physiology.
A: Atrium; BA: Bulbus arteriosus; LAD: Left anterior descending artery; MI: Myocardial infarction; ROS: Reactive oxygen species; SN: Sinus venosus; V: Ventricle.
Images created with BioRender.com.

Cardiomyocyte-intrinsic mechanisms affecting cardiac regeneration

Cardiomyocyte polyploidization reduces their regenerative ability

Differences in cell cycle activity and ploidy levels between neonatal and adult mammalian cardiomyocytes may cause loss of regenerative ability in adult cardiomyocytes [24]. In mice, cardiomyocytes exit the cell cycle due to failed cytokinesis or karyokinesis in the absence of cytokinesis, thus terminally differentiating into bi-/multi-nucleated or polyploid cells [25]. In neonatal mice, the transition from diploid to polyploid cardiomyocytes coincides with the loss of regenerative potential at P7 [25]. Around this time period, mice cardiomyocytes also exhibit a shift toward binucleation. At P2, 93.2% of cardiomyocytes are mononucleated, whereas 78.4% of them are binucleated at P11 [25]. Furthermore, 99% of the adult zebrafish cardiomyocytes are normally mononucleated and diploid [26]. Induction of polyploidization in >45% of cardiomyocytes in the adult zebrafish resulted in loss of regenerative capacity postresection injury [26]. Accordingly, cardiomyocytes of nonregenerative species are often polyploid. However, patterns of ploidy and multinucleation vary significantly across mammalian species [24,27], thus limiting translatability of these findings to humans. Across experiments, 22.5 to 33.4% of human cardiomyocytes were found to be diploid, with the rest being polyploid and the majority of those being tetraploid [28,29]. Most of those cardiomyocytes are mononucleated cells that exhibit increasing polyploidization with age and after myocardial infarction [29–31]. Nevertheless, although ploidy does not hinder regeneration in other tissues, such as the liver [32], modulating cardiomyocyte polyploidy may be a useful therapeutic target in cardiac regenerative medicine.

Multiple miRNAs regulate cardiac regeneration

The cell cycle of cardiomyocytes is also regulated by certain miRNAs that control cardiomyocyte proliferation via mRNA degradation [33]. Important relevant miRNAs identified in murine hearts include miR-17-92, which can enhance cardiomyocyte proliferation in neonatal mice and in adult mice post-coronary artery occlusion [33], and miR-195, which limits cardiac regeneration in neonatal mice after left anterior descending artery (LAD) ligation [34]. Upregulation of miR-195, part of the miR-15 family, leads to cardiomyocyte binucleation via inhibition of *Check1*, a mitosis gene controlling cytokinesis and chromosomal segregation [35]. Also, various miRNAs from the miR-15 family were upregulated during P7 and P14 in mice, the time period associated with the loss of their cardiomyocyte regenerative capacity [35]. In addition, when zebrafish regenerate their cardiomyocytes post-resection injury, miR-133 levels are suppressed [36]. However, elucidating the role of different miRNAs in cardiac regeneration is limited by the vast number of targets they each possess [36]. For example, multiple downstream mediators of miR-133 have been identified in zebrafish, such as the cell cycle regulator mps1 and the cell junction cx43 [36]. A recent study on 96 miRNAs on human-derived induced pluripotent stem cell cardiomyocytes showed that they impact the regulation of the Hippo pathway and specifically its mediator YAP, but their role was not essential for cardiac proliferation to occur [37]. Overall, despite uncontrollable cell division being a potential risk, promoting cell cycle re-entry through the modulation of miRNA remains a potential therapeutic option for cardiac regeneration that merits further research [33,34,36].

C-Myc expression may induce cardiomyocyte regeneration

C-Myc is a proto-oncogene that controls differentiation and proliferation in multiple tissues [38]. Under physiological conditions, *c-Myc* is not expressed in the adult murine heart, but it can be upregulated in the presence of pathological signals, such as ischemia and pressure overload [39]. Wang *et al.* found that upregulating the *c-Myc*/FoxM1 pathway increased cardiomyocyte proliferation after apical resection injury to the neonatal murine heart and post-LAD ligation in adult mice [40]. Kisby *et al.* also increased regeneration by inducing cardiomyocytes in mice to a pluripotent dedifferentiated state via *c-Myc* and three other transcription factors [41]. However, their administration via an adenoviral vector resulted in limited cardiac repair in the myocardium following LAD ligation [41], highlighting the necessity of improving available delivery methods. A recent study identified a positive correlation between *c-Myc* activity and P-TEFβ, thus proposing increasing P-TEFβ levels as a way of achieving *c-Myc*-induced cardiomyocyte proliferation [42]. P-TEFβ can act as a mediator of CDK9, with the levels of the latter remaining relatively high throughout the adult life of the zebrafish [43]. However, they decrease significantly in adult mice [43], further suggesting a possible role of P-TEFβ, and hence *c-Myc*, in cardiac regeneration. Research on the role of *c-Myc* in zebrafish cardiomyocyte proliferation has been limited and has not yet yielded definitive, clear-cut findings. Miklas *et al.* suggested that *c-Myc* targets, including oxidative phosphorylation pathways, are overexpressed in zebrafish hearts 7 days after chemical ablation injury and generally in young zebrafish hearts [44]. Another study, however, demonstrated downregulation of *c-Myc* targets during cardiac regeneration in zebrafish after cryoinjury [45]. It

is important to highlight that overexpression of *c-Myc* is carcinogenic [46] because its targets are upregulated in many tumors [45,46]. Therefore, its potential therapeutic expression in future studies should be transient to limit malignancy [46].

The Hippo-YAP pathway modulates cardiac regeneration

The Hippo pathway has been extensively studied in regenerative medicine because it regulates cell cycle activity and interacts with the NRG1 and ErbB2 pathway [47]. The Hippo pathway cascade consists of core kinases – namely, MST1/2, MAP4Ks and LATS1/2 [48]. Activation of this pathway leads to inactivation of the most important downstream effectors – namely, YAP and TAZ [49]. Hence, many targets exist in the pathway to affect the ultimate downstream mediators. When the *YAP* gene, a critical regulator of the Hippo pathway, was directly knocked out in neonatal mice, minimal cardiac regeneration and extensive fibrosis were observed after MI-induced injury [50]. Furthermore, *Salvador*, an upstream inhibitor of *YAP*, when knocked out of mice, promoted cardiomyocyte proliferation [51]. Because studies in mice found that YAP decreases with age and is undetectable by week 12 [52], more research is needed to determine whether a similar pattern occurs in humans and whether potentiating *YAP* expression is safe therapeutically, given that *YAP* is a proto-oncogene [53]. A study in zebrafish, however, demonstrated that YAP deficiency did not reduce cardiomyocyte proliferation in cryoinjured myocardium [53,54]. It may be that other or additional proteins contribute to cardiac proliferation in zebrafish via the Hippo pathway [48,54]; these differences emphasize the need for further research in large mammals to extrapolate accurately the therapeutic potential of modulating the Hippo pathway in humans.

Oxidative DNA damage can reduce cardiac regeneration

Adult zebrafish and neonatal mammals use anaerobic glycolysis as their energy source because they exist in a relatively hypoxic environment [55,56]. In contrast, adult mammals have a higher oxygenation status and perform aerobic respiration. The switch from glycolysis to oxidative phosphorylation causes reactive oxygen species (ROS) production, which damages proteins, lipids and DNA [57]. The detrimental effects of ROS were noted in a study on postnatal mice, which found an increase in oxidized DNA and an upregulation of the DNA damage response pathway by P7 compared with P0 [58]. Another study on neonatal mice showed that mild hypoxic conditions increased cardiomyocyte replication, whereas exposure to high oxygen levels increased oxidative stress and consequently decreased cardiomyocyte replication [59]. To further emphasize the causal role of ROS in cardiomyocyte cell cycle arrest, Puente *et al.* [59]. injected mice with diquat, an ROS generator, and found that cardiomyocyte replication decreased compared with control. Conversely, N-acetylcysteine, an ROS scavenger, administered on P14, increased the number of cardiomyocytes. Notably, cardiomyocyte proliferation gradually decreased over 1 month, suggesting that ROS scavenging may have limited benefits in long-term cardiac regeneration. Further research has shown that *in vitro* administration of HIF-1α, a transcription factor that mediates metabolic responses to hypoxia, correlated with an increase in cardiomyocyte cell cycle activity [60]. However, forced activation of HIF-1α signaling in cardiomyocytes was shown to cause dilated cardiomyopathy and HF [61]. Given the vital importance of aerobic metabolism, ways to minimize oxidative DNA damage should be the primary focus of research rather than reduction in mitochondrial oxidative phosphorylation.

In contrast to neonatal mice, recent studies on zebrafish suggest that elevated levels of ROS can promote a regenerative response [62]. Using RNA *in situ* hybridization, a recent study found that ROS production increased in zebrafish after metronidazole-induced cardiac injury due to the activity of duox/nox2, a family of ROS-generating NADPH oxidases [63]. ROS were found to promote cardiac myocyte proliferation because inhibiting them with apocynin resulted in decreased cardiac regeneration [63]. The study concluded that a likely cause for the pro-regenerative effect of ROS in zebrafish is its inhibitory effect on Dusp6, a PTP, resulting in derepression of MAP kinase signaling, favoring the ERK1/2 pathway and therefore promoting cardiac repair [63]. In fact, treatment of adult zebrafish with a selective inhibitor of PTP-1B increased cardiac regeneration rate by twofold to threefold [64]. Given the harmful effects of ROS on DNA, a means of increasing cardiac regeneration without inducing oxidative damage may be via modulation of substrates downstream of ROS, such as PTPs. Studies have also found that glycolysis is an important determinant of cardiac regeneration in zebrafish because blocking glycolysis decreases cardiac regeneration [56]. Both glycolysis and oxidation of PTPs may contribute to cardiac regeneration independently; however, more research is needed to confirm these findings.

The adult mammalian heart has limited cardiomyocyte replication, with annual regeneration rates of 0.5–1% [23]. Interestingly, fate mapping demonstrated that the majority of dividing cardiomyocytes in the adult mammalian

heart originated from a population of hypoxic cardiomyocytes [6,65]. Additionally, studies have shown that mortality rates from ischemic heart disease decrease linearly with increasing altitudes, thus suggesting a potential link between hypoxia and cardiovascular health [66]. A study using human cardiomyocytes found that moderate hypoxia increased cardiomyocyte proliferation [67]. However, there are conflicting results in the literature, as a recent randomized controlled trial found that ischemic conditioning did not improve outcomes post-MI [68].

Fatty acid oxidation is the main source of energy in the adult human heart, whereas embryonic and neonatal cardiomyocytes produce energy mostly through glycolysis [69]. The shift toward fatty acid oxidation occurs to sustain the metabolic needs of the heart, but this may also contribute to loss of regenerative capacity in the human heart as cardiomyocytes exit the cell cycle [69]. Favoring glycolysis instead of fatty acid oxidation may improve cardiac regeneration. For instance, studies have found that in HF and dilatative cardiomyopathy, there is a shift from fatty acid oxidation to glycolysis, which may be a compensatory mechanism to improve cardiac function [57,70]. However, a different study found that glycolysis may favor cardiac fibrosis post-MI, and thus inhibiting glycolysis may result in reduced fibrosis. Promoting glycolysis to regenerate cardiomyocytes post-MI may therefore be complicated by increased fibrosis and the arrhythmogenic propensity of fibrotic substrates [71]. The risks and benefits of glycolysis upregulation should be further studied [72]. Further exploring the modulation of mitochondrial oxidative metabolism as a potential therapeutic target in cardiac regeneration may be an important step toward developing safe and effective regenerative therapies. Overall, more research is needed to define the impact of oxygen metabolism in cardiomyocyte proliferation and to elucidate potential therapeutic targets that are both safe and effective.

Cardiomyocyte-extrinsic mechanisms affecting cardiac regeneration

The autonomic nervous system regulates cardiomyocyte proliferation

Research has highlighted the importance of the sympathetic nervous system (SNS) in mediating cardiac regeneration. For instance, ablating sympathetic nerves in the subepicardium, using 6-OHDA inhibited regeneration in neonatal mice [73]. However, 6-OHDA has been shown to induce ROS production [74], which may have damaged the epicardium, an important regulator of cardiac regeneration [75]. Nevertheless, a study using a neural chemorepellent to disrupt sympathetic fibers without injuring the epicardium still impaired ventricular regeneration in zebrafish [76], thus suggesting that the sympathetic nervous system affects cardiac regeneration. Interestingly, reduction of parasympathetic supply by vagotomy in neonatal mice also impaired cardiomyocyte proliferation, indicating that the autonomic nervous system plays a broad role in cardiac regeneration [76]. Therefore, because MI results in sympathetic denervation in large mammals [77], future studies should assess if reinnervation can support cardiac regeneration.

Epicardium & cardiac regeneration

Over the past decade, there has been substantial evidence around the importance of the epicardium on cardiac regeneration via a huge array of mechanisms. Studies in zebrafish show that after ventricular resection, the epicardium becomes further activated and the epicardial cells undergoing epithelial–mesenchymal transition have further proliferation and more directly migrate toward the injured zone [78]. Much evidence exists around the array of growth factors secreted and stimulated by the activated epicardium. For example, epicardial IGF signaling in zebrafish was shown to increase cardiomyocyte proliferation after resection [79]. In zebrafish, it was shown that the subepicardial cardiomyocytes that primarily contribute to the regenerating myocardium have increased expression of the intracellular transcription factor gata4, which may be responsible for regeneration after ventricular resection [80]. Additionally, the activation and upregulation of epicardial Notch receptors in zebrafish were shown to be crucial in cardiomyocyte proliferation after ventricular resection [81].

NRG1 increases cardiac regeneration

Many molecular pathways have been found to regulate cardiac regeneration; for instance, the growth factor NRG1 was found to support cardiomyocyte proliferation by binding to ErbB2/ErbB4 receptors [82]. Activation of *NRG1* was also shown to induce glycolysis, which, as previously discussed, favors cardiac regeneration [83]. Furthermore, NRG1 was shown to reverse the regenerative impairment caused by vagotomy in neonatal mice, suggesting that NRG1 also acts through the autonomic nervous system pathway [76]. A study using zebrafish identified an 11-fold increase in NRG1 after cardiomyocyte genetic ablation injury and even in the absence of injury, NRG1 treated neonatal mice had increased cardiomyocyte proliferation [84]. In humans, targeted NRG1 administration

induced cardiomyocyte proliferation in cultured myocardial samples from infants with HF [85]. Older patients were unaffected, possibly due to reducing ErbB2 expression during ageing as seen in mice [85,86]. This suggests a potential therapeutic window for NRG1 administration, which may be overcome by methods of ErbB2 upregulation. Future research should explore methods to increase NRG1 and ErbB2 expression and their therapeutic potential.

IGF increases regenerative potential

IGF is an anabolic hormone contributing to the growth of tissues [87]. In zebrafish, for instance, inhibition of IGF1 receptors, resulted in impaired cardiomyocyte proliferation after ventricular resection [79,87]. Furthermore, in neonatal mice with induced MI, administration of IGFBP3 restored regenerative capacity after it was shown that knockout of *IGFBP3* impaired regeneration [88]. These findings suggest the role of IGF in regeneration is conserved across species. Interestingly, when cortisol levels were increased in zebrafish with cryoinjured myocardium, there was a notable downregulation of *IGF*, leading to cardiac scarring [89]. When psychological stress was ameliorated, pharmacologically with propranolol/fluoxetine, regeneration was subsequently rescued [89]. Future research should therefore explore pathways modulating stress responses and whether these can be exploited therapeutically to promote regeneration.

The role of the extracellular matrix in cardiomyocyte regeneration

The regenerative ability of the zebrafish and neonatal mice heart may also be attributed to differences in extracellular matrix (ECM) components. ECM remodeling is a major contributor to scar formation and fibrosis, which hinders cardiac function [90]. Adult mammalian hearts exhibit a dominant fibrotic response of the ECM components to injury, which may contribute to their inability for cardiac regeneration [91].

Notari *et al.* found that in neonatal mice P2 hearts are ~50% stiffer than P1 hearts, coinciding with a loss of regenerative potential by P2 [92]. Similarly, in zebrafish a decrease in collagens is observed 7 days after amputation via resection injury, correlating with a decrease in cardiac stiffness [91]. Indeed, when cardiomyocytes from neonatal mice were cultured on less stiff matrices, they dedifferentiated [93]; cell rounding was observed, indicating cell division [94]. This evidence suggests that increase in ECM stiffness reduces the capacity for cardiac regeneration [93] and may therefore be a potential therapeutic target.

The differential composition of ECM between species influences cardiac regeneration. In mouse ECM, collagens predominate, encouraging tissue differentiation [91]. By contrast, in zebrafish ECM (zECM), elastin content is high, promoting cell proliferation whereas downregulation of fibroblasts restricts fibrosis [94]. Regeneration was stimulated when zECM was injected into adult mice hearts following acute MI via intramyocardial injection, suggesting that zECM has pro-proliferative effects [94]. However, it is unknown whether these results will be reproduced in subacute or chronic HF patients [94]. The effects of zECM did not persist beyond 6 weeks, suggesting a limited therapeutic window. Furthermore, repeated zECM injections may result in uncontrollable proliferation, via NRG1 signaling, thereby inducing HF [95].

The ECM gene *Ccn2a* is known to be a positive regulator of heart regeneration in zebrafish and neonatal mice. *Ccn2a* is highly expressed in injured zebrafish cardiac tissue, and loss of *Ccn2a* expression diminishes cardiomyocyte proliferation after cryoinjury [10,96]. *Ccn2a* also regulates expression of downstream ECM proteins such as fibronectins and collagens, which modulate heart regeneration by directing cardiomyocyte migration [10,97–99]. Further ECM molecules associated with cell proliferation include tenascin C and periostin. Upregulation of tenascin C corresponds with an increase in tissue remodeling and encourages regenerative growth in zebrafish following amputation via resection injury, whereas periostin has been shown to stimulate cardiomyocyte cell cycle reentry after cryoinjury [100,101]. Thus, a variety of ECM components work together to promote cardiac regeneration in zebrafish and neonatal mice.

A final key component of the ECM associated with the heart's regenerative capacity is Agrin, an extracellular proteoglycan [90]. In mice, postnatal reduction of its expression coincides with loss of regenerative capacity [90]. Genetic deletion of Agrin in neonatal mice increases fibrosis, while reducing cardiomyocyte proliferation, demonstrating its critical role in heart regeneration [95]. In the neonatal mouse heart, Agrin binds to *Dag1* and favors cardiac regeneration through destabilization of the dystrophin–glycoprotein complex and by triggering downstream YAP signaling [90]. Postnatally, Agrin levels drop, forcing *Dag1* to bind to alternative ECM molecules such as Laminin, which indirectly promotes cardiomyocyte cell cycle arrest [90]. Intramyocardial injection of recombinant mouse Agrin into adult mice stimulated cardiomyocyte cell cycle reentry [90]. Furthermore, similar studies using recombinant human Agrin improved hallmark signs of HF in porcine hearts after infarction by LAD ligation and

showed additional antiinflammatory properties [102]. However, Notari *et al.* found no difference between the levels of Agrin and *Dag1* in P1 and P2 mice, suggesting Agrin does not influence neonatal heart regeneration to a great extent [92]. Additionally, Agrin-based therapies should be considered with caution, given its carcinogenic role in the liver via YAP signaling [103].

Therapeutically, targeting ECM handling presents multiple novel approaches to induce cardiomyocyte proliferation following acute MI. Intramyocardial injection of zECM, Agrin or derivatives of periostin or fibronectins may promote cardiac regeneration while preventing progression to HF. Additionally, the success of human recombinant Agrin in larger mammals encourages further research into therapeutic avenues. Nonetheless, these studies are not entirely comparable; MI induced through ligation of the left anterior descending artery does not always stimulate complete regeneration, whereas results obtained through ventricular resection methods are often reproducible [92]. Hence, there are promising results suggesting that ECM modulation can promote cardiac regeneration; however, more research is needed.

The immune system modulates cardiac regeneration

A powerful inflammatory response occurs post-MI, triggering cell death and leading scar-formation in adult mice [6,104,105]. An equally vigorous inflammatory response occurs following apical resection injury to the myocardium in neonatal mice; however, this precipitates regeneration instead [6,104,105]. That inflammation can simultaneously be detrimental and crucial for the repair of injured cardiac tissue is a concept that was only recently heeded; the mechanism underlying this dichotomy has since been researched extensively [106]. There are two distinct subsets of macrophages that mediate inflammatory (CCR2+) and reparative (CCR2-) functions, respectively [107–112]. One study induced cardiomyocyte ablation via a diphtheria-toxin system to simulate injury in adult mice [112]. Although both types of macrophages were present within the heart [113], the resident pro-regenerative CCR2- macrophages were proportionally replaced following injury by previously circulating, scar-forming CCR2+ macrophages. However, these findings should be approached cautiously because both vascular and interstitial cells are preserved, which would have succumbed to hypoxia postinfarction, making it possible that the reparative responses observed in this model differ from what truly ensues following ischemia. Nevertheless, another study found that depleting subpopulations of CCR2+ macrophages derived from blood monocyte reservoirs in neonatal mice resulted in improved regeneration [114]. Given that CCR2- macrophages predominate in the adult myocardium, it may therefore be possible to improve cardiac repair by therapeutically inhibiting monocyte recruitment following MI. Despite this, patients on steroids with lower monocyte counts exhibit impaired wound healing post-MI [115], and macrophage-depleted neonatal mice lost their ability to regenerate [104], suggesting that a finely calibrated macrophage-mediated response is important for regeneration.

Two subsets of macrophages have also been discovered in adult zebrafish, which exhibit similar features as in mice [116]. Further insights into the role of the immune system in regeneration employed by adult zebrafish were ascertained through experiments on a phylogenetically related species: the Medaka [117]. Unlike the adult zebrafish, Medaka are unable to regenerate their myocardia [118]. Comparisons of bulk RNA-Seq data between the two fish species following resection injury revealed their respective immune systems are distinguished by a triad of diverging responses: within the Medaka, a smaller number of macrophages were activated, fewer localized to the site of injury and neutrophils remained at the resection site for significantly longer [119]. Strikingly, modulating the Medaka's immune response to expedite macrophage recruitment and neutrophil clearance so that it more closely resembled the adult zebrafish led to increased cardiomyocyte regeneration and scar resolution [120]. Although convenient, resection injury may not recapitulate the immune response to ischemic injury because it eliminates fragmented ECM proteins, dying cells and the sources of inflammatory signals, which would have been otherwise present in the context of infarction. Other immune factors and cell types have also been shown to play important roles in mediating regeneration in both species. For instance, IL-13 knockout mice fail to regenerate after apical resection, and administration of recombinant IL-13 increases cardiomyocyte proliferation [121]. Furthermore, ablation of zebrafish Treg-like cells compromised myocardial regeneration via inhibition of IL-10-independent secretion of *NRG1* [122]. However, other studies have shown perivascular cells as another source of *NRG1* within the heart [84], suggesting successful regeneration may require multiple sources of growth factors. These studies provide insights into how IL-13 and human Treg cells may potentially be targeted to facilitate regeneration of injured myocardium therapeutically, and together they highlight the complexity of immune regulation of regeneration in both neonatal mice and adult zebrafish. Ultimately, however, the extent to which these findings can be used to infer knowledge about humans remains unknown.

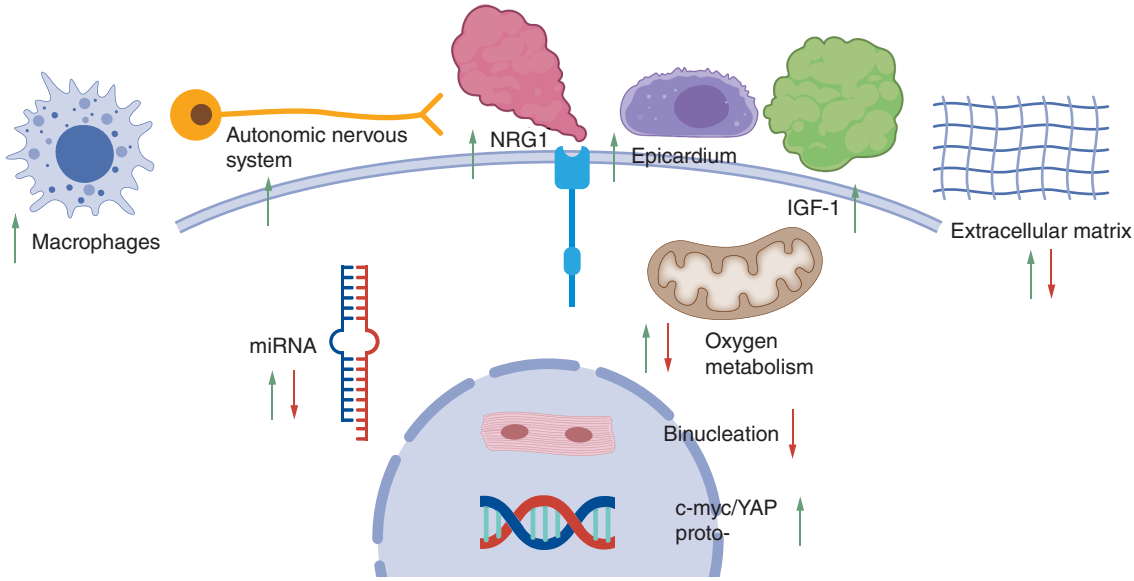


Figure 2. Illustration summarizing the main molecular and cellular pathways mediating cardiac regeneration in neonatal mice and adult zebrafish. Arrows pointing up (in green) demonstrate upregulation of cardiac regeneration, whereas arrows pointing down (in red) indicate downregulation of cardiomyocyte regeneration. In both species, macrophage recruitment to the site of myocardial injury promotes proliferation. Cardiac regeneration may be decreased by bi-/multi-nucleation (and polyploidy), whereas IGF-1, NRG1 and autonomic nervous system signaling can increase it. Furthermore, the expression of certain miRNAs and extracellular matrix components can both enhance or suppress cardiomyocyte regeneration. Factors such as c-Myc, YAP signalling, and the activated epicardium can increase cardiomyocyte proliferation. Finally, oxidative phosphorylation in neonatal mice promotes ROS production limiting cardiac proliferation through DNA damage, while Reactive oxygen species in zebrafish may promote cardiomyocyte proliferation, thus highlighting the heterogeneity of regenerative pathways between species. Image created with BioRender.com

Conclusion

The aforementioned studies have highlighted multiple cellular and molecular pathways contributing to the regenerative ability of zebrafish and neonatal mice, as illustrated in Figure 2. Metabolically, anaerobic glycolysis promotes cardiac regeneration in zebrafish, whereas in adult mice, aerobic respiration results in ROS production, inducing cardiomyocyte cell cycle arrest. Hippo-YAP and IGF signaling are essential pathways that regulate cardiomyocyte proliferation, whereas decreased NRG1 and autonomic nerve signaling are associated with reduced regenerative capacity. The activated epicardium increases cardiac regeneration via secretion of various growth factors and further activation of genes such as gata4 and Notch. Furthermore, both species have higher proportions of diploid cardiomyocytes, which favor proliferation and healing postinjury. Regulation of gene expression through miRNAs and the proto-oncogene c-Myc also play a critical role in controlling cardiomyocyte proliferation. Finally, changes in ECM handling, and the immune response – in particular, monocyte recruitment – are imperative to postnatal hypertrophic growth and adverse tissue remodeling. These mechanisms provide insight into cardiac regeneration in smaller vertebrates, but because of the lack of knowledge in larger mammals, it is still difficult to test these concepts therapeutically. Ultimately, as discussed in the future perspective section, targeting these pathways to promote cardiomyocyte proliferation remains a promising approach to regenerate adult myocardium.

Future perspective

Recent advances & clinical applications

Previously, there has been limited success in translating regenerative mechanisms for clinical application. Nevertheless, a diverse range of opportunities have recently arisen for novel therapies that could stimulate cardiomyocyte proliferation, including but not limited to drugs targeting PTP inhibition, modulation of IGF stability, increasing cardiomyocyte sensitivity to c-Myc, intramyocardial injection of Agrin or zECM and NRG1 administration [85,88,94,102,123,124]. The new therapeutic avenues explored in this review have the potential to improve recovery post-MI, thereby surpassing current treatment strategies that aim merely to minimize pathological

remodeling and the progression to HF following MI. However, further research is needed to determine the extent to which these approaches can be translated to clinical practice.

Future concerns

Despite the promise of these therapies, there is concern regarding their possible detrimental effects. For instance, using c-Myc therapeutically carries a carcinogenic risk, and modulating the immune system could result in a hypo- or hyper-sensitive response, thereby compromising healing post-MI. Moreover, hypertrophic hearts in HF patients have fewer mononucleated cells and reduced capacity for proliferation and may hence not respond as favorably to potential treatments due to the barriers of polyploidization [125]. Finally, given the vast evolutionary distance between zebrafish and humans, we cannot be certain that the pathways pertaining to the adult zebrafish outlined in this review are conserved in humans.

Therefore, future studies should aim to shift from smaller vertebrates to large mammals such as pigs, dogs and sheep. Fine-tuning our understanding of the mechanisms underlying regeneration in these species will facilitate the design of studies for therapies targeting cardiac regeneration in humans.

Executive summary

Cardiomyocyte polyploidization reduces their regenerative ability

- Polyploidization and binucleation are associated with decreased cardiac regenerative ability across multiple species, including humans.
- Zebrafish and neonatal mice cardiomyocytes are mostly mononucleated and diploid, with adult mice then experiencing a transition to high numbers of binucleated and polyploid cardiomyocytes.

Multiple miRNAs regulate cardiac regeneration

- miRNAs control multiple downstream pathways involved in cardiomyocyte cell cycle and proliferation.
- The miR-15 family, and specifically miR-195, in neonatal mice and the miR-133 in zebrafish have been found to suppress proliferation of cardiomyocytes, whereas the miR-17-92 cluster in mice can promote cardiac regeneration.

C-Myc expression may induce cardiomyocyte regeneration

- The proto-oncogene c-Myc has various targets, including FoxM1 and P-TEFβ, which can potentially increase cardiomyocyte regeneration in neonatal mice.
- Modulation of c-Myc pathways should be transient and thoroughly studied to avoid carcinogenic complications.

Oxidative DNA damage can reduce cardiac regeneration

- In humans, sustained reactive oxygen species (ROS) production can cause damage to proteins, lipids and DNA molecules.
- Fatty acid oxidation occurs to sustain the metabolic needs of the adult human heart, but this may contribute to loss of regenerative capacity as cardiomyocytes exit the cell cycle. Favoring glycolysis instead of fatty acid oxidation may improve cardiac regeneration.

The autonomic nervous system regulates cardiomyocyte proliferation

- The sympathetic/parasympathetic nervous system has been shown to be important in mediating cardiac regeneration because its inhibition reduces cardiac regeneration. Neuregulin-1 growth factor upregulation, which is also linked with the autonomic nervous system, also increases cardiac regeneration.

Hippo pathway mediates cardiac regeneration

- Inhibition of the Hippo pathway activates the TAZ/YAP proteins, which are important promoters of cardiac regeneration.

Epicardium & cardiac regeneration

- The epicardium has been shown to be important in cardiac regeneration via various studied mechanisms, some of which include the secretion of growth factors and further activation of genes such as gata4 and Notch.

The role of the extracellular matrix in cardiomyocyte regeneration

- Zebrafish and neonatal mice upregulate several extracellular matrix (ECM) components after myocardial injury that minimize fibrosis and encourage cardiac regeneration.
- Differences in composition of ECM between these species and adult mammalian hearts may contribute to the loss of regenerative potential in the latter.
- ECM proteins such as Fibronectin 1, Agrin or Ccn2a stimulate cardiomyocyte proliferation following injury
- Therapeutic use of these proteins or injection of zebrafish ECM intramyocardially may promote cardiac regeneration in adult mammalian hearts

The immune system modulates cardiac regeneration

- A finely calibrated macrophage-mediated response is important for cardiac regeneration
- Successful cardiac regeneration may require multiple sources of growth factors (including IL-10, IL-13 and NRG1)

Author contributions

All authors have contributed equally to the conception, design, drafting and final approval of the version to be published. The authors are all accountable for the accuracy and integrity of this work.

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References

Papers of special note have been highlighted as: • of interest

1. Laflamme MA, Murry CE. Heart regeneration. *Nature* 473(7347), 326–335 (2011).
2. Torabi A, Cleland JG, Rigby AS, Sherwi N. Development and course of heart failure after a myocardial infarction in younger and older people. *J. Geriatr. Cardiol.* 11(1), 1–12 (2014).
3. Drexler H, Fuchs M. Current limitations in treatment of heart failure: new avenues and treatment options. *J. Cardiovasc. Electrophysiol.* 13(Suppl. 1), S53–6 (2002).
4. Tonsho M, Michel S, Ahmed Z *et al.* Heart transplantation: challenges facing the field. *Cold Spring Harb. Perspect. Med.* 4(5), a015636 (2014).
5. Poss KD, Wilson LG, Keating MT. Heart regeneration in zebrafish. *Science* 298(5601), 2188–90 (2002).
6. Porrello ER, Mahmoud AI, Simpson E *et al.* Transient regenerative potential of the neonatal mouse heart. *Science* 331(6020), 1078–1080 (2011).
7. González-Rosa JM, Martín V, Peralta M *et al.* Extensive scar formation and regression during heart regeneration after cryoinjury in zebrafish. *Development* 138(9), 1663–1674 (2011).
8. González-Rosa JM, Mercader N. Cryoinjury as a myocardial infarction model for the study of cardiac regeneration in the zebrafish. *Nat. Protoc.* 7(4), 782–788 (2012).
9. Wang J, Panáková D, Kikuchi K *et al.* The regenerative capacity of zebrafish reverses cardiac failure caused by genetic cardiomyocyte depletion. *Development* 138(16), 3421–3430 (2011).
10. Mukherjee D, Wagh G, Mokalled MH *et al.* Ccn2a is an injury-induced matricellular factor that promotes cardiac regeneration in zebrafish. *Development* 148(2), dev193219 (2021).
11. Raya A, Koth CM, Büscher D *et al.* Activation of Notch signaling pathway precedes heart regeneration in zebrafish. *Proc. Natl Acad. Sci. USA*100, 11889–95 (2003).
12. Chablais F, Veit J, Rainer G, Jaźwińska A. The zebrafish heart regenerates after cryoinjury-induced myocardial infarction. *BMC Dev. Biol.* 11(1), 1–13 (2011).
13. Schnabel K, Wu C-C, Kurth T, Weidinger G. Regeneration of cryoinjury induced necrotic heart lesions in zebrafish is associated with epicardial activation and cardiomyocyte proliferation. *PLOS ONE* 6 (4), e18503 (2011).
14. Curado S, Anderson RM, Jungblut B *et al.* Conditional targeted cell ablation in zebrafish: a new tool for regeneration studies. *Dev. Dyn.* 236(4), 1025–1035 (2007).
15. Sun F, Shoffner AR, Poss KD. A genetic cardiomyocyte ablation model for the study of heart regeneration in zebrafish. *Cardiac Regeneration* 2158, 71–80 (2021).
16. Missinato MA, Zuppo DA, Watkins SC *et al.* Zebrafish heart regenerates after chemoptogenetic cardiomyocyte depletion. *Dev. Dyn.* 250(7), 986–1000 (2021).
17. Kolk MVV, Meyberg D, Deuse T *et al.* LAD-ligation: a murine model of myocardial infarction. *J. Vis. Exp.*32(14), 1438 (2009).
18. Haubner BJ, Adamowicz-Brice M, Khadayate S *et al.* Complete cardiac regeneration in a mouse model of myocardial infarction. *Aging* 4(12), 966 (2012).
19. Haubner BJ, Schuetz T, Penninger JM. A reproducible protocol for neonatal ischemic injury and cardiac regeneration in neonatal mice. *Basic Res. Cardiol.* 111(6), 64(2016).
20. Liu C, Wang L, Wang X, Hou X. A complete heart regeneration model with inflammation as a key component. *Exp. Anim.* 70(4), 479–487 (2021).

21. Li F, Wang X, Capasso JM, Gerdes AM. Rapid transition of cardiac myocytes from hyperplasia to hypertrophy during postnatal development. *J. Mol. Cell. Cardiol.* 28(8), 1737–1746 (1996).
22. Yutzey KE. Cardiomyocyte Proliferation: Teaching an Old Dogma New Tricks. *Circ. Res.* 120(4), 627–629 (2017).
23. Bergmann O, Bhardwaj RD, Bernard S *et al.* Evidence for cardiomyocyte renewal in humans. *Science* 324(5923), 98–102 (2009).
24. Patterson M, Barske L, Van Handel B *et al.* Frequency of mononuclear diploid cardiomyocytes underlies natural variation in heart regeneration. *Nat. Genet.* 49(9), 1346–1353 (2017).
25. Alkass K, Panula J, Westman M *et al.* No evidence for cardiomyocyte number expansion in preadolescent mice. *Cell*163(4), 1026–1036 (2015).
26. González-Rosa JM, Sharpe M, Field D *et al.* Myocardial polyploidization creates a barrier to heart regeneration in zebrafish. *Dev. Cell* 44(4), 433–446.e7 (2018).
27. Adler CP, Friedburg H, Herget GW *et al.* Variability of cardiomyocyte DNA content, ploidy level and nuclear number in mammalian hearts. *Virchows Arch.* 429(2-3), 159–164 (1996).
28. Herget GW, Neuburger M, Plagwitz R, Adler CP. DNA content, ploidy level and number of nuclei in the human heart after myocardial infarction. *Cardiovasc. Res.* 36(1), 45–51 (1997).
29. Bergmann O, Zdunek S, Felker A *et al.* Dynamics of cell generation and turnover in the human heart. *Cell* 161(7), 1566–1575 (2015).
30. Meckert PC, Rivello HG, Vigliano C *et al.* Endomitosis and polyploidization of myocardial cells in the periphery of human acute myocardial infarction. *Cardiovasc. Res.* 67(1), 116–123 (2005).
31. Mollova M, Bersell K, Walsh S *et al.* Cardiomyocyte proliferation contributes to heart growth in young humans. *Proc. Natl Acad. Sci. USA* 110(4), 1446–1451 (2013).
32. Miyaoka Y, Ebato K, Kato H *et al.* Hypertrophy and unconventional cell division of hepatocytes underlie liver regeneration. *Curr. Biol.* 22(13), 1166–1175 (2012).
33. Chen J, Huang Z-P, Seok HY *et al.* mir-17-92 cluster is required for and sufficient to induce cardiomyocyte proliferation in postnatal and adult hearts. *Circ. Res.* 112(12), 1557–1566 (2013).
34. 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. USA* 110(1), 187–192 (2013).

- **This study investigates the important role of the miR-15 family in mice cardiac regeneration and its potential therapeutic use following cardiac injury.**

35. Porrello ER, Johnson BA, Aurora AB *et al.* MiR-15 family regulates postnatal mitotic arrest of cardiomyocytes. *Circ. Res.* 109(6), 670–679 (2011).
36. Yin VP, Lepilina A, Smith A, Poss KD. Regulation of zebrafish heart regeneration by miR-133. *Dev. Biol.* 365(2), 319–327 (2012).
37. Diez-Cuñado M, Wei K, Bushway PJ *et al.* MiRNAs that induce human cardiomyocyte proliferation converge on the Hippo pathway. *Cell Rep.* 23(7), 2168–2174 (2018).
38. Jackson T, Allard MF, Sreenan CM *et al.* The c-myc proto-oncogene regulates cardiac development in transgenic mice. *Mol. Cell. Biol.* 10(7), 3709–3716 (1990).
39. Ahuja P, Zhao P, Angelis E *et al.* Myc controls transcriptional regulation of cardiac metabolism and mitochondrial biogenesis in response to pathological stress in mice. *J. Clin. Invest.* 120(5), 1494–1505 (2010).
40. Wang Y, Li Y, Feng J *et al.* Mydgf promotes cardiomyocyte proliferation and neonatal heart regeneration. *Theranostics* 10(20), 9100–9112 (2020).
41. Kisby T, Lázaro I, Fisch S *et al.* Adenoviral mediated delivery of OSKM factors induces partial reprogramming of mouse cardiac cells in vivo. *Adv. Ther.* 4(2), 2000141 (2021).
42. Bywater MJ, Burkhart DL, Straube J *et al.* Reactivation of Myc transcription in the mouse heart unlocks its proliferative capacity. *Nat. Commun.* 11(1), 1827 (2020).

- **This study explores c-Myc-induced cardiac regeneration and proposes an innovative way of enhancing it by manipulating P-TEFβ levels in mice.**

43. Sano M, Abdellatif M, Oh H *et al.* Activation and function of cyclin T–Cdk9 (positive transcription elongation factor-b) in cardiac muscle-cell hypertrophy. *Nat. Med.* 8(11), 1310–1317 (2002).
44. Miklas JW, Levy S, Hofsteen P *et al.* Amino acid primed mTOR activity is essential for heart regeneration. *iScience* 25(1), 103574 (2022).
45. Dicks S, Jürgensen L, Leuschner F *et al*. Cardiac regeneration and tumor growth-what do they have in common? *Front. Genet.* 11, 586658 (2020).
46. Chen Y, Lüttmann FF, Schoger E *et al.* Reversible reprogramming of cardiomyocytes to a fetal state drives heart regeneration in mice. *Science*.373(6562), 1537–1540 (2021).
47. Aharonov A, Shakked A, Umansky KB *et al.* ERBB2 drives YAP activation and EMT-like processes during cardiac regeneration. *Nat. Cell Biol.* 22(11), 1346–1356 (2020).

48. Plouffe SW, Lin KC, Moore JL 3rd *et al.* The Hippo pathway effector proteins YAP and TAZ have both distinct and overlapping functions in the cell. *J. Biol. Chem.* 293(28), 11230–11240 (2018).
49. Xiao Y, Dong J. The Hippo signaling pathway in cancer: a cell cycle perspective. *Cancers* 13(24), 6214(2021).
50. Xin M, Kim Y, Sutherland LB *et al.* Hippo pathway effector Yap promotes cardiac regeneration. *Proc. Natl Acad. Sci. USA* 110(34), 13839–13844 (2013).
51. Heallen T, Morikawa Y, Leach J *et al.* Hippo signaling impedes adult heart regeneration. *Development*140(23), 4683–4690 (2013).
52. Von Gise A, Lin Z, Schlegelmilch K *et al.* YAP1, the nuclear target of Hippo signaling, stimulates heart growth through cardiomyocyte proliferation but not hypertrophy. *Proc. Natl Acad. Sci. USA* 109(7), 2394–2399 (2012).
53. Nishimoto M, Uranishi K, Asaka MN *et al.* Transformation of normal cells by aberrant activation of YAP via cMyc with TEAD. *Sci. Rep.* 9(1), 10933 (2019).
54. Flinn MA, Jeffery BE, O'Meara CC, Link BA. Yap is required for scar formation but not myocyte proliferation during heart regeneration in zebrafish. *Cardiovasc. Res.* 115(3), 570–577 (2019).
55. Bo B, Li S, Zhou K, Wei J. The regulatory role of oxygen metabolism in exercise-induced cardiomyocyte regeneration. *Front. Cell Dev. Biol.* 9, 664527 (2021).
56. Fukuda R, Marín-Juez R, El-Sammak H *et al.* Stimulation of glycolysis promotes cardiomyocyte proliferation after injury in adult zebrafish. *EMBO Rep.* 21(8), e49752 (2020).
57. Lopaschuk GD, Karwi QG, Tian R *et al.* Cardiac energy metabolism in heart failure. *Circ. Res.* 128(10), 1487–1513 (2021).
58. Elhelaly WM, Lam NT, Hamza M *et al.* Redox regulation of heart regeneration: an evolutionary tradeoff. *Front. Cell. Dev. Biol* 4, 137 (2016).
59. Puente BN, Kimura W, Muralidhar SA *et al.* The oxygen-rich postnatal environment induces cardiomyocyte cell-cycle arrest through DNA damage response. *Cell* 157(3), 565–579 (2014).
60. Bai C-G, Liu X-H, Liu W-Q, Ma D-L. Regional expression of the hypoxia-inducible factor (HIF) system and association with cardiomyocyte cell cycle re-entry after myocardial infarction in rats. *Heart Vessels* 23(3), 193–200 (2008).
61. Moslehi J, Minamishima YA, Shi J *et al.* Loss of hypoxia-inducible factor prolyl hydroxylase activity in cardiomyocytes phenocopies ischemic cardiomyopathy. *Circulation* 122(10), 1004–1016 (2010).
62. Gauron C, Rampon C, Bouzaffour M *et al.* Sustained production of ROS triggers compensatory proliferation and is required for regeneration to proceed. *Sci. Rep.* 3, 2084 (2013).
63. Han P, Zhou X-H, Chang N *et al.* Hydrogen peroxide primes heart regeneration with a derepression mechanism. *Cell Res.* 24(9), 1091–1107 (2014).

- **This study provides evidence for the role of reactive oxygen species in cardiac repair in zebrafish and identifies downstream molecules such as PTPs, which may be potential therapeutic targets for cardiac regeneration.**

64. Smith AM, Maguire-Nguyen KK, Rando TA *et al.* The protein tyrosine phosphatase 1B inhibitor MSI-1436 stimulates regeneration of heart and multiple other tissues. *NPJ Regen. Med.* 2, 4 (2017).
65. Kimura W, Xiao F, Canseco DC *et al.* Hypoxia fate mapping identifies cycling cardiomyocytes in the adult heart. *Nature* 523(7559), 226–230 (2015).
66. Faeh D, Moser A, Panczak R *et al.* Independent at heart: persistent association of altitude with ischaemic heart disease mortality after consideration of climate, topography and built environment. *J. Epidemiol. Community Health* 70(8), 798–806 (2016).
67. Ye L, Qiu L, Feng B *et al.* Role of blood oxygen saturation during post-natal human cardiomyocyte cell cycle activities. *JACC Basic Transl. Sci.* 5(5), 447–460 (2020).
68. Francis R, Chong J, Ramlall M *et al.* Effect of remote ischaemic conditioning on infarct size and remodelling in ST-segment elevation myocardial infarction patients: the CONDI-2/ERIC-PPCI CMR substudy. *Basic Res. Cardiol.* 116(1), 59 (2021).
69. Bae J, Paltzer WG, Mahmoud AI. The role of metabolism in heart failure and regeneration. *Front. Cardiovasc. Med.*8, 702920 (2021)
70. Tan J, Yang M, Wang H *et al.* Moderate heart rate reduction promotes cardiac regeneration through stimulation of the metabolic pattern switch. *Cell Rep.* 38(10), 110468 (2022).
71. Ali RL, Hakim JB, Boyle PM *et al.* Arrhythmogenic propensity of the fibrotic substrate after atrial fibrillation ablation: a longitudinal study using magnetic resonance imaging-based atrial models. *Cardiovasc. Res.* 115(12), 1757 (2019).
72. Chen Z-T, Gao Q-Y, Wu M-X *et al.* Glycolysis inhibition alleviates cardiac fibrosis after myocardial infarction by suppressing cardiac fibroblast activation. *Front. Cardiovasc. Med.*8, 701745 (2021).
73. White IA, Gordon J, Balkan W, Hare JM. Sympathetic reinnervation is required for mammalian cardiac regeneration. *Circ. Res.* 117(12), 990–994 (2015).
74. Rodriguez-Pallares J, Parga JA, Muñoz A *et al.* Mechanism of 6-hydroxydopamine neurotoxicity: the role of NADPH oxidase and microglial activation in 6-hydroxydopamine-induced degeneration of dopaminergic neurons. *J. Neurochem.* 103(1), 145–156 (2007).

75. Del Campo CV, Liaw NY, Gunadasa-Rohling M *et al.* Regenerative potential of epicardium-derived extracellular vesicles mediated by conserved miRNA transfer. *Cardiovasc. Res.* 118(2), 597–611 (2022).
76. Mahmoud AI, O'Meara CC, Gemberling M *et al.* Nerves regulate cardiomyocyte proliferation and heart regeneration. *Dev. Cell* 34(4), 387–399 (2015).
77. Dae MW, Herre JM, O'Connell JW *et al.* Scintigraphic assessment of sympathetic innervation after transmural versus nontransmural myocardial infarction. *J. Am. Coll. Cardiol.* 17(6), 1416–1423 (1991).
78. Lepilina A, Coon AN, Kikuchi K *et al.* A dynamic epicardial injury response supports progenitor cell activity during zebrafish heart regeneration. *Cell* 127(3), 607–619 (2006).
79. Huang Y, Harrison MR, Osorio A *et al.* Igf signaling is required for cardiomyocyte proliferation during zebrafish heart development and regeneration. *PLOS ONE*.8(6), e67266 (2013).
80. Kikuchi K, Holdway JE, Werdich AA *et al.* Primary contribution to zebrafish heart regeneration by gata4(+) cardiomyocytes. *Nature* 464(7288), 601–605 (2010).
81. Zhao L, Borikova AL, Ben-Yair R *et al.* Notch signaling regulates cardiomyocyte proliferation during zebrafish heart regeneration. *Proc. Natl Acad. Sci. USA* 111(4), 1403–1408 (2014).
82. D'Uva G, Aharonov A, Lauriola M *et al.* ERBB2 triggers mammalian heart regeneration by promoting cardiomyocyte dedifferentiation and proliferation. *Nat. Cell Biol.* 17(5), 627–638 (2015).
83. Honkoop H, De Bakker DE, Aharonov A *et al.* Single-cell analysis uncovers that metabolic reprogramming by ErbB2 signaling is essential for cardiomyocyte proliferation in the regenerating heart. *Elife* 8, e50163 (2019).
84. Gemberling M, Karra R, Dickson AL, Poss KD. Nrg1 is an injury-induced cardiomyocyte mitogen for the endogenous heart regeneration program in zebrafish. *Elife* 4, e05871 (2015).
85. 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.* 7(281), 281ra45 (2015).
86. Ma H, Yin C, Zhang Y *et al.* ErbB2 is required for cardiomyocyte proliferation in murine neonatal hearts. *Gene* 592(2), 325–330 (2016).
87. Takahashi T, Ishida K, Itoh K *et al.* IGF-I gene transfer by electroporation promotes regeneration in a muscle injury model. *Gene Ther.* 10(8), 612–620 (2003).
88. Ali SR, Elhelaly W, Nguyen NUN *et al.* Angiocrine IGFBP3 spatially coordinates IGF signaling during neonatal cardiac regeneration. *bioRxiv*460522(2021).
89. Sallin P, Jaźwińska A. Acute stress is detrimental to heart regeneration in zebrafish. *Open Biol.* 6(3), 160012(2016).
90. Bassat E, Mutlak YE, Genzelinakh A *et al.* The extracellular matrix protein agrin promotes heart regeneration in mice. *Nature* 547(7662), 179–184 (2017).
91. Garcia-Puig A, Mosquera JL, Jiménez-Delgado S *et al.* Proteomics analysis of extracellular matrix remodeling during zebrafish heart regeneration. *Mol. Cell. Proteomics* 18(9), 1745–1755 (2019).

- **This study explores the composition of extracellular matrix (ECM) in zebrafish and demonstrates that there is a sharp increase in specific ECM proteins following amputation. It highlights the differences in these ECM protein levels before and after resection and outlines their individual roles in cardiac regeneration.**

92. Notari M, Ventura-Rubio A, Bedford-Guaus SJ *et al.* The local microenvironment limits the regenerative potential of the mouse neonatal heart. *Sci. Adv.* 4(5), eaao5553 (2018).

- **The study shows that the composition of ECM in zebrafish undergoes an abundance of changes following amputation and highlights the influence of ECM handling on cardiac regeneration. The authors also highlight crucial changes in ECM stiffness during regeneration.**

93. Yahalom-Ronen Y, Rajchman D, Sarig R *et al.* Reduced matrix rigidity promotes neonatal cardiomyocyte dedifferentiation, proliferation and clonal expansion. *Elife*.4, e07455 (2015).
94. Chen WCW, Wang Z, Missinato MA *et al.* Decellularized zebrafish cardiac extracellular matrix induces mammalian heart regeneration. *Sci. Adv.* 2(11), e1600844 (2016).
95. Yutzey KE. Regenerative biology: Neuregulin 1 makes heart muscle. *Nature* 520(7548), 445–446 (2015).
96. Pfefferli C, Jaźwińska A. The careg element reveals a common regulation of regeneration in the zebrafish myocardium and fin. *Nat. Commun.* 8, 15151 (2017).
97. Chablais F, Jazwinska A. The regenerative capacity of the zebrafish heart is dependent on TGFβ signaling. *Development* 139(11), 1921–1930 (2012).
98. George EL, Baldwin HS, Hynes RO. Fibronectins are essential for heart and blood vessel morphogenesis but are dispensable for initial specification of precursor cells. *Blood* 90(8), 3073–3081 (1997).
99. Trinh LA, Stainier DYR. Fibronectin regulates epithelial organization during myocardial migration in zebrafish. *Dev. Cell* 6(3), 371–382 (2004).
100. Jaźwińska A, Sallin P. Regeneration versus scarring in vertebrate appendages and heart. *J. Pathol.* 238(2), 233–246 (2016).

101. Kühn B, del Monte F, Hajjar RJ *et al.* Periostin induces proliferation of differentiated cardiomyocytes and promotes cardiac repair. *Nat. Med.* 13(8), 962–969 (2007).

102. Baehr A, Umansky KB, Bassat E *et al.* Agrin promotes coordinated therapeutic processes leading to improved cardiac repair in pigs. *Circulation* 142(9), 868–881 (2020).

103. Chakraborty S, Njah K, Pobbati AV *et al.* Agrin as a mechanotransduction signal regulating YAP through the Hippo pathway. *Cell Rep.* 18(10), 2464–2479 (2017).

104. Han C, Nie Y, Lian H *et al.* Acute inflammation stimulates a regenerative response in the neonatal mouse heart. *Cell Res.* 25(10), 1137–1151 (2015).

105. Aurora AB, Porrello ER, Tan W *et al.* Macrophages are required for neonatal heart regeneration. *J. Clin. Invest.* 124(3), 1382–1392 (2014).

- **This study highlights the importance of the role of the immune system in regulating cardiac regeneration and specifically the differential role of macrophages in regeneration of myocardium of neonatal mice. It shows that macrophage depletion removes the ability of neonatal mice to regenerate their myocardium.**

106. Glaros T, Larsen M, Li L. Macrophages and fibroblasts during inflammation, tissue damage and organ injury. *Front. Biosci.* 14(10), 3988–3993 (2009).

107. Schulz C, Gomez Perdiguero E, Chorro L *et al.* A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* 336(6077), 86–90 (2012).

108. Hashimoto D, Chow A, Noizat C *et al.* Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity* 38(4), 792–804 (2013).

109. Yona S, Kim K-W, Wolf Y *et al.* Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* 38(1), 79–91 (2013).

110. Ginhoux F, Greter M, Leboeuf M *et al.* Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 330(6005), 841–845 (2010).

111. Hoeffel G, Wang Y, Greter M *et al.* Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *J. Exp. Med.* 209(6), 1167–1181 (2012).

112. 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. U. S. A.* 111(45), 16029–16034 (2014).

113. Epelman S, Lavine KJ, Beaudin AE *et al.* Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. *Immunity* 40(1), 91–104 (2014).

114. Bajpai G, Bredemeyer A, Li W *et al.* Tissue resident CCR2- and CCR2+ cardiac macrophages differentially orchestrate monocyte recruitment and fate specification following myocardial injury. *Circ. Res.* 124(2), 263–278 (2019).

115. Roberts R, DeMello V, Sobel BE. Deleterious effects of methylprednisolone in patients with myocardial infarction. *Circulation* 53(Suppl. 3), I204–6 (1976).

116. Bevan L, Lim ZW, Venkatesh B *et al.* Specific macrophage populations promote both cardiac scar deposition and subsequent resolution in adult zebrafish. *Cardiovasc. Res.* 116(7), 1357–1371 (2020).

117. Kirchmaier S, Naruse K, Wittbrodt J, Loosli F. The genomic and genetic toolbox of the teleost medaka (Oryzias latipes). *Genetics* 199(4), 905–918 (2015).

118. Ito K, Morioka M, Kimura S *et al.* Differential reparative phenotypes between zebrafish and medaka after cardiac injury. *Dev. Dyn.* 243(9), 1106–1115 (2014).

119. Lai S-L, Marín-Juez R, Moura PL *et al.* Reciprocal analyses in zebrafish and medaka reveal that harnessing the immune response promotes cardiac regeneration. *Elife*.6, 6 (2017).

120. Potts HG, Stockdale WT, Mommersteeg MTM. Unlocking the secrets of the regenerating fish heart: comparing regenerative models to shed light on successful regeneration. *J. Cardiovasc. Dev. Dis.* 8(1), 4 (2021).

121. Wodsedalek DJ, Paddock SJ, Wan TC *et al.* IL-13 promotes in vivo neonatal cardiomyocyte cell cycle activity and heart regeneration. *Am. J. Physiol. Heart Circ. Physiol.* 316(1), H24–H34 (2019).

122. Hui SP, Sheng DZ, Sugimoto K *et al.* Zebrafish regulatory T cells mediate organ-specific regenerative programs. *Dev. Cell* 43(6), 659–672.e5 (2017).

123. Miller DM, Thomas SD, Islam A *et al.* c-Myc and cancer metabolism. *Clin. Cancer Res.* 18(20), 5546–5553 (2012).

124. Tanner JJ, Parsons ZD, Cummings AH *et al.* Redox regulation of protein tyrosine phosphatases: structural and chemical aspects. *Antioxid. Redox Signal.* 15(1), 77–97 (2011).

125. Adler CP, Friedburg H. Myocardial DNA content, ploidy level and cell number in geriatric hearts: post-mortem examinations of human myocardium in old age. *J. Mol. Cell. Cardiol.* 18(1), 39–53 (1986).