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Publication Date

2016

DOI

10.1016/j.phrs.2015.11.001

HHS Public Access

Author manuscript

Pharmacol Res. Author manuscript; available in PMC 2017 January 01.

Published in final edited form as:

Pharmacol Res. 2016 January ; 103: 13–16. doi:10.1016/j.phrs.2015.11.001.

Personalizing cardiac regenerative therapy: at the heart of Pim1 kinase

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Abstract

During cardiac aging, DNA damage and environmental stressors contribute to telomeric shortening and human cardiac progenitor cells acquire a senescent phenotype that leads to decreased stem cell function. Reversion of this phenotype through genetic modification is essential to advance regenerative therapy. Studies in the cardiac specific overexpression and subcellular targeting of Pim1 kinase demonstrate its influence on regeneration, proliferation, survival, metabolism and senescence. The cardioprotective effects of Pim1 modification can be picked apart and enhanced by targeting the kinase to distinct subcellular compartments, allowing for selection of specific phenotypic traits after molecular modification. In this perspective, we examine the therapeutic implications of Pim1 to encourage the personalization of cardiac regenerative therapy.

Keywords

Pim1; aging; apoptosis; heart failure; human cardiac progenitor cell; senescence

1) Introduction

Following a myocardial infarction (MI)[1] in the human heart, catastrophic consequences result from failure of endogenous survival and reparative responses to moderate damage caused by cardiomyocyte death. As the heart attempts to heal, activated fibroblasts form scar tissue at the site of injury, contributing to maladaptive remodeling and progression toward heart failure. As a leading cause of death in the United States, treatment of patients with heart failure has very limited options and a profound need for more adept cardiac regenerative therapies.

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[1]*Abbreviations:* MI, myocardial infarction; CPC, cardiac progenitor cell; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; STAT, signal transducers and activators of transcription; PI3K, phosphatidylinositide 3-kinases; Klf5, kruppel-like factor 5; EGF, epidermal growth factor; LIF, leukemia inhibitory factor; Pim1-KO, Pim1 knock-out; TAC, trans-aortic constriction; hCPC, human cardiac progenitor cell; TERT, telomerase reverse transcriptase; CDK, cyclin-dependent kinase; NuMA, nuclear mitotic apparatus; PTP, permeability transition pore; PimWT, whole-cell Pim1 overexpression; Nuc-Pim1, nuclear-targeted Pim1; Mito-Pim1, mitochondrial-targeted Pim1.

Interventional strategies to manipulate the heart's endogenous system of repair suggest there is significant room for improvement (1). Ability to replace damaged and aging myocardium with newly formed, functional cardiomyocytes is at the forefront of cardiovascular regenerative research and the potential lies in molecular modification of the cells that make up the myocardium including the stem cells in the heart known as cardiac progenitor cells (CPCs).

2) Manipulating Cardiac Regeneration with Pim1

Pim1 is a highly conserved proto-oncogene in a three-member family of serine-threonine kinases. As the main isoform of the kinase in the heart, Pim1 expression is regulated both at transcriptional and posttranslational levels. Interleukins and transcription factors like p53 and NFκB regulate signal transducers and activators of transcription (STAT) factors, which bind the Pim1 promoter and activate transcription (2). The PI3K/Akt signaling pathway, pathological stimuli, kruppel-like factor 5 (Klf5) and hypoxic conditions all elevate Pim1 expression levels (3). Expression is also stimulated by factors such as epidermal growth factor (EGF), leukemia inhibitory factor (LIF) and interferon-alpha (4). Pim1 is constitutively active, therefore expression can also be controlled posttranslationally by a variety of proteins that inhibit or drive its phosphorylation, ubiquitination, dephosphorylation and degradation (5).

As a downstream target of cardioprotective nuclear Akt accumulation and regulator of MYC transcriptional activity Pim1 influences cellular processes such as cell cycle progression, survival signaling, telomere preservation and senescence by interaction, stabilization and phosphorylation of countless downstream targets (6–16). Studies in the cardiac specific overexpression and/or global knockdown of Pim1 have demonstrated how, where and when Pim1 influences these cellular processes. Myocardial specific overexpression of Pim1 in transgenic mice results in decreased infarct size and maintenance of contractility after MI, enhanced calcium dynamics, sarcomeric shortening and prevention of mitochondrial fission in cardiomyocytes (11, 16). Pim1 mice display elevated expression of anti-apoptotic proteins, attenuation of mitochondrial swelling after calcium overload, prevention of cytochrome c release from mitochondria, and preservation of mitochondrial integrity in cardiomyocytes (7). Conversely, knock out of Pim1 (Pim-KO) results in reduced contractility, ejection fraction and fractional shortening, increased cell death and impaired adaptation to pressure overload after trans-aortic constriction (TAC) (16).

Similarly, Pim1-modified human cardiac progenitor cells (hCPC) exhibit enhanced mitochondrial activity and cardiac commitment, and when delivered into the mouse model of MI there is reduced infarct size, improved cardiac function and enhanced myocyte formation and neovascularization (15). Pim1 overexpression rejuvenates aged progenitors by elongating telomeres coincident with increased expression and activity of telomerase reverse transcriptase (TERT), the enzyme required to preserve telomeres. Additionally, Pim1 increases proliferation, metabolic activity and survival, upregulates genes necessary for the maintenance of stemness, delays the acquisition of senescence and partially reverses the senescent phenotype of hCPCs from multiple patients (14). Importantly, Pim1-mediated rejuvenation of hCPCs occurs without changes in ploidy or oncogenic transformation (15).

Modification of stem cells with Pim1 has been thoroughly studied in our lab, and extensive documentation of cardioprotection suggests that Pim1 is capable of restoring hCPCs into the ideal candidate for cardiovascular regenerative therapy.

3) Subcellular Localization and Functional Effect of Pim1

Fluctuations in subcellular localization and expression level of Pim1 are evident during cardiogenesis (16). The kinase is highly expressed in the neonatal heart, declines during postnatal development, and is induced pursuant to pathological injury (16). Predominantly localized to the nuclei of cardiomyocytes in the neonatal heart, Pim1 mediates rapid proliferation during cardiac development (Figure 1) (16). As a cell cycle mediator, Pim1 drives G1-S progression by stabilizing the interaction of Cyclin D with Cyclin-Dependent Kinases (CDKs) and by hindering various cell cycle inhibitors (9, 17–19). Pim1 also plays a role in mitosis by phosphorylating and activating nuclear mitotic apparatus (NuMA), which associates with a protein complex that drives spindle pole formation, promotes segregation of the chromosomes, and facilitates cell division (20).

Following cardiogenesis, Pim1 expression decreases and translocates to the cytosol of cardiomyocytes. Cardiac injury reactivates Pim1 and shuttles the kinase to mitochondria where it plays a role in cell survival (16). Upon stress stimulus in the heart, activated pro-apoptotic proteins translocate to the outer mitochondrial membrane to form a permeability transition pore (PTP) and catalyze the release of pro-apoptotic signaling molecules (21). This activates a caspase cascade that results in DNA damage and cell death. Anti-apoptotic proteins Bcl-2 and Bcl-X_L work to sequester and inactivate the formation of the PTP, thereby preventing membrane permeability and inhibiting the apoptotic signaling cascade. Pro-apoptotic protein Bad forms a heterodimer with and inactivates Bcl-2 and Bcl-X_L, allowing formation of the PTP (5, 16, 22). Pim1 directly interferes with apoptosis by elevating Bcl-2 and Bcl-X_L levels at the mitochondria and phosphorylating and inactivating Bad (4). Overall, Pim1 antagonizes apoptosis at the mitochondria by modulating pro- and anti-apoptotic regulator proteins, thereby promoting preservation of mitochondrial integrity and structure, and inhibiting the release of apoptotic signaling molecules in the adult heart (Figure 1) (7). Localization, expression level and functional effect of Pim1 differs during fetal and postnatal cardiac development, which suggests that the specific role of the kinase is influenced by location within the cell.

4) “Fine-Tuning” Pim1 Potential

Recent studies from our lab show that organelle-specific overexpression of Pim1 preferentially modifies cellular characteristics of human CPCs (hCPCs) and reinforces cardioprotective effects based upon internal localization (23). Adult hCPCs were isolated from patients undergoing left ventricular assist device implantation and were engineered to overexpress Pim1 throughout the cell (PimWT) or targeted to either nuclear (Nuc-Pim1) or mitochondrial (Mito-Pim1) compartments. Similar to what is seen during cardiogenesis, the location of Pim1 in hCPCs orchestrates the downstream cardioprotective performance of the kinase.

The senescent phenotype of hCPCs isolated from heart failure patients is readily apparent in *ex vivo* culture. Reversal of senescence, returning aged adult stem cells to a more youthful phenotype, is essential to support regeneration after autologous transplantation into a failing heart. Nuc-Pim1 preferentially enhances stem cell youthfulness associated with reduced senescence associated β-galactosidase activity, increased TERT expression, preserved telomere length, decreased expression of p53 and p16 and upregulation of nucleostemin relative to PimWT hCPCs (Figure 1) (23). Nuc-Pim1 hCPCs also have decreased flattened morphology and the ability to undergo several successive passages indicative of a more youthful cellular phenotype. Nuc-Pim1 specifically supports both phenotypic and molecular changes in senescent hCPCs to enhance stem cell youthfulness associated with increased growth potential, telomere maintenance and reduced markers of senescence.

Adult hCPCs exhibit low proliferation rate and increased sensitivity to apoptotic stimuli (14, 15, 23). Targeting Pim1 expression to mitochondria promotes increased interaction with anti-apoptotic proteins, inhibiting apoptosis in aged hCPCs. Mito-Pim1 hCPCs have increased resistance to H_2O_2 induced cell death, coincident with enhanced expression of Bcl-2 and Bcl-X_L, which suggests superior preservation of mitochondrial integrity as compared to PimWT hCPCs. In addition, Mito-Pim1 is more effective than PimWT at promoting proliferation as evidenced by increased expression of cell cycle modulators Phospho-Rb, CDK4 and Cyclin D (Figure 1). Improvement in proliferative capacity of Mito-Pim1 hCPCs is supported by collective maintenance of energy metabolism, with increased ATP levels and upregulation of mitochondrial biogenesis gene regulators. This study differentiates cardioprotective roles of Pim1 based on compartmental expression and further reinforces the potential of Pim1 in the context of stem cell based cardiac regeneration.

5) The Future of Cardiovascular Regeneration

As the heart ages, DNA damage and environmental stressors contribute to telomeric shortening, and hCPCs acquire a senescent phenotype that leads to decreased stem cell function in the diseased heart (24). Reversion of this phenotype through genetic modification is essential to advance regenerative therapy. Response to genetic modification varies from patient to patient, requiring a more personalized form of regenerative medicine (14, 23). Numerous influences, both genetic and environmental, result in biological aging of hCPCs despite chronological age. Factors such as disease etiology, alcohol and cigarette consumption, medication and diabetes contribute to the variability in hCPCs isolated from multiple patients, as evident by subtle differences in proliferation rate, susceptibility to apoptotic stimuli and telomere lengths (14, 15, 23). Future directions of the field will distinguish attributes that qualify heart failure patients as potential candidates for Pim1 modification before autologous hCPC therapy. Controlled localization of Pim1 allows for preferential enhancement of specific stem cell properties, customizing the benefits of modification. Our laboratory aims to extend rejuvenation of hCPCs through modification with targeted Pim1 kinase.

Although Pim1 may be used to personalize and enhance cardiac regeneration based on our studies in hCPCs, these effects are not merely restricted to mitotic cell types; they can be

extended to cardiomyocytes. Findings from various laboratories suggest that cardiac renewal is not only dependent upon differentiation of progenitors, but that new cardiomyocytes can be derived from the division of pre-existing cardiomyocytes in the adult mammalian heart (25–27). Studies by Shapiro *et al.* support the possibility of genetically manipulating cardiac regeneration to jump-start cytokinesis of adult cardiomyocytes after MI in the porcine model (28). Mito-Pim1 and PimWT hold therapeutic potential to increase cardiomyocyte cell cycle re-entry and positively influence cardiac regeneration after injury.

In the future, molecular interventions can be tailored to the individual needs of the patient. Pim1 differentially regulates cellular processes based on subcellular localization, allowing for selection of desired cellular properties and making it an ideal molecule for customization of cardiovascular therapy.

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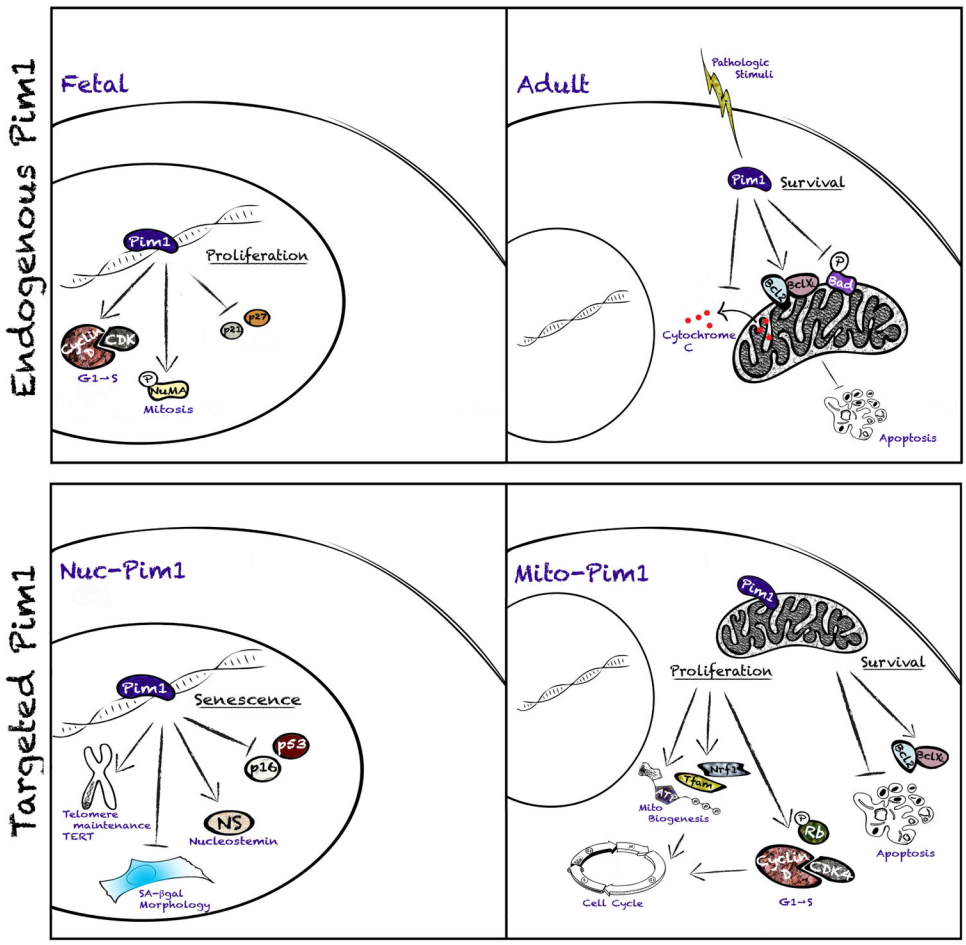

Fig. 1.

Differences between endogenous Pim1 expression in the fetal and adult heart (top) versus overexpression of nuclear- and mitochondrial-targeted Pim1 in hCPCs (bottom).