Induced Cardiomyocyte Proliferation

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Contributor: Riham Abouleisa

Spontaneous cardiomyocyte regeneration has been demonstrated in embryonic and neonatal mammals after genetic ablation, apical resection, or myocardial infarction. Adult cardiomyocyte proliferation and turnover have been reported to be minimal in human heartsand rodents. Cardiomyocyte division was also demonstrated to occur at a very low rate after acute and chronic infarction in humans.

cardiomyocyte proliferation cardiac regeneration cell cycle

1. Introduction

Heart failure is the most prominent cause of hospitalization and one of the leading causes of mortality globally. During heart failure progression, damaged cardiomyocytes are replaced by fibrotic tissue, since cardiomyocytes are not able to regenerate themselves when damaged. This subsequently triggers cardiac remodeling and heart failure [1][2]. All the current treatment strategies for heart failure are symptomatic treatments to slow down its progression. Hence, heart transplants, which are eminently scarce and not without complications, are as yet the only cure for heart failure [3].

2. Induction of Cardiomyocyte Cell Cycle Entry through Direct Cell Cycle Activation

Overexpression of cell cycle activators or knocking down of cell cycle inhibitors have been widely used to induce cardiomyocyte cell cycle entry. The cyclin D family and its related kinases (CDKs) are known to regulate the transition of the cell cycle from G1 to S phase, induce DNA synthesis, and maintain cell cycle activity [4][5][6]. Cyclins A, D, and E, as well as the related kinases, are also involved in DNA synthesis, while cyclin B is crucial for cytokinesis [7]. It has been shown that cyclin A and B disappear in adult hearts, while cyclin D, E, CDK2, and CDK4 expression and activity are very low in adult hearts [8][9].

Cyclin A2 is expressed during embryonic development of the heart and the early postnatal period and silenced afterward [8]. Many studies have investigated the effect of cyclin A2 expression on cell cycle resumption. Chaudhry et al. showed cardiac enlargement with hyperplasia of cardiomyocytes in adult transgenic mice persistently expressing cyclin A2 [10]. Woo et al. used an adenoviral vector to deliver cyclin A2 to mice hearts following left anterior descending (LAD) artery ligation. Cyclin A2-treated rats showed cardiac cell cycle activation associated with an improvement in hemodynamics, preload-adjusted maximal power, and cardiac output compared to control-treated rats [11]. Similar findings were reported by Cheng et al. in cyclin A2 overexpression transgenic mice

subjected to LAD artery ligation [12]. Shapiro et al. confirmed these findings in large animals, namely pigs. Cyclin A2-treated pigs showed an ~18% increase in ejection fraction compared to control-treated pigs. Cultured porcine adult cardiomyocytes showed a more than threefold increase in the phospho-histone H3 (PHH3) in cyclin A2-expressing animals compared to controls [13].

Mohamed et al. showed efficient induction of stable cell cycle division, assessed by EdU and PHH3, in 15–20% of adult mouse, rat, and hiPS cardiomyocytes overexpressing a combination of four cell cycle genes; cyclin-dependent kinase 1 (CDK1), CDK4, cyclin B1, and cyclin D1 (termed 4F). Cell cycle induction using 4F also showed robust complete cytokinesis in vivo using MADM mice [14]. In a trial for preclinical application of 4F, Abouleisa et al. cloned a polycistronic non-integrating lentivirus encoding 4F in which each factor was driven by TNNT2 promotor (TNNT2-4F-NIL) to induce cell cycling transiently and specifically in cardiomyocytes. Intramyocardial injection of TNNT2-4F-NIL improved systolic functions and reduced the scar size after ischemic reperfusion in rats and pigs compared to control-treated rats and pigs [15].

Another idea for the activation of cell cycle genes is to target cell cycle inhibitors. Sirt-1 is a member of the histone deacetylase (HDAC) family, which deacetylate multiple targets including P21 protein. Hence, the deacetylated form of P21 is less stable and more prone to ubiquitination. Sirt-1 overexpression showed a significant reduction in active P21 acetylation, which resulted in a release of the inhibitory effect on the cell cycle genes and a significant increase in the mitotic markers, including PHH3 and Aurora B kinase-positive nuclei [16].

3. MicroRNA Regulates Cardiomyocyte Cell Cycle Entry

The miR-302-367 cluster is expressed in the early developmental stage in mouse hearts and it is involved in modulating cardiomyocyte proliferation during the embryonic stage. Expression of miR-302-367 led to continuous cardiomyocyte proliferation and reduction of scar size after MI, probably through inhibition of the Hippo signaling pathway. However, it also led to immature cardiomyocyte dedifferentiation and heart failure, while transient expression of miR-302-367 results in more controlled proliferation and does not deteriorate cardiac function [17].

miR-128 is a microRNA the upregulation of which coincides with cell cycle arrest. Neonatal mice overexpressing miR-128 showed impaired function and attenuated proliferation capacity, while miR-128 KO mice showed a prolonged postnatal proliferation window through suppressing CDK inhibitor p27 and activation of cell cycle regulator cyclin E and of CDK2. Adult KO mice showed improved scar size and cardiac function after MI [18]. In this study, the authors suggested that the reduction in scar size was attributed to the dedifferentiation of the adult cardiomyocytes and their ability to proliferate, hence preventing the development of fibrosis.

miR-294 is another microRNA that is expressed during prenatal development and its expression is lost in adult cardiomyocytes. miR-294 expression in neonatal myocytes showed a significant increase in cell cycle markers Ki69, PHH3, and Aurora kinase-positive nuclei compared to control neonatal myocytes. miR-294–treated mice showed a significant improvement in scar size and apoptosis after MI compared to control mice. miR-294

increased cardiomyocyte proliferation through repression of Wee1 activity, which led to the activation of the cyclin B1/CDK1 complex [19].

Furthermore, another microRNA, miR-499 , has been found to regulate cardiomyocyte cell cycle entry and apoptosis via modulation of Sox6 and cyclin D1 activity [20].

4. Signaling Pathways That Control Cardiomyocyte Proliferation

Many signaling pathways interact to balance the progression and arrest of the cell cycle and apoptosis in mammalian cells [21]. Several pathways interact with cyclin-dependent kinases inhibitors (CDKIs) or directly with cyclins and CDKs to activate or inhibit cell cycle progression. Understanding the basic bio-physiology of these signaling pathways can provide a tool for controlled induction of cardiomyocyte proliferation in the hope for a regenerative niche [21].

PI3K-AKT is one of the major signaling pathways controlling the cell cycle. Increased AKT activity is associated with a longer half-life of cyclin D, increased CDK2 and CDK7 levels, and decreased expression of cell cycle inhibitors P21/P27/P57, which in turn promotes cell cycle progression [22][23][24]. Studies of small molecules activating the PI3K-AKT signaling pathway showed a successful induction of mitosis and cytokinesis in neonatal and adult cardiomyocytes [25]. PI3K-AKT interacts with two other fundamental pathways to facilitate progression into the cell cycle: the Hippo signaling pathway and Wnt/β-catenin [26]. Interestingly, the PI3K-AKT pathway could act as a survival pathway [27][28], which might imply that the beneficial effects post-infarction represent a salvaging of cell death in addition to the induction of the cardiomyocyte cell cycle.

The Hippo signaling pathway regulates cell proliferation mainly through phosphorylation/dephosphorylation of the transcriptional co-activator yes-associated protein (YAP). YAP is known to be required for normal fetal heart growth and it regulates insulin-like growth factor-1 (IGF-1) in developing hearts [29][30]. YAP interacts directly with the Myb–MuvB (MMB) complex to facilitate G2/M transition [31]. Monroe et al. showed that an active version of the Hippo pathway effector YAP (YAP5SA) can program adult mice cardiomyocytes to a fetal heart program with high proliferation capacity and increased chromatin accessibility [32]. Other studies have shown that YAP activation or overexpression in mice hearts enhances cardiomyocyte proliferation and improves outcomes after myocardial infarction. Xin et al. showed that cardiac-specific deletion of YAP in mice using α-myosin heavy chain C (αMHC-c)-Cre was associated with impaired cardiac functions with ventricular wall fibrosis, development of dilated cardiomyopathy, and death by 9 weeks of age. LAD artery ligation in these P2 day neonatal mice showed limited regenerative capacity compared to controls. Transgenic mice overexpressing a constitutively active YAP showed enhanced proliferative capacity and increased PHH3 expression after LAD artery ligation in P7 day old neonatal mice [33]. Lin et al. reported similar findings after MI induction in adult mouse hearts and YAP overexpression using adenoviral vector [34].

The **Notch signaling** pathway controls cellular proliferation and trabeculation in developing hearts [35]. Notch signaling activates bone morphogenic protein-10 (BMP-10), a protein involved in cardiomyocyte growth and maturation during development [35][36]. BMP10 has been shown to increase myocyte cell cycle entry by downregulation of cell cycle inhibitors P21, P27, and P75 [36]. Notch signaling indirectly increases neuregulin expression. Neuregulin is a paracrine agonist of the ErbB1-4 tyrosine kinase receptor which stimulates intracellular PI3K-AKT signaling [35][37]. Furthermore, another study demonstrated the direct interaction between Notch signaling and the PI3K-AKT survival pathway through c-Met-mediated activation of AKT [38]. Zhao et al. showed that suppression of Notch signaling in zebrafish impairs their capacity to regenerate their heart after ventricular resection [37].

The Wnt/β-catenin signaling pathway is known to be active during cardiac development and during adult left ventricular remodeling [39]. In normal adult hearts, Wnt is under continuous negative regulation by upstream soluble frizzled-related proteins (sFRPs). Cardiac injury—for example, after MI—removes the inhibitory effect of sFRPs, allowing Wnt to bind to frizzled receptors and activate the complex canonical pathway leading to increased expression and nuclear localization of the transcription factor β-catenin, as well as non-canonical downstream pathways [39]. sFRP2 protein knockout mice showed reduced fibrosis and improved cardiac functions after experimental MI. The mechanisms underlying these findings are complex and wnt/β-catenin can be involved [40]. The Wnt/β-catenin pathway is also subject to an inhibitory effect of GSK3β, which markedly increases during the postnatal period and is thought to be involved in the postnatal cell cycle exit. GSK3β inhibition or knockout activates Wnt/β-catenin, which increases cyclin D and allows the cardiomyocytes to proceed into S phase [41][42].

The Jak/Stat signaling pathway is a novel and promising pathway for cell cycle induction. Cytokines binding to their receptors lead to dimerization of interleukin-6 signal transducer (IL6st), which activates Jak1. In return, Jak1 phosphorylates IL6st to form a docking site for Stat3 phosphorylation and activation. Active Stat3 translocate to the nucleus to stimulate the expression of several transcription factors involved in cell proliferation [43]. A study of myocarditis models showed that Stat3 facilitates cell cycle re-entry, while its inhibition is associated with limited ability of cardiomyocytes to proliferate and increased scarring in response to injury [43][44].

The Hedgehog(Hh) signaling pathway is currently emerging as a promising target for cell cycle modulation. Hedgehog proteins are known to interact with specific transmembrane receptor proteins, named Patched receptors, which in turn regulates the activity of the Glioma-associated oncogene homolog (Gli) family of transcription factors [45]. Hedgehog is known to be active during embryogenesis, controlling cellular proliferation and differentiation [46]. Studies of cardiac regeneration in lower vertebrates, including zebrafish and newts, showed a role for Hh signaling in cardiomyocytes proliferation [47][48][49]. Sonic Hedgehog (SHh) protein reconstitutes the embryonic signaling pathways, so it has been introduced to promote cardiomyocyte cell cycle re-entry [50][51][52]. Mechanistically, Hh signaling stimulates the expression of cyclin D2 and cyclin E1 and inhibits P27 through Gli1–Mycn network interaction [53].

Two studies tried to investigate the relationship between **autonomic innervation** and cardiomyocyte proliferation. White et al. have shown that sympathetic innervation is required to maintain neonatal mammalian heart

regenerative capacity. Chemical sympathectomy in P2 neonatal mice hearts significantly inhibited cardiac regeneration after apical resection [54]. While Liu et al. showed that β-adrenergic receptor (β-AR) stimulation in patients with tetralogy of Fallot and pulmonary stimulation is linked to cardiomyocyte cell cycle arrest through repression of the cytokinesis gene epithelial cell transforming 2 (ECT2). Inactivation of β-AR genes in mouse models, and β-AR blocking using propranolol in both mouse models and human subjects with tetralogy of Fallot, increased cardiomyocyte proliferative capacity [55]. Other studies on zebrafish and neonatal mice hearts showed that both pharmacological inhibitions of cholinergic nerve functions and mechanical denervation diminished cardiomyocyte proliferation. Cholinergic stimulation by carbachol extended the postnatal cardiomyocyte regeneration window [56]. In brief, a balanced autonomic drive of the heart is required to maintain the proliferative capacity of cardiomyocytes.

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