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Gene therapy for cardiomyocyte renewal: cell cycle, a potential therapeutic target

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Abstract

Heart disease is the primary cause of death worldwide. Even though enormous research has been done, and many pharmacological and surgical treatments have been introduced to treat heart disease, the mortality rate still remains high. Gene therapy is widely used to understand molecular mechanisms of myocadiac infarction and to treat cardiomyocyte loss. It was reported that adult cardiomyocytes proliferate at a very low rate, thus targeting their proliferation has become a new regenerative therapeutic target. Currently, re-activating cardiomyocyte proliferation seems one of the most promising methods to promote adult cardiomyocyte renewal. In this review, we highlight gene therapeutic targets of cell proliferation presently being pursued to re-activate the cell cycle of cardiomyocytes, including cell cycle regulators, transcription factors, micro RNAs, signal transduction, and other contributing factors. We also summarize gene delivery vectors that have been used in cardiac research and ongoing major challenges to be overcome in the translation to the clinical approach and future directions.

1. Introduction

Heart disease is the primary cause of death in developed countries, accounting for approximately 20% of total deaths in the United States in 2020 [1]. Heart disease results in the loss of cardiomyocytes, causing ventricular dysfunction and heart failure. Tremendous research has been conducted to treat heart disease for decades; however, it still remains the leading cause of mortality in the world. A major reason is that following myocardial infarction, the lost cardiomyocytes are replaced with fibrotic scar tissue because the heart is one of the least regenerative organs in the body [2, 3]. Unlike neonatal hearts where cardiomyocytes divide at the first a few days, adult cardiomyocytes exit cell cycle and lack the ability to recover impaired cardiac function [4–7]. In the past decades, it seemed impractical to replace the lost cardiomyocytes or restore the function of cardiomyocytes, despite the existence of conventional treatments. Therefore, heart transplantation had been considered the only treatment to cure heart disease at the end stage of heart failure [8]. Even though the outcome of heart transplantation results in an improvement in patient survival, there are serious limitations facing the field such as the number of available donor hearts, rejection of the donor heart, and primary graft failure [9]. For these reasons, there have

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been increasing efforts in the development of alternative strategies to treat the damaged myocardium including achieving cardiomyocyte renewal [10–12].

Gene therapy is being explored to treat heart disease by transferring potential therapeutic genetic information to cardiomyocytes and modifying the expression and levels of proteins. Over the past 30 years, the field of cardiac gene therapy has developed significantly, and it is one of the extensively investigated methods to improve cardiomyocyte renewal [13]. Many previous studies have investigated gene therapy for cardiac regeneration *in vitro* and *in vivo*, however, it has been challenging when it comes to clinical settings due to the low efficiency of gene transference and the lack of significant clinical results [14, 15]. In this review, we discuss gene therapeutic strategies presently being pursued to promote cardiomyocyte proliferation. We also discuss ongoing major challenges to be overcome in the translation to the clinical approach and future directions. The unique feature of the current review is to present current cardiac gene therapy strategies specifically to promote cell proliferation, targeting cell cycle re-entry. A literature search was performed using PubMed using the keywords “cardiomyocyte proliferation”, “gene therapy”, and “cell cycle”.

2. Activators of cardiomyocyte proliferation are potential therapeutic targets for heart regeneration

2.1 Cell cycle regulators determine the proliferative capacity

In mammals, the heart grows via the cell division of cardiomyocytes during embryonic and fetal development [16]. Shortly after birth, cardiomyocytes exit the cell cycle, resulting in the enlargement of myocardial volume via cardiac hypertrophy instead of hyperplasia [16]. In detail, mice have cardiac regeneration capacity within the first seven days after birth, and pigs can regenerate from preexisting cardiomyocytes within the first two days postnatally [6, 7, 17]. In humans, it was generally believed that cardiomyocytes exit the cell cycle after birth and stay in a quiescent state, completing mitosis. However, the integration of ^{14}C demonstrated that the cardiomyocyte turnover rate is approximately 1% at the age of 20, constantly decreasing annually to 0.3% at the age of 75 [18]. Even though it is minimal, cell cycle re-activation in postnatal cardiomyocytes has become a potential therapeutic intervention for promoting cardiomyocyte proliferation after cardiac injury. Therefore, cell cycle regulators were targeted to promote cell cycle re-entry and endogenous cardiomyocyte proliferation (Figure 1).

The cell cycle is tightly regulated by multiple factors such as cyclins and the cyclin-dependent kinases (CDKs) at different phases, thus it is expected that manipulating the expression of the cell cycle regulators would induce cell cycle re-entry. For instance, cyclin A2-induced cell cycle activation increases border zone myofilament density and myocardial function in the damaged rat hearts [19] as well as mediating mitosis and hyperplasia in mice [20]. Cyclin D1 overexpression increased cardiomyocyte DNA synthesis and multinucleation in adult mice [21]. In addition, cyclin D2 overexpressing adult transgenic mice maintained cell cycle activity and reduced scar size after myocardial infarction [22]. Furthermore, a combination of cell cycle activators including CDK1, CDK4, cyclin B1, and cyclin D1 efficiently improved cell division and cardiac function in mouse, rat, and

human cardiomyocytes after myocardial infarction [23]. The researchers reported that a combination of the cell cycle regulators might efficiently activate the proliferative potential in adult cardiomyocytes. In addition to cell cycle activators, cell cycle inhibitors including p21, p27, and p57 have been used to manipulate the cell cycle in adult cardiomyocytes, and silencing the expression of the inhibitors induces re-entry of the cell cycle in cardiomyocytes [24, 25].

2.2 Transcription factors control the expression of cell cycle regulators

There are several transcription factors that regulate the cell cycle (Figure 1), and various research have been done on these transcription factors as potential targets to activate cardiomyocyte renewal. First, myeloid ecotropic viral integration site 1 (Meis1) regulates cell cycle arrest and is necessary to induce transcription of the CDK inhibitors including p15, p16, and p21 [26]. Meis1 knockout in mice showed improvement in postnatal cardiomyocyte proliferation capacity. Forkhead box M1 (Foxm1), a major proliferation-associated transcription factor, regulates S-phase and M-phase entry, and it is essential for mitosis and progression in cardiomyocytes [27]. A recent study reported that Foxm1 deletion in adult zebrafish resulted in a decrease in cardiomyocyte proliferation and significantly increased scar area after ventricular amputation [28]. In addition, the E2f family is known to regulate many cell cycle genes, with E2f1, E2f2, and E2f4 being the most investigated [29–31]. Intramyocardial injection of E2f1 via adenoviral delivery in adult mice re-activated the cell cycle and completed DNA synthesis in postmitotic cardiac muscle [29]. Similarly, E2f2 overexpression in the heart re-activated proliferation by inducing cyclin A and cyclin E in adult mouse cardiomyocytes [30]. Another study found that adenovirus-mediated delivery of E2f2 and E2f4 but not E2f1 and E2f3 induced S-phase entry, activating DNA synthesis without apoptosis in neonatal cardiomyocytes from both rats and mice [31]. Additionally, overexpression of the T-box transcription factor (Tbx20) in adult mouse cardiomyocytes promoted cardiomyocyte proliferation, cardiac function, and survival rate after myocardial infarction by activating the expression of proliferation positive regulators [32]. Gata binding protein 4 (Gata4) plays a significant role in neonatal cardiac development, promoting cardiac hypertrophy and angiogenesis and the maintenance of postnatal cardiac function [33, 34]. Cardiomyocyte Gata4 knockout mice showed reduced angiogenesis and cardiomyocyte proliferation after cryoinjury whereas Gata4 overexpression induced cardiomyocyte proliferation and cardiac regeneration in mice 7 days after cryoinjury [35]. The paired related homeobox 1 (Prrx1b) is a transcription factor that participates in wound healing and limb regeneration [36]. Prrx1b knockout resulted in excessive fibrosis and impaired proliferation in zebrafish cardiomyocytes, suggesting that Prrx1b is necessary for balancing fibrosis and cardiomyocyte renewal.

2.3 microRNAs regulate cell cycle in cardiomyocytes

Various microRNAs (miRNAs) play a pivotal role in regulating the cell cycle in cardiomyocytes (Figure 1). Eulalio et al. screened various functional miRNAs regulating neonatal cardiomyocyte proliferation, and they found that forty miRNAs significantly enhanced DNA synthesis as well as cytokinesis in both mouse and rat cardiomyocytes [37]. Among them, has-miR-590 and has-miR-199a significantly promoted postnatal cardiomyocyte renewal by increasing proliferation and reducing infarct size in adult mice

after myocardial infarction. In addition, overexpression of miR-204, miR-17–92, miR-302–367, and miR-294 promoted cardiomyocyte proliferation in adult mice [38–41]. On the other hand, inhibition of miR-195 and miR-128 improved cardiomyocyte proliferation of preexisting cardiomyocytes and left ventricular systolic function in adult mice after myocardial infarction [42–44].

2.4 The signaling pathways activate postnatal cardiomyocyte proliferation

Various intracellular developmental signaling pathways play a pivotal role in regulating cardiomyocyte proliferation (Figure 1). Hippo/Yes-associated protein (YAP) signaling is essential in embryonic heart growth as well as postnatal cardiac function [45]. Cardiac specific YAP knockout in mice resulted in impairment of neonatal cardiac regeneration post-myocardial infarction [46]. In addition, YAP overexpression in transgenic mice enhanced cardiomyocyte proliferation in the postnatal heart after cardiac injury [46]. The mitogen-activated protein kinases (MAPK) signaling is a core pathway that controls cellular proliferation, differentiation, development, and apoptosis in mammalian cells [47]. In 2015, Zhao and Peng found that MAPK1 promotes the proliferation of H9C2 rat cardiomyocytes by activating phosphoinositide-3-kinase (PI3K)/AKT signaling pathway [48]. PI3K/AKT signaling pathway is linked to the Hippo/YAP signaling through PI3K catalytic subunit beta (Pi3kcb), a critical enzyme that regulates cell growth and metabolic activity to promote adult cardiomyocyte proliferation and survival [49]. The Wnt/β-catenin signaling pathway is known to participate in embryonic development and adult tissue homeostasis [50]. Cardiomyocyte-specific deletion of low-density lipoprotein receptor-related protein 6 (LRP6), a Wnt co-receptor, increased cardiomyocyte proliferation in mice after myocardial infarction [51]. Liver kinase B1 (LKB1), a major upstream kinase for AMP-activated protein kinase, was also identified as a potential therapeutic target, showing that LKB1 knockdown improves postnatal cardiomyocyte renewal [52]. Furthermore, thyroid hormone signaling may regulate cardiac regeneration [53]. Cardiomyocyte-specific inhibition of thyroid hormone signaling postponed cell cycle exit, restoring cardiac regenerative potential [53, 54]. Additionally, fibroblast growth factor (FGF) 10 is known to promote cardiomyocyte renewal [55, 56]. A previous study found that conditional overexpression of FGF10 in adult mice hearts promoted cardiomyocyte cell cycle re-entry [55]. The study suggested that FGF10 signaling induces Forkhead Box O3 (FOXO3) phosphorylation, resulting in the inhibition of CDK inhibitor such as P27 and thus the activation of proliferation.

2.5 Other contributing factors

Besides the direct cell cycle regulators, there are indirect factors that regulate the cell cycle (Figure 1). Myeloid-derived growth factor (Mydgf) improved cardiomyocyte proliferation as well as heart regeneration in adult mice after myocardial infarction, reducing fibrotic area and increasing the survival rate by activating c-Myc/FoxM1 signaling pathway compared to the control group [57]. Neuregulin 1 (NRG1), FGF1, and periostin significantly increased DNA synthesis in primary adult rat cardiomyocytes, inducing cell-cycle reentry [58, 59]. The enhancer of zeste homolog 2 (Ezh2) is required to activate the platelet-derived growth factor receptor- β (PDGFR-β) to induce cardiomyocyte proliferation, and the activation of PDGFR-β resulted in the improvement of adult heart regeneration after myocardial infarction by decreasing scar size as well as increasing left ventricular systolic

function [60]. Interestingly, metabolic activity also affects the cardiomyocyte cell cycle after myocardial infarction. Neonatal cardiomyocytes switch metabolism from glycolysis to oxidative phosphorylation, resulting in reactive oxygen species production and DNA damage and eventually causing cardiomyocyte cell cycle exit [61]. Bae et al. reported that malonate to inhibit succinate dehydrogenase can activate adult cardiomyocyte proliferation, revascularization, and heart regeneration by reprogramming the metabolism in the adult cardiomyocytes [62]. Lastly, an anti-inflammatory cytokine, IL-13 stimulated the cell cycle and heart regeneration in neonatal mice cardiomyocytes by activating ERK1/2 and AKT downstream signaling [63]. Furthermore, after myocardial infarction, it appears that promoting IL-13 binding to type II IL4Rα/IL13Rα1 receptor increased cardiomyocyte DNA synthesis and recovery from myocardial infarction at the age when heart regeneration is not active [64].

3. Gene therapy vectors

Gene therapy is to treat and/or improve a patient's disease condition by manipulating the expression of a gene in the patient's target cells. Since naked DNA molecules have a relatively large size and hydrophilic characteristics, they are not able to enter the cells efficiently [65]. Therefore, in gene therapy, a gene can be delivered by a carrier, called a vector. Generally, gene delivery to target cells can be achieved using either viral vectors or non-viral vectors (Figure 2).

3.1 Non-viral vectors

The non-viral gene delivery employs synthetic or natural compounds to deliver a gene into cells instead of viruses. The non-viral vectors carry plasmid DNA, mRNA, and nucleic acid complexes [66]. Non-viral gene delivery has critical limitations such as low gene transfection efficiencies due to the limited cellular uptake, low transient expression, and short-term expression period [65]. Nevertheless, there are many beneficial features of non-viral vectors including less toxicity, low immunogenicity, no packaging limitations, and low cost of production [67]. In heart disease research, various non-viral vectors have been used (Table 1). The easiest non-viral transfection method is the direct transfer with DNA plasmids. Direct intramyocardial injection of a plasmid encoding vascular endothelial growth factors (VEGF) A-165 was successful to improve heart conditions in injured hearts in pigs, sheep, and humans [68–70].

To increase the stability of plasmid DNA, the biomedical engineering approach is introduced to carry the naked plasmid DNA to the heart. Previously, nanoparticles have shown safe and effective gene delivery in cardiovascular disease models [71]. In particular, ionizable lipid nanoparticles have shown high transfection efficacy in cardiomyocytes with less toxicity *in vitro* as well as *in vivo* [71], and many studies have used lipid nanoparticles to deliver their target genes to repair infarcted myocardial tissue [72–74]. Liposomes or lipoplex nanoparticles surround negatively charged DNA with positively charged lipids and detergents to increase cellular uptake through endocytosis [75]. However, the liposomal DNA complexes are rapidly removed from the systemic circulation and do not prevent intracellular degradation in endosomes [76]. In addition, the liposome

delivery method presents critical limitations such as high toxicity because of the interaction with the cell membrane and the release of the DNA from the endosomes to the cytoplasm [77, 78]. To reduce the aggregation of lipid nanoparticles and nonspecific endocytosis, polyethylene glycol-modified (PEGylated) lipid nanoparticle conjugates were synthesized and showed a promising delivery capacity in the myocardial ischemia model [79–81]. Another nanoparticle that has been used in a heart disease model is poly-lactide/glycolide acid (PLGA). Ikeda et al. successfully delivered their target gene, cyclosporine A, to cardiomyocyte mitochondria through intravenous injection of PLGA nanoparticles in a murine myocardial ischemia-reperfusion model [82]. Furthermore, PLGA-mediated intravenous delivery of irbesartan and pitavastatin ameliorated left ventricular remodeling after myocardial ischemia-reperfusion injury in various animal models [83–86]. Besides intravenous injection, PLGA-mediated intramyocardial delivery of VEGF and insulin-like growth factor 1 (IGF1) to the mice hearts promoted recovery from myocardial infarction by improving vascular density and left ventricular contractile function and reducing infarct size [87, 88].

Another plasmid DNA delivery strategy is to use polymers. Various polymers have been introduced such as poly-L-lysine (PLL) and polyethylenimine (PEI) to treat heart disease because of their low immunogenicity; however, the poor circulatory half-lives and transfection efficiency are the major drawbacks in the gene therapy field [77]. Thus, many researchers have tried to overcome the obstacles. Previously, a copolymer conjugate chitosan-graft-PEI-eprosartan to VEGF plasmid showed strong therapeutic impacts in cardiomyocytes in myocardial ischemia rats [89]. To increase the transfection efficiency and reduce the toxicity of polymers, Xu et al. synthesized a cationic poly (β-amino ester) with a degradable backbone (PDMA), and the PDMA carrying plasmid EGFP showed a higher transfection rate and cell viability than PEI alone in neonatal mouse cardiomyocytes [90]. Additionally, dexamethasone-conjugated PEI was suggested as a potential therapeutic gene carrier in cardiomyocytes since it showed strong transfection efficiency and anti-apoptotic effect in rat cardiomyocytes [91].

To increase gene delivery sustainability and localization with minimal disturbance in the cardiac function, injectable hydrogel encapsulation was introduced. Hydrogels are crosslinked hydrophilic polymers and have great benefits for the heart because they show similar features to the heart including viscoelasticity and structures, resulting in less immune response [92]. Intramyocardial delivery of miR-302 via a hydrogel promoted cardiomyocyte proliferation and regeneration after myocardial infarction in mice [93]. Polymeric nanoparticles with a shear-thinning hydrogel encapsulating miR-199a-3p showed lower toxicity than lipid nanoparticles, and the injection of hydrogel with miR-199a-3p significantly improved cardiac functions after myocardial infarction in rats [94]. miR-21–5p carrying mesoporous silica nanoparticles encapsulated into hydrogel matrix improved vascularization and reduced the scar size after myocardial infarction in pig hearts [95].

As a physical delivery method, the ultrasound-targeted microbubble (UTM) was introduced, and the method showed increased cell membrane permeability, less toxicity, and low immunogenicity. UTM delivery of VEGF, which is an angiogenic gene, into the heart increased capillary and arteriolar density, myocardial perfusion, and cardiac function

after myocardial infarction in mice [96]. However, there are still challenges such as low transfection efficiency and toxicity that must be overcome for using non-viral vectors in large animal models and eventually clinical trials [97, 98]. As we discussed in this section, there are only a few studies that investigated the effects of non-viral vector delivery on cardiomyocyte renewal [69, 70, 93]. More research needs to be undertaken into the use of non-viral vectors as a therapeutic method in large animals and then clinical settings.

3.2 Viral vectors

In gene therapy, most research has focused on viral gene delivery rather than non-viral vectors over the past years. As the efficiency of gene transfer is the most challenging obstacle in gene therapy, their high gene transfer efficiency took the spotlight. Specifically many studies in heart disease have used viral vectors to explore cardiomyocyte function owing to longer transgene expression and higher gene transfer capacity than non-viral vectors [99]. We summarize the advantages and disadvantages of major viral vectors that are used in cardiomyocyte research such as adenovirus, adeno-associated viruses (AAVs), and lentivirus (Table 2).

Adenovirus—The adenoviral vector consists of non-enveloped, double-stranded DNA (dsDNA), and the dsDNA is translocated into the nucleus followed by gene transduction. Adenovirus has a high DNA packaging capacity up to 36 kb [76]. The adenovirus delivery method was widely used in clinical trials at the beginning of gene therapy. The expression of transgene through adenoviral vectors is very vigorous for the first few days, however, it decreases within 2 weeks [100]. A major disadvantage of the adenovirus vector is that it promotes high antibody and inflammatory responses [76]. Adenovirus-mediated immunogenicity was further increased with myocardial ischemia-reperfusion [101]. Nevertheless, several animal and clinical trials have been done with an adenoviral vector carrying angiogenic factors. A few showed positive therapeutic effects by inducing angiogenesis and vasculogenesis, but most of the trials showed no significant difference compared to the placebo group [102–108]. Cardiomyocyte renewal has recently gained attention as a therapeutic target for heart failure, thus there are only a handful number of studies that used adenovirus to improve cardiomyocyte renewal. In 2014, Shapiro et al. found that intramyocardial injection of replication-deficient adenovirus vector with cyclin A2 induced cardiac regeneration after myocardial infarction by increasing cytokinesis of cardiomyocytes in adult pigs [109]. In addition, adenoviral delivery of cyclin D1 with cyclin-dependent kinase 4 (CDK4) into the myocardium induced cell cycle re-entry, resulting in cell division of cardiomyocytes in adult rats [110]. Similarly, adenoviral transfection of a combination of CDK1, CDK4, cyclin B1, and cyclin D1 improved cardiac function by increasing cardiomyocyte proliferation after myocardial infarction in mice [23].

Adeno-associated viruses—Adeno-associated viruses (AAVs) are nonenveloped, single-stranded DNA viruses and require a helper virus such as adenovirus to replicate [111]. AAVs enter the cytoplasm of the target cells through endocytosis by binding to receptors. Then, the virus translocates into the nucleus followed by transcription after synthesizing the double-strand genome. Since AAVs use a helper virus, it produces rapid and persistent AAV capsids, resulting in the degradation of the infected cells [112].

Therefore, AAVs are considered relatively reliable when it comes to immunogenicity and transfection compared to other viral vectors [113, 114]. However, AAVs have limited packaging capacity (~4.7 kb), which limits the size of the transgene [76]. There are more than 100 serotypes of AAVs have been identified, and AAV6 and AAV9 have been shown to be the most efficient AAV serotype in the heart [115, 116]. Despite positive results *in vitro* and *in vivo* studies that targeted angiogenic cardiac gene therapy using AAVs to treat ischemic heart disease, the translation into clinical trials has been disappointing. In the first AAV clinical trial, epicardial coronary artery infusion of AAV1 with sarcoplasmic/endoplasmic reticulum Ca2+-ATPase (SERCA2a) showed no significant clinical outcomes compared to the placebo group [117]. Ever since then, no further clinical trials with cardiac AAV gene therapy have been reported, except for the one currently ongoing (ClinicalTrials.gov Identifier: NCT04179643). The study is a phase 1, sequential dose escalation study to test the safety and effectiveness of intracoronary infusion of a new chimeric AAV vector, BNP116, in patients with heart failure.

In preclinical research, a previous study reported that the AAV9 infection of glycoprotein 130, which is necessary for neonatal cardiomyocyte proliferation, enhanced cardiomyocyte proliferation and regeneration after myocardial infarction by activating the Yap pathway in adult mice [118]. Moreover, overexpression of AAV9-mediated miRNA-low-density lipoprotein receptor-related protein 6 (LRP6) and lysophosphatidic acid 3 reduced myocardial infarction size and ameliorated left ventricular systolic function by increasing cardiomyocyte proliferation in mice [119, 120]. In addition, Gabisonia et al. found that injection of AAV6-mediated human miRNA-199a increased cardiomyocyte proliferation after myocardial infarction in pigs [121]. Robust research has investigated gene therapy through AAVs for the therapeutic purpose of heart repair and regeneration *in vitro* and in small animals. As the results seem promising in cardiomyocyte renewal, investigation in large animal models should be undertaken to allow the move forward into clinical trials.

Lentivirus—Lentiviruses are enveloped, single-stranded RNA retroviruses and have up to 10 kb packaging capacity [76]. A unique characteristic of lentivirus is that it can transduce non-dividing cells without an immune response against the transduced cells and present long-term gene expression with high transduction efficiency [122]. Because cardiomyocytes are non-replicating cells after birth, lentiviral gene delivery has the potential as an effective transfection method in cardiomyocytes. A few clinical studies have used lentiviral vectors in patients with HIV, Wiskott-Aldrich syndrome, β-hemoglobinopathies, and leukodystrophies [123–127]. Even though there is no clinical study that has used lentiviral vectors in cardiac tissue as yet, many researchers have used lentiviral gene transfection to investigate cardiomyocyte proliferation *in vitro* as well as *in vivo* models [128]. Lentiviral delivery of miR302–367 induced cardiomyocyte renewal in adult mice after myocardial infarction by promoting cardiomyocyte proliferation and reducing scar formation [129]. Intramyocardial injection of lentivirus encoding four cell cycle factors including CDK1, CDK4, CCNB1, and CCND1 after myocardial infarction in rats and pigs improved left ventricular ejection fraction and scar size [130]. In addition, lentiviral vector-mediated overexpression of integrin β1 increased cardiomyocyte survival after myocardial infarction in rats [131]. The biggest concern of using lentivirus vectors is their safety. Lentivirus has a potential risk

to cause insertional oncogenesis because it integrates into the target cell randomly with a preference to the coding regions of the gene [132]. Therefore, lentiviral vectors need further improvements in safety matter before they can be used in cardiomyocytes in a clinical setting.

4. Conclusive summary, limitations, and future directions

Understanding the underlying mechanism behind cardiomyocyte proliferation has become significantly important to treat myocardial infarction and heart failure. After heart injury, the heart significantly loses cardiomyocytes and thus functions. Recent research proved that using gene therapy, cardiomyocytes can proliferate by re-entering the cell cycle. Therefore, genetically editing genes involved in the cell cycle may improve adult cardiomyocyte proliferation and renewal. Thus, gene therapy targeting the cell cycle to restore the function of cardiomyocytes can now be considered a potential therapeutic method for heart failure. However, there is limited evidence that shows the cell cycle activation results in the generation of new cardiomyocytes restoring the lost function [13, 133]. Cell proliferation is necessary for the renewal of cardiomyocytes, yet it remains to be confirmed if those proliferated cells differentiate into and regenerate fully functioning cardiomyocytes.

The current review intended to provide comprehensive gene therapeutic strategies that have been used to activate the cell cycle. We mainly focused on the genetic targets specifically to induce cardiomyocyte proliferation along with gene delivery vectors. The limitation is that we have not discussed other contributing factors that are known to affect cardiomyocyte proliferation such as angiogenesis, gender, and age. However, the current review is unique since it provides a comprehensive discussion on gene therapeutic targets of the cell cycle to activate cardiomyocyte proliferation as there is growing evidence that targeting the cell cycle enables regeneration in injured hearts.

The past and ongoing clinical trials that used gene therapy to treat heart failure are mostly focusing on the regulation of intracellular calcium and angiogenesis [134]. Even though preclinical studies showed promising results in the improvement of cardiac function by delivering genes that are involved in angiogenesis and calcium regulation, similar results were not shown in humans. As stated in this review, recently, multiple studies have shown promising results in activating cell proliferation in small animal models using gene therapy. A clinical trial has been done with a cell cycle activator, recombinant human neuregulin1, via intravenous infusion in chronic heart failure patients [135]. The patients who received recombinant human neuregulin1 showed increased left ventricular function and structure compared to the placebo group even though it was not statistically significant. Although promoting the cell cycle provides hope to treat heart disease, it is yet premature to administrate gene therapy that promotes the cell cycle into clinical practice due to the lack of sufficient preclinical studies to assess efficacy and safety. Even though small animal models play significant roles in translational medicine, there are still physiological and anatomical differences between the small animals and humans, therefore meticulous consideration has to be given during data analysis. Taken together, more intensive research with large animal models and proper preclinical study design are required to establish a more practical translational method to reduce the risk of the failure of clinical translation.

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Data availability:

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Key Points

- It is important to understand the underlying mechanism behind cardiomyocyte cell cycle exits in adult mammalian hearts.
- Gene therapy targeting the cell cycle genes increases adult cardiomyocyte proliferation.
- Promoting the cell cycle appears to be a viable therapeutic strategy for cardiomyocyte renewal.

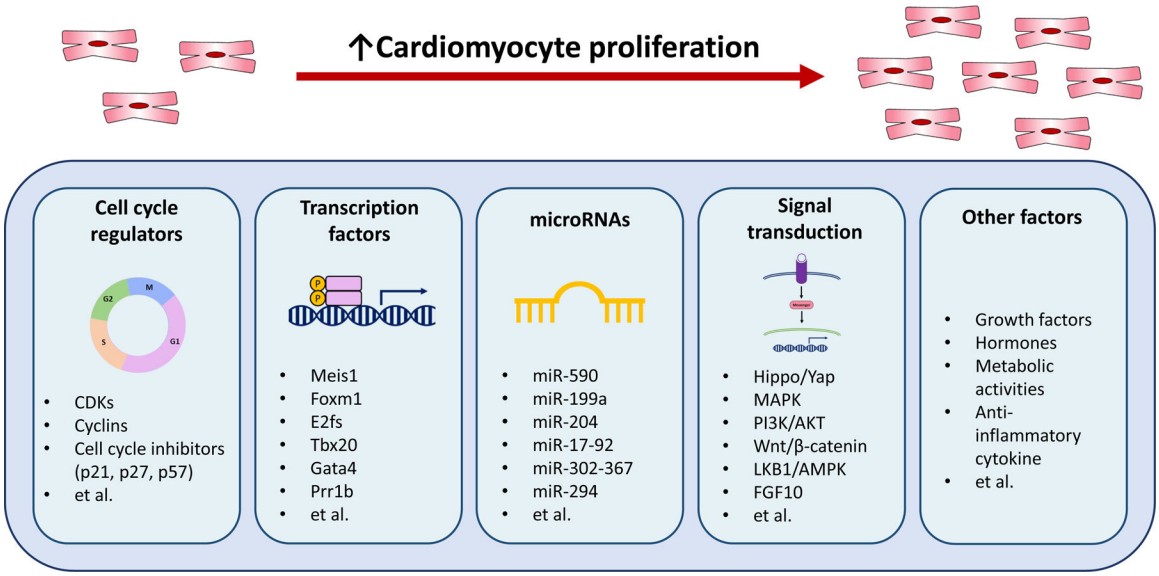

Figure 1.

Molecular targets that promote cardiomyocyte cell cycle and proliferation. The figure summarizes the molecules that have been shown to regulate cardiomyocyte cell cycle and proliferation.

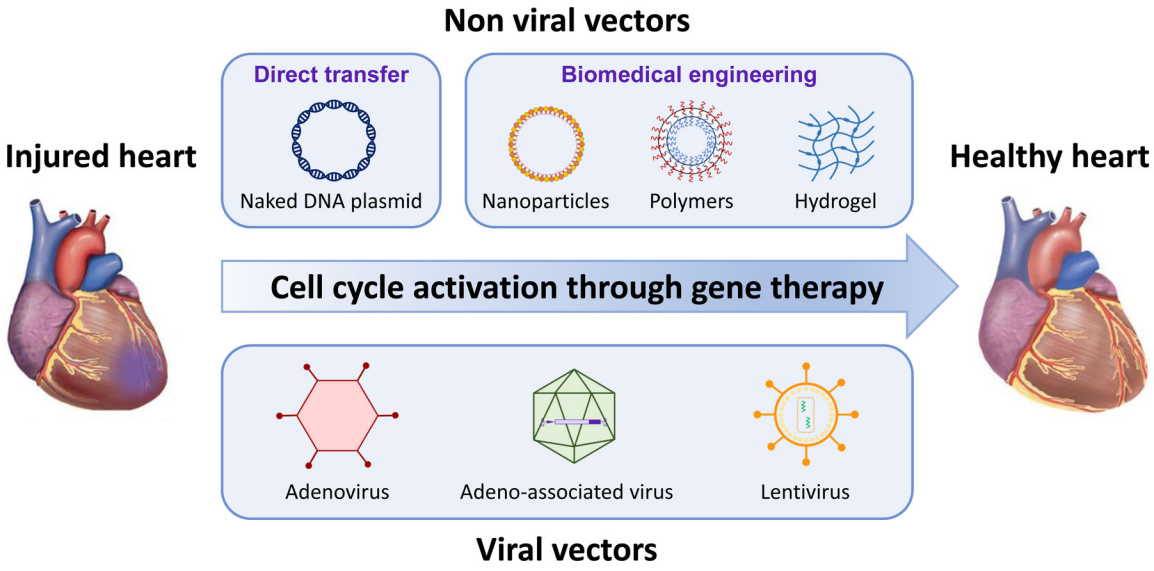

Figure 2.

Approaches for cardiac gene therapy. The figure outlines the viral- and nonviral-based approaches to deliver genes and cardioprotective agents for promoting cell cycle activation.

Table 1.

Non-viral vectors to deliver genes into injured hearts.

Delivery method	Target gene	Species	Reference
DNA plasmid	VEGF A-165	Human, Pig, Sheep	[66–68]
PEGylated-NP	Schisandrin B	Rat	[77]
PEGylated-NP	Baicalin	Rat	[78]
PEGylated-NP	Puerarin	Rat	[79]
PLGA-NP	Cyclosporine A	Mouse	[80]
PLGA-NP	Irbesartan	Mouse	[81]
PLGA-NP	Pitavastatin	Mouse, Rat, Pig	[82–84]
PLGA-NP	VEGF	Mouse	[85]
PLGA-NP	IGF1	Mouse	[86]
Conjugate chitosan-graft-PEI-eprosartan	VEGF	Rat	[87]
PDMA	EGFP	Mouse	[88]
Hydrogel	miR-302	Mouse	[91]
Hydrogel	miR-199a-3p	Rat	[92]
Hydrogel	miR-21–5p	Pig	[93]
UTM	VEGF	Mouse	[94]

PEGylated-NP, nanoparticles with polyethylene glycol; PLGA-NP, a copolymer (lactic-co-glycolic acid); PEI, polyethylenimine; PDMA, a cationic poly(beta-amino ester); UTM, the ultrasound-targeted microbubble; VEGF, vascular endothelial growth factor; IGF, insulin-like growth factor; EGFP, enhanced green fluorescent protein.

Table 2.

Viral vectors to deliver genes into injured hearts.

Virus	Target gene	Species	Reference
Adenovirus	VEGF-$D^{\Delta N \Delta C}$	Human	[100]
	Adenylyl cyclase 6	Human	[101]
	VEGF, Angiopoietin-1	Pig	[102]
	Proline/arginine-rich peptide 39	Pig	[103]
	FGF4	Pig, Human	[104, 105]
	VEGF121	Human	[106]
	Cyclin A2	Pig	[107]
	Cyclin D1, CDK4	Rat	[108]
	CDK1, CDK4, Cyclin B1, Cyclin D1	Mouse	[21]
AAV	SERCA2a	Human	[115]
	BNP116	Human	ClnicalTrials.gov Identifier: NCT04179643
	Glycoprotein 130	Mouse	[116]
	miRNA-LRP6	Mouse	[117]
	Lysophosphatidic acid 3	Mouse	[118]
	miR-199a	Pig	[119]
Lentivirus	miR302–367	Mouse	[127]
	CDK1, CDK4, CyclinB1, CyclinD1	Rat, Pig	[128]
	Integrin β1	Rat	[129]

AAV, adeno-associated virus; VEGF, vascular endothelial growth factor; FGF, fibroblast growth factor; CDK, cyclin-dependent kinases; SERCA2a, sarcoplasmic/endoplasmic reticulum Ca2+-ATPase; LRP6, low-density lipoprotein receptor-related protein 6.