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Placental Growth Factor (PGF) as a Protective Paracrine Effector in the Heart

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Abstract

In response to injury the myocardium hypertrophies in an attempt to maintain or augment function, which is associated with ventricular remodeling and changes in capillary density. During the compensatory phase of the hypertrophic response the myocardium maintains output and is characterized by a coordinated neo-angiogenic and fibrotic response that supports cardiomyocyte health and survival. Emerging evidence shows that paracrine-mediated cross-talk between cardiac myocytes and non-myocytes within the heart is critical for cardiac adaptation to stress, including the extent of hypertrophy and angiogenesis. This review will discuss recent results indicating that Placental Growth Factor (PGF, also called PlGF), a secreted factor within the VEGF superfamily, is a pivotal mediator of adaptive cardiac hypertrophy and beneficial angiogenesis through its ability to coordinate the intercellular communication between different celltypes in the heart.

Introduction

The heart is a dynamic organ able to respond to environmental insults or stress stimuli by remodeling and hypertrophy. These events are typically compensatory as a means of maintaining cardiac output and reducing ventricular wall stress after myocardial infarction, with myocarditis, aortic stenosis, hypertension, valve defects, or mutations in genes that affect myocyte contractility. Although the origin of the injury often varies, the remodeling of the stressed heart is commonly associated with cardiac hypertrophy and, over extended periods of time, this can lead to heart failure and death (Kehat et al. 2010). Cardiac hypertrophy is accompanied by an increase in the production and release of catecholamines, growth factors, and cytokines that together contribute to the growth of cardiomyocytes, blood vessels and capillaries, as well as interstitial connective tissue through activation of fibroblasts. In fact, although the growth of the cardiomyocyte is the most prominent feature associated with hypertrophy, it is now evident that this process intimately involves non-myocytes within the heart such as fibroblasts and endothelial cells (Baudino et al. 2006). The growth of fibroblasts and extracellular matrix (ECM) deposition, a process termed fibrosis, is considered to be a maladaptive response that contributes to ventricular arrhythmia and left ventricular dysfunction (Sabbah et al. 1995). Yet, fibroblasts may also play an adaptive role in hypertrophy, underlying the importance of understanding the

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mechanisms leading to a balanced response. Indeed, the extracellular matrix of the heart is a complex milieu containing structural proteins, growth factor binding proteins, and signaling factors that orchestrate the communication between myocytes, fibroblasts and endothelial cells in coordinating hypertrophy, fibrosis and angiogenesis. For example, a coordinated increase in vessel/capillary density is typically necessary to provide oxygen and nutrients as the cardiomyocytes enlarge, which in turn requires increased deposition of extracellular matrix to provide mechanical support and elasticity for contraction (Walsh et al. 2007). Impaired vascular growth and abnormal extracellular matrix deposition are common maladaptive features that accelerate the transition from compensated to decompensated hypertrophy (Bowers et al. 2010). Due to the importance of paracrine communication between these celltypes in the heart during stress stimulation, much effort has been put forth towards identifying secreted factors in the heart that function as central regulators of adaptive and maladaptive remodeling. This review focuses on the recent finding that Placental Growth Factor (PGF, also referred to as PlGF), a secreted factor from the VEGF superfamily, is a crucial paracrine mediator between cardiomyocytes and endothelial cells that coordinates the hypertrophic and angiogenic response with stress stimulation.

Intercellular communication in the heart

Within a tissue all the resident celltypes coordinate their activity to guarantee the proper function and stress responsiveness. Coordinated activity amongst celltypes is achieved by cell-cell and cell-matrix interactions, as well as by the secretion and reception of paracrine factors (growth factors and cytokines), many of which initiate intracellular signaling pathways in the target cells allowing them to interpret cues from neighboring cells (Kakkar et al. 2010). In the heart, myocytes, fibroblasts and endothelial cells are the most abundant celltypes that account for most of the requisite functions of the organ, and interactions between these cells are necessary for stress responsiveness and hypertrophic growth. Co-culture experiments with endothelial cells and myocytes, as well as fibroblasts and myocytes show that each of these 2 non-myocyte celltypes is able to induce hypertrophic growth of cardiomyocytes *in vitro* (Bowers et al. 2010). Indeed, the profile of cytokines and growth factors present within the ECM of the heart communicates myocyte growth, fibroblast transformation to myofibroblasts to make ECM, and to endothelial cells if neo-angiogenesis is needed. Finally, secretion of chemoattractant factors and cytokines during cardiac injury also recruits inflammatory and progenitor cells, which can each participate in the healing process and affect disease through inflammation. Well established factors responsible for paracrine intercellular communication amongst the cells within the heart are transforming growth factor β (Tgf β), fibroblast growth factors (FGF) growth and differentiation factor 15 (GDF15), natriuretic peptides, angiotensin II, aldosterone, thymosin β , chemokines, and the interleukins (i.e. IL-6), among many others (Souders et al. 2009, Tsuruda et al. 2010, Ottaviano et al. 2011). In addition, PGF has emerged as a central mediator responsible for both coordination of cardiomyocyte growth as well as neo-angiogenesis.

PGF, VEGF and the VEGF receptors

PGF is a secreted factor from the VEGF superfamily originally discovered in the placenta, where it was proposed to control trophoblast growth, differentiation and vascular development (Maglione et al. 1991). The expression of PGF is induced by hypoxia and the hypoxia-inducible transcription factor Hif1 α can directly activate its transcription (Jiang et al. 2011). PGF acts as a dimeric glycoprotein that, like VEGF, can bind with high affinity to the tyrosine kinase receptor, VEGF receptor 1 (VEGFR-1/Flt-1) (Park et al. 1994). By comparison, VEGFR-2 (Flk1) is specific for VEGF, is not bound by PGF, and is the main effector of VEGF proangiogenic activity (Persico et al. 1999). PGF homodimers, presumably acting through VEGFR-1, are chemotactic for cultured endothelial cells and

monocytes and hence probably function in concert with VEGF and the VEGFR-2 in affecting angiogenesis (Clauss et al. 1996). Importantly, the VEGFR-1 is expressed more widely than just endothelial cells and monocytes, such as in cardiac fibroblasts, meanwhile its expression by cardiomyocytes is still under debate (Chintalgattu et al. 2003, Herz et al. 2012). Although both VEGF and PGF can bind VEGFR-1, PGF homodimers uniquely stimulate the phosphorylation of specific VEGFR-1 tyrosine residues and elicit a distinct response biologically (Autiero et al. 2003). In addition to direct stimulation of VEGFR1 and signaling there after, another important mechanism of action for PGF is by binding to VEGFR-1 as a means of directly displacing VEGF, permitting greater VEGFR-2 occupancy by VEGF and a more potent pro-angiogenic effect since this later receptor is more central in mediating endothelial cell activity and capillary formation. Indeed, deletion of VEGFR-1 leads to embryonic lethality with too much endothelial cell activity and capillary formation, while deletion of VEGFR-2 leads to embryonic lethality with too few capillaries and endothelial cells (reviewed in (Shibuya 2006). Hence, PGF can signal directly through VEGFR-1 to affect biologic processes, or it can enhance the efficiency of proximal VEGF signaling through VEGFR-2 to enhance angiogenesis.

PGF and its role in cardiac adaptation

Surprisingly, unlike *Vegf* deletion in cardiomyocytes that resulted in capillary rarefaction and reduced cardiac contractility (Giordano et al. 2001), inactivation of the *Pgf* gene did not have an effect on vascular development in mice (Carmeliet et al. 2001) or otherwise affect capillary density in the heart (Accornero et al. 2011), although one report did suggest a mild reduction at baseline in cardiac capillaries (Gigante et al. 2004). Mice deleted for *Pgf* did show retarded pathological angiogenesis and collateral growth during ischemia (Carmeliet et al. 2001). Similarly, systemic high-dose production of PGF in a cardiac left coronary artery ligation model for 4 weeks stimulated angiogenesis in the infarct border and promoted vessel enlargement in the remote myocardium that improved recovery (Roncal et al. 2008). Similarly, direct injection of PGF protein into the infarct region of the rat heart enhanced border-zone angiogenesis, attenuated maladaptive ventricular remodeling, and preserved cardiac function (Kolakowski et al. 2006). Additional evidence that PGF functions as a stress response factor came from a genetic model of cardiac-specific PGF overexpression in transgenic mice, which showed no effect on the heart at baseline but with improved angiogenesis in mice subjected to pressure-overload stimulation, as well as preserved cardiac function when also stimulated with a neuroendocrine agonist (Accornero et al. 2011). By comparison, studies in *Pgf* gene-deleted mice showed that endogenous PGF is required for adaptive angiogenesis and the prevention of heart failure in mice after pressure-overload stimulation (Accornero et al. 2011). These results suggest that PGF is a stress-response factor that functions to suppress disease in the heart by maintaining capillary/vessel density in the heart as well as providing a protective trophic effect to cardiomyocytes.

In the absence of external stress PGF is weakly expressed in the heart. However, PGF levels dramatically increase after myocardial infarction (MI) as demonstrated by clinical studies as well as in murine models of injury (Iwama et al. 2006, Nakamura et al. 2009). Similarly, PGF expression increases in the heart after pressure overload stimulation, and high levels are maintained during the compensatory phase of hypertrophy, although they return to basal levels in heart failure (Accornero et al. 2011). The observations that high levels of PGF overexpression by either a cardiac-specific transgene or administration of a recombinant adenovirus encoding PGF each showed cardioprotection suggests a therapeutic approach with PGF in heart failure (Roncal et al. 2008, Accornero et al. 2011).

PGF as a cardiac paracrine factor

Disassociation studies from the heart demonstrate that all composing celltypes express PGF, although induction of PGF mRNA and protein after pressure overload stimulation appears to be predominantly from the cardiomyocytes (Accornero et al. 2011, Carnevale et al. 2011). PGF is then secreted where it accumulates in the ECM to signal to the target cells. As discussed earlier, both cardiac fibroblasts and endothelial cells express VEGFR-1, the receptor for PGF, while it still remains unclear if cardiomyocytes themselves express this receptor and if so, what the signaling effect intrinsic to the myocyte might be. While mice overexpressing PGF in the heart with a cardiomyocyte-specific promoter driven transgene showed increased compensatory hypertrophy in response to pressure overload, treatment of isolated cardiomyocytes in culture failed to show hypertrophy with PGF application (Accornero et al. 2011). However, treatment of cultured myocytes with supernatant from cardiac fibroblasts or endothelial cells overexpressing PGF induced a greater hypertrophic response indicating that the pro-hypertrophic properties of PGF observed *in vivo* are likely due to the secretion of secondary paracrine factors from non-myocytes to promote hypertrophy (Accornero et al. 2011). More specifically, PGF induced expression of IL6, IL1 β , and Cxcl1 in non-myocytes, all of which can have a pro-hypertrophic effect (Accornero et al. 2011). The results suggest that PGF activates a specific adaptive program in non-myocytes that favors the beneficial or supportive properties of these cells in the heart.

Studies in *Pgfr* gene-deleted mice (*Pgfr*^{-/-}) showed that this factor is necessary for efficient cardiac adaptation following pressure overload stimulation, as these mice developed heart failure and died more readily (Accornero et al. 2011). This more rapid and fulminant heart failure phenotype in *Pgfr*^{-/-} mice after pressure overload was associated with an impaired angiogenic, inflammatory and fibrotic response. Moreover, *Pgfr*^{-/-} mice also showed an inability to induce the known physiologic response whereby capillary density is increased in the heart by pressure overload stimulation during the more acute and compensated phase of the hypertrophic program (Accornero et al. 2011, Carnevale et al. 2011). Another important protective mechanism may actually be a temporary trophic effect on fibroblasts that facilitates compensatory remodeling, as PGF was also sufficient to stimulate cardiac fibroblast proliferation, in addition to the known angiogenic effect on endothelial cells with enhanced capillary tube formation (Figure 1).

PGF and the recruitment of inflammatory and progenitor cells

The recruitment of circulating progenitor cells into the heart actively participates in the remodeling of vessels and myocardium as part of the healing process (Li et al. 2005). PGF can also stimulate the chemotaxis of monocytes that could also impact cardiac remodeling and the fibrotic response, as well as have effects on angiogenesis during healing (Clauss et al. 1996). Indeed, monocyte recruitment was shown to be necessary for the PGF-mediated enhancement of neo-angiogenesis during pathological states (Pipp et al. 2003). In addition, hematopoietic progenitor cells (HPCs) residing within specified niches of the bone marrow express VEGFR-1. During pathology, VEGFR1-positive cells are mobilized from the bone marrow in association with increased PGF levels where they presumably migrate to the site of injury and the perivascular area to contribute to neo-angiogenesis (Kaplan et al. 2005, Li et al. 2006). PGF can also induce cardiac fibroblasts to secrete other factors with known chemoattractant properties such as tumor necrosis factor α (TNF α), IL1 α , IL1 β and Cxcl1 (Accornero et al. 2011, Carnevale et al. 2011). Indeed, cardiac overexpression of PGF improved bone marrow cell and endothelial progenitor cell invasion into the injured heart, which may have helped with preserving cardiac function after injury (Iwasaki et al. 2011).

Conclusions

Genetic and pharmacologic studies have identified PGF as a novel cardioprotective factor. The upregulation and secretion of PGF in the heart after injury is necessary for a coordinated adaptive and compensatory response between myocytes, fibroblasts, and endothelial cells, as well as progenitor cells that might aid in regeneration and neo-angiogenesis. The recent advances in the understanding of PGF function as a pivotal paracrine factor in the heart suggest that it could have a beneficial effect in human heart failure and hence could be an interesting biologic agent for clinical application in the near future. For example, the infusion of PGF peptides during percutaneous coronary intervention could improve the outcomes of coronary heart disease treatments, or possibly some sort of subcutaneous injection regimen (daily) of PGF active protein days and weeks after a myocardial infarction to help with revascularization.

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Figure 1. Role of PGF as a cardiac paracrine factor

PGF is upregulated in the heart during stress, most of which comes from cardiomyocytes. Endothelial cells and fibroblasts directly respond to PGF that is secreted into the extracellular matrix (ECM), where they then affect ventricular remodeling and angiogenesis. In addition, PGF stimulates fibroblasts and endothelial cells to release growth factors (GFs), which secondarily support cardiomyocyte hypertrophy and adaptation to stress.