Platelet-derived growth factor-C and -D in the cardiovascular system and diseases

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Abstract

The cardiovascular system is among the first organs formed during development and is pivotal for the formation and function of the rest of the organs and tissues. Therefore, the function and homeostasis of the cardiovascular system are finely regulated by many important molecules. Extensive studies have shown that platelet-derived growth factors (PDGFs) and their receptors are critical regulators of the cardiovascular system. Even though PDGF-C and PDGF-D are relatively new members of the PDGF family, their critical roles in the cardiovascular system as angiogenic and survival factors have been amply demonstrated. Understanding the functions of PDGF-C and PDGF-D and the signaling pathways involved may provide novel insights into both basic biomedical research and new therapeutic possibilities for the treatment of cardiovascular diseases.

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1. The platelet-derived growth factor (PDGF) family

1.1. The “classic” PDGFs: PDGF-A and PDGF-B

Platelet-derived growth factors (PDGFs) were first identified in the 1970s and have been extensively studied since then (Antoniades et al., 1979; Heldin et al., 1977, 1979; Kohler and Lipton, 1974; Ross et al., 1974; Westermark and Wasteson, 1976). Intensive studies for more than four decades have demonstrated their critical roles in many tissues and organs, such as cardiovascular, digestive, hematopoietic, reproductive, respiratory, nervous, and immune systems. In addition, studies on the PDGFs have made critical contributions to the advancement of many research fields, including signal transduction. Therefore, although the PDGFs were discovered a long time ago, they still attract considerable attention in the field of biomedical research and remain to be a highly active research area for drug development.

Currently, there are four PDGF family members: PDGF-A, PDGF-B, PDGF-C, and PDGF-D. PDGF-A and PDGF-D were discovered about four decades ago (Antoniades et al., 1979; Heldin et al., 1977, 1979; Westermark and Wasteson, 1976). For more than 20 years, PDGF-A and PDGF-B were thought to be the only two PDGFs. However, in 2000 and 2001, two new members of the family, PDGF-C and PDGF-D, were unexpectedly discovered, leading to new breakthroughs in numerous disciplines of biomedical research (Bergsten et al., 2001; Kazlauskas, 2000; Larochelle et al., 2001; Li and Eriksson, 2003; Li et al., 2000).

PDGFs exert their biological effects through two PDGF receptor tyrosine kinases, PDGFR-α and PDGFR-β, which can form homo- or heterodimers. Traditionally, PDGFs are considered to be potent mitogens for cells of mesenchymal origin, such as fibroblasts, smooth muscle cells (SMCs) and glial cells. However, more recent studies have shown that the functions of PDGFs are much broader than previously thought and that these proteins have direct effects on a wide range of cell types, including stem/progenitor cells, neuronal cells, vascular endothelial cells and inflammatory cells. Therefore, PDGFs play important roles in a wide variety of developmental, physiological and pathological conditions. Moreover, due to their potent effects, PDGFs and their receptors are considered as promising therapeutic targets.

1.2. The “new” PDGFs: PDGF-C and PDGF-D

Two genes encoding PDGF-C and PDGF-D were identified in a search of mouse expressed-sequence tag (EST) databases. PDGF-C and PDGF-D show high similarities to the growth factor family of PDGF/vascular endothelial growth factor (VEGF), PDGF-C and PDGF-D are 323 and 348 amino acids each, with predicted molecular weights of 36.7 and 40.2 kDa, respectively (Bergsten et al., 2001; Li et al., 2000). PDGF-C and PDGF-D are produced as
secreted homodimers: PDGF-CC and PDGF-DD. Both PDGF-C and PDGF-D contain two protein domains: a C-terminal PDGF/VEGF core domain, and an N-terminus CUB (complement subcomponents C1r/C1s, urchin EGF-like protein and bone morphogenic protein 1) domain (Fredriksson et al., 2004; Lee et al., 2013; Li et al., 2008; Andrae, 2008; Borkham-Kamphorst and Weiskirchen, 2016; Jones and Cross, 2004). PDGFR-a and PDGFR-b have about 30% amino acid sequence homology and belong to the class III subtype of receptor tyrosine kinases (RTKs), including c-Kit (stem cell factor receptor), c-Fms (macrophage-stimulating factor receptor), and Flt3 (FMS-like tyrosine kinase 3 receptor) (Chen et al., 2013; Jones and Cross, 2004).

PDGF-AAA, -BB, -AB, and -CC bind to and activate the PDGFR-a homodimer, and PDGF-BB and -DD bind to and activate PDGFR-b homodimer. In addition, PDGF-AB, -BB, -CC, and -DD also bind to and activate PDGFR-a/b heterodimer when PDGFR-a and PDGFR-b are co-expressed in the same cells. The binding of PDGF ligands to their receptors results in receptor dimerization and trans-autophosphorylation between the subunits of PDGFR-a and PDGFR-b. Upon activation of PDGFRs, multiple tyrosine residues are phosphorylated and the phosphorylated PDGFRs recruit the interacting molecules to elicit downstream effectors, such as Src, PI3K, Shc, Grb2, Akt, SHP2, and other signaling molecules to induce a variety of downstream signaling pathways. The activation of these signaling pathways leads to cell proliferation, migration, survival, and differentiation.

Moreover, the PDGF signaling can be modulated by their association with other cell surface receptors. For example, PDGFR-b and epidermal growth factor receptor (EGFR) can form heterodimers under basal level conditions, and disruption of this heterodimerization abolishes EGFR transactivation and inhibits PDGF-BB-mediated extracellular signal-regulated kinase (Erk1/2) activation (Saito et al., 2001). Moreover, integrin α1 can induce early and transient phosphorylation and internalization of PDGFR-β in fibroblasts independent of the PDGFs (Sundberg and Rubin, 1996).

1.3. PDGF receptor tyrosine kinases: PDGFR-a and PDGFR-b

The dimeric (homodimers or heterodimers) forms of PDGF ligands (PDGF-AA, -AB, -BB, -CC, and -DD) activate and bind to two tyrosine kinase receptors, PDGFR-a and PDGFR-b (Fig. 2). PDGFRs are membrane-anchored proteins composed of five extracellular immunoglobulin-like (Ig) domains, a single transmembrane domain, a juxtamembrane domain (JMD), a kinase insert domain (KID), and an intracellular kinase domain (KD) (Andrae, 2008; Chen et al., 2013; Jones and Cross, 2004). PDGFR-a and PDGFR-b have about 30% amino acid sequence homology and belong to the class III subtype of receptor tyrosine kinases (RTKs), including c-Kit (stem cell factor receptor), c-Fms (macrophage-stimulating factor receptor), and Flt3 (FMS-like tyrosine kinase 3 receptor) (Chen et al., 2013; Jones and Cross, 2004).

PDGF-AAA, -BB, -AB, and -CC bind to and activate the PDGFR-a homodimer, and PDGF-BB and -DD bind to and activate PDGFR-b homodimer (Fig. 2). In addition, PDGF-AB, -BB, -CC, and -DD also bind to and activate the PDGFR-a/b heterodimer when PDGFR-a and PDGFR-b are co-expressed in the same cells. The binding of PDGF ligands results in receptor dimerization and trans-autophosphorylation of the subunits of PDGFR-a and PDGFR-b. Upon activation of PDGFRs, multiple tyrosine residues are phosphorylated in the cytoplasmic domains, including JMD, KID, and KD. The phosphorylated PDGFRs recruit interacting molecules to elicit downstream signaling pathways, such as Src, FAK, Akt, SHP2, and other signaling molecules to induce a variety of downstream signaling pathways. The activation of these signaling pathways leads to cell proliferation, migration, survival, and differentiation.
Furthermore, PDGFR-β has been shown to be associated with the low density lipoprotein receptor-related protein (LRP) and functions to enhance PDGF-dependent LRP signaling and to suppress the hyaluronan receptor CD44 pathway (Li et al., 2006; Loukino et al., 2002; Newton et al., 2005). In addition, PDGFR-β and sphingosine 1-phosphate receptor 1 (S1P1) were found to form a complex in airway SMCs and mouse embryonic fibroblasts to regulate cell migration via Erk1/2 activation (Long et al., 2006; Waters et al., 2006). Taken together, these data suggest that the crosstalk, complex formation, and transactivation among different receptors of different signaling pathways can result in a further level of complexity and flexibility in terms of their spatial and temporal regulation and context-dependent responses. Further studies are necessary to better elucidate the complexity of these pathways and to determine the biological consequences of these crosstalk in normal physiology and diseases.

2. PDGF-C and PDGF-D expression in development and adults

The expressions of PDGF-C and PDGF-D are considerably broad in many different types of cells and tissues. Human PDGF-C is expressed as two transcripts of 3.9 and 2.8 kb (Li et al., 2000). Abundant PDGF-C expression is observed in the heart, liver, kidney, pancreas, ovary, vascular SMCs, and endothelial cells, whereas lower levels of PDGF-C expression is found in the brain, placenta, lung, skeletal muscle, thymus, prostate, testis, and small intestine (Karvinen et al., 2009; Li et al., 2000; Uutela et al., 2001). Mouse and rat PDGF-C are expressed as two transcripts (2.9 and 3.6 kb) in the kidney, testis, liver, heart, and brain. In the testis, a shorter transcript of PDGF-C (1.8 kb) is observed (Aase et al., 2002; Hamada et al., 2002). During development, PDGF-C is widely expressed in many types of cells and organs, including somites and their derivatives, craniofacial mesenchyme, cardiomyocytes, some vascular SMCs, cartilage, hypertrophic chondrocytes, cerebellum, and neurogenic derivatives of the neural crest cells and central nervous system (Aase et al., 2002; Ding et al., 2000; Hamada et al., 2002). In Gekko japonicas, a 2.8-kb PDGF-C transcript is abundantly expressed in the heart, lung, kidney, and ovary (Liu et al., 2009).

Compared with that of PDGF-C, the expression of PDGF-D appears to be less abundant and more restricted spatially and temporally, although there is some overlap. The human PDGF-D gene is expressed as two transcripts of 4 and 2 kb in the heart, pancreas, ovary, brain, lung, and skeletal muscle due to alternative splicing (Fredriksson et al., 2004; Hamada et al., 2001). Notable expression of PDGF-D has been found in human umbilical vein and microvascular endothelial cells (Uutela et al., 2001). During mouse development, PDGF-D is expressed in the heart, lung, kidney, and muscle derivatives (Fredriksson et al., 2004). In rats, PDGF-D is widely expressed as a 3.8-kb transcript during development and in adults, with a higher level of expression in the kidney and eye (Hamada et al., 2002).

3. Regulation of PDGF-C and PDGF-D expression

The promoter region of PDGF-C has several putative recognition elements for early growth response-1 (EGR-1), Sp1, and Smad, indicating a regulatory role of them in PDGF-C expression. Indeed, in vascular SMCs, fibroblast growth factor (FGF)-2 upregulates PDGF-C expression via EGR-1 and Erk (Midgley and Khachigian, 2004). In pulmonary fibroblasts, interleukin (IL)-13 increases PDGF-C expression by regulating signal transduction and activation of transcription (STAT) 5 and STAT1 activation and EGR-1 expression (Ingram et al., 2006). Another study showed that the peptide hormone angiotensin (ATII) induces PDGF-C expression through angiotensin type 1 receptor and EGR-1 in SMCs (Sanchez-Guerrero et al., 2008). Similarly, PDGF-D expression can be induced by ATII via ETS-1 and Sp1 in response to oxidative stress (Liu et al., 2006). In addition, Erk phosphorylation and Krüppel-like factor-4 are required for PDGF-D expression in SMCs (Thomas et al., 2009). PDGF-C has been shown to be upregulated by the EWS/ETS transcription factor, which has a central role in the development of Ewing family sarcoma (Zwerner and May 2001). PDGF-D expression can be induced by ATII and involves protein kinase C ζ-dependent Sp1 phosphorylation in SMCs (Tan et al., 2008). Moreover, deletion of INK4a/ARF in KRAS (G12D)-expressing mice upregulates PDGF-D expression in tumors and tumor-derived Rink-1 cells (Wang et al., 2013). Further studies are needed to delineate the regulation of PDGF-C and PDGF-D expression in other types of cells.

4. The angiogenic effects of PDGF-C and PDGF-D

The PDGs and their receptors are well known for their critical roles in blood vessel maturation and stabilization (Betscholtz, 2004; Millette et al., 2006; Raines, 2004). In addition, numerous studies have shown that PDGF-C and PDGF-D are potent angiogenic factors that function through multiple mechanisms (Jones et al., 2006; Li et al., 2005; Rolny et al., 2006; Xiao et al., 2007). These include direct effects on vascular stem/progenitor cells (Li et al., 2005; Tang et al., 2013), upregulation of other potent angiogenic factors, including VEGF, FGF2, cyclooxygenase-2, angiopoietin (Ang) 1, matrix metalloproteinases (MMPs), and integrins (Chao et al., 2006; Holburn et al., 2006; Machens et al., 2006; Pohlers et al., 2006; Wang et al., 2006). Moreover, PDGF-C and PDGF-D can have different secreted levels in newly formed blood vessels are markedly upregulated (Aase et al., 2002; Ding et al., 2000; Hamada et al., 2002). In Gekko japonicas, a 2.8-kb PDGF-C transcript is abundantly expressed in the heart, lung, kidney, and ovary (Liu et al., 2009).

Compared with that of PDGF-C, the expression of PDGF-D appears to be less abundant and more restricted spatially and temporally, although there is some overlap. The human PDGF-D gene is expressed as two transcripts of 4 and 2 kb in the heart, pancreas, ovary, brain, lung, and skeletal muscle due to alternative splicing (Fredriksson et al., 2004; Hamada et al., 2001). Notable expression of PDGF-D has been found in human umbilical vein and microvascular endothelial cells (Uutela et al., 2001). During mouse development, PDGF-D is expressed in the heart, lung, kidney, and muscle derivatives (Fredriksson et al., 2004). In rats, PDGF-D is widely expressed as a 3.8-kb transcript during development and in adults, with a higher level of expression in the kidney and eye (Hamada et al., 2002).

Fig. 3. Blood vessel sprouting. PDGF-C and PDGF-D are potent angiogenic factors functioning through multiple mechanisms, including direct effects on vascular stem/progenitor cells, upregulation of other potent angiogenic factors, such as VEGF, FGF-2, COX-2, Ang1, and MMPs. The expression levels of PDGFRs in vascular endothelial cells are relatively low under normal conditions. However, in the presence of high levels of angiogenic factors, such as FGF-2, PDGF-B, and PDGF-C, the expression levels of PDGF-α and PDGFR-β in newly formed blood vessels are markedly upregulated. PDGF-C and PDGF-D are also expressed by vascular endothelial cells, suggesting that PDGF-C and PDGF-D have direct effects on vascular endothelial cells. In addition to vascular endothelial cells, PDGFRs and their receptors are major regulators of the proliferation, migration, and survival of vascular SMCs and pericytes. PDGFRs and their receptors are also expressed by monocytes, macrophages, and fibroblasts. Sprouting angiogenesis is mediated by multiple angiogenic factors, such as EGF, FGF-2, VEGF, PDGF produced by various types of cells or tumors. These angiogenic factors trigger many sequential processes such as endothelial cell proliferation, migration, survival, and neovessel formation.
recruit inflammatory cells and downregulate anti-angiogenic genes such as Ang2 (Cheon et al., 2004; Phelps et al., 2006; Uutela et al., 2004) (Fig. 3). Indeed, increased expression of the PDGFs can lead to pathological angiogenesis in different diseases, such as ocular neovascular disorders and tumors (Kono et al., 2012; Mori et al., 2002; Seo et al., 2000; Vinores et al., 2003). Importantly, inhibition of the PDGFs can suppress neovascularization under different conditions (Akiyama et al., 2006; Jo et al., 2006). Due to their critical roles in angiogenesis and blood vessel maturation, the PDGFs have become critical targets in anti-angiogenic therapy (Petrillo et al., 2012).

5. Effects of PDGF-C and PDGF-D on vascular endothelial cells

Traditionally, PDGFs are known as major regulators of SMCs and fibroblasts since PDGFRs are highly expressed in these cells. By contrast, the expression levels of PDGFRs in vascular endothelial cells are relatively low under normal conditions. Therefore, the effects of PDGFs on vascular endothelial cells have not been extensively studied and are in fact under appreciated. However, under other conditions, such as in the presence of high levels of angiogenic factors, such as FGF-2, PDGF-B, and PDGF-C, the expression levels of PDGFR-α and PDGFR-β in newly formed blood vessels are markedly upregulated, indicating important roles of the PDGFs and PDGFRs in the making of new blood vessels (Cao et al., 2002).

Indeed, many studies have reported the expression of PDGFRs in vascular endothelial cells (Battagay et al., 1994; Cao et al., 2002; Hermansson et al., 1988; Li et al., 2005; Marx et al., 1994). During mouse cornea neovascularization, PDGFR-α is highly expressed in vascular endothelial cells, whereas PDGFR-β is mostly expressed in vascular SMCs (Cao et al., 2002). PDGF-C and PDGF-D are also expressed by different types of vascular endothelial cells, including human umbilical vein endothelial cells, human microvascular endothelial cells, renal artery endothelial cells, and human endothelial cells exposed to an atherosclerosis-prone pattern (Eitner et al., 2003; Floege et al., 2008; Li et al., 2005; Thomas et al., 2009). These findings thus suggest that PDGF-C and PDGF-D have direct autocrine effects on vascular endothelial cells (Fig. 3). Consistently, it has been shown that under ischemic conditions, PDGF-C treatment mobilized endothelial progenitor cells, induced the differentiation of bone marrow stem/progenitor cells into vascular endothelial cells and stimulated their migration (Li et al., 2005). Moreover, PDGF-D has been shown to be able to induce tube formation of human umbilical vein endothelial cells (Wang et al., 2007). Thus, PDGF-C and PDGF-D have direct effects on vascular endothelial cells and thereby contribute to angiogenesis.

6. Effects of PDGF-C and PDGF-D on blood vessel permeability

The effects of PDGF-C and PDGF-D on blood vessel permeability have not been well studied and limited reports have shown varying results. One study showed an inhibitory effect of PDGF-C on vascular permeability by promoting blood vessel maturation (di Tomaso et al., 2009). This is consistent with previous reports showing the anti-permeability effects of PDGFs by maintaining vascular integrity through pericytes, which suppress the expression of many vascular permeability genes. Indeed, loss of pericytes increases vascular permeability due to elevated transcytosis (Andrae, 2008; Daneman et al., 2010; Lindahl et al., 1997; Semela et al., 2008; Soriano, 1994; Winkler et al., 2011; Zymek et al., 2006). It has been shown that PDGFC deficiency does not alter Evan’s blue extravasation in the retina and brains, suggesting a negligible role of PDGF-C in inducing blood vessel permeability. Moreover, injection of PDGF-C protein into mouse vitreous, substantia nigra, normal, or ischemic brains increased neuronal survival without affecting vascular permeability (Tang et al., 2010). On the other hand, intraventricular injection of PDGF-C protein increases cerebrovascular permeability in mice by activating PDGFR-α on perivascular astrocytes and inhibition of PDGFR-α after ischemic stroke reduces cerebrovascular permeability (Su et al., 2008). The reasons for these varying observations are currently unclear. However, they may be explained by a study showing that the effects of PDGFR-β on vascular permeability depend on the levels of its ligand PDGF-B, which induces pericyte detachment by downregulating the expression of integrins (Hosaka et al., 2013). The potential effect of PDGF-D on blood vessel permeability remains largely unknown. One study reported that increased expression of PDGF-D may be related to sinonasal polyp formation by increasing vascular permeability and infiltration of inflammatory cells (Bayar Muluk et al., 2013). Thus, the roles of PDGF-C and PDGF-D in the regulation of blood vessel permeability remain to be better addressed and further studies are required to gain better insights into the mechanisms involved.

7. Effects of PDGF-C and PDGF-D on vascular SMCs, pericytes, and other mesenchymal cells

PDGFs and their receptors are major regulators of the proliferation, migration, and survival of vascular SMCs and pericytes, which are the prototypic cellular targets of PDGFs. PDGFR-α and PDGFR-β are highly expressed by vascular smooth muscle cells and pericytes (Armulik et al., 2011). PDGFR-β deficiency by genetic deletion leads to loss of vascular pericytes and subsequent endothelial hyperplasia, indicating a role of PDGF-B, PDGF-C and PDGF-D in this process since they both bind to PDGFR-β (Hellström et al., 2001). PDGF-C is highly expressed in vascular SMCs, and pdgfc deficiency led to abnormal vascular SMC coverage in the brains of pdgf-c-deficient mice in a C57B/L6 background (Fang et al., 2004; Fredriksson et al., 2012; Midgley and Khachigian, 2004; Sanchez-Guerrero et al., 2008; Uutela et al., 2001). In a mouse model of glioblastoma, PDGF-C produced by tumor cells recruits pericytes to blood vessels, resulting in increased blood vessel stabilization and drug resistance to anti-VEGF treatment (di Tomaso et al., 2009). Moreover, transgenic overexpression of PDGF-C in mouse hearts increases the density of SMC-coated blood vessels (Pontén et al., 2003). Studies have shown that PDGF-D is highly expressed in vascular SMCs and colocalizes with PDGFR-β (Pontén et al., 2005; Uutela et al., 2001). In vivo, overexpressing the core domain of PDGF-D in mouse hearts leads to hyperproliferation of vascular SMCs, increased SMC-coated blood vessels, and thickening of arterial walls (Pontén et al., 2005).

In addition to vascular SMCs and pericytes, PDGF-C and PDGF-D are also highly expressed by other types of mesenchymal cells. PDGF-C is detected in the mesonephric and frontonasal mesenchyme, cortical and metanephric mesenchyme in the kidney, and lateral mesenchyme of the otic vesicle in mice (Aase et al., 2002; Fang et al., 2004; Gilbertson et al., 2001). PDGF-D has been shown to be expressed in the mesenchyme of normal prostate tissue, suggesting a role of PDGF-D in regulating the functions of different types of mesenchyme cells (Ostendorf et al., 2006; Ustach and Kim, 2005).

8. Expression of PDGF-C, PDGF-D, and PDGFRs in the cardiovascular system

The mammalian heart is composed of chambers (atria and ventricles), vessels, valves, coronary circulation, and a conduction system. On embryonic day 8 (E8) in mice, heart progenitor cells begin to develop the heart tube from the epithelial cardiac crescent.
PDGF-A expression is found in the epicardium, PDGF-B in endocardial cells, PDGF-C in cardiomyocytes, and PDGF-D in the epicardium and myocardium (Richarte et al., 2007; Smith and Tallquist, 2010). In the human fetal heart, PDGFR-α expression is found in interstitial cells of the epicardium, endocardium, and myocardium of the atria and ventricles (Chong et al., 2013). A recent study on pdgf-α/− mice showed no major heart defects. However, systemic alterations with elevated blood pressure and serum levels of Ca²⁺ and Cl⁻ were detected (Gladh et al., 2016) (Table 1). Furthermore, mouse and zebrafish embryos of pdgfr-α mutant displayed cardiac fusion defects and disrupted heart tube assembly (Bloomekatz et al., 2017). Further analysis of the mutant embryos revealed that PDGFR-α regulates the medial direction of the movement of cardiomyocytes during cardiac fusion, indicating a possible link between PDGFR-α mutations and congenital heart diseases, particularly, those with disruption of heart tube assembly.

Neural crest cells (NCCs) are a population of multipotent and migratory stem cells derived from the embryonic dorsal neural tube to form craniofacial skeleton, smooth muscles, sensory neurons, and connective tissues (Smith and Tallquist, 2010; Williams and Bohnsack, 2015). There are several subpopulations of NCCs, including cranial, cardiac, trunk, vagal, and sacral NCCs, which yield a broad range of tissues. Cardiac NCCs contribute to outflow tract septation and parasympathetic innervation of the developing heart (Harvey, 2002). They migrate ventrally to form a component of the vascular smooth muscles of the aortic arch, ventricular, and aortico-pulmonary septum.

Migrating cardiac NCCs express PDGFR-α and PDGFR-β, and their expression is segregated after they arrive at the developing outflow tract. Gene ablation of pdgfr-α in mice leads to defects in cardiac and cranial NCC populations, including pigmentation, palatogenesis, and the aortic arch (Richarte et al., 2007; Soriano, 1997; Tallquist and Soriano, 2003). Additionally, studies using mice of pdgfr-α deletion or Patch (Ph) mutation (a naturally occurring allele with pdgfr-α deletion) demonstrated that cell survival and matrix deposition are the main functions regulated by PDGFR-α. However, in mice with NCC-specific deletion of pdgfr-α, cell proliferation and apoptosis are not affected. This suggests that PDGFR-α regulates cell survival and matrix deposition in other cells than NCCs (Richarte et al., 2007). Moreover, mice with conditional deletion of PDGFR-α and PDGFR-β (Pdgfr-α<sup>fl/fl</sup>; Pdgfr-β<sup>-/−</sup>; Wnt1-Cre) in NCCs show severe defects in aortic arch and ventricular septum, indicating that signaling from both receptors is necessary for the recruitment of cardiac NCCs and the formation of the aortic-pulmonary and ventricular septum (Richarte et al., 2007).

9. PDGF-C and PDGF-D in cardiovascular diseases

9.1. Cardiac fibrosis

Cardiac fibrosis is the pathological expansion of cardiac
extracellular matrix (ECM) proteins, which occurs in most types of heart diseases (Gourdie et al., 2016; Tallquist and Molkentin, 2017). This condition is commonly found in many cardiac diseases, such as post-myocardial infarction, heart failure, hypertension, and diabetic cardiomyopathy. Myocardial infarction leads to sudden loss of cardiomyocytes and triggers inflammation, consequently inducing the replacement of the dead myocardium with ECM (Porter and Turner, 2009; Shinde and Frangogiannis, 2014). Moreover, a persistent pressure overload induced by hypertension results in extensive cardiac fibrosis, which may lead to ventricular dilation and combined diastolic and systolic heart failure. In addition, metabolic stress, obesity, and toxic insults, such as alcohol, can induce progressive fibrotic changes in the myocardium.

Histological analysis of pdgfb and pdgfr-β-knockout embryos demonstrated the presence of cardiac abnormalities, such as cardiac hypotrophy, capillary microaneurysm, and placental defects (Andrae et al., 2008; Hellstrom et al., 1999; Ollis et al., 1999). The endothelium-restricted pdgfb-deficient mutant showed defective blood vessels similar to those in pdgfb-null mice. Moreover, cardiac microvascular communication is mediated by PDGF signaling (Bjarnegard et al., 2004). These findings show that the PDGF/PDGFR signaling pathway plays a central role in the development of the cardiovascular system. The normal myocardium of the rat heart expresses all the PDGF isoforms, which are primarily expressed in interstitial fibroblasts, indicating that PDGFs have important roles in the regulation of fibroblasts and contribute to the maintenance of connective tissues in the heart.

α-Myosin heavy chain (MHC) promoter-driven overexpression of pdgfc in mouse hearts induces cardiac fibrosis, cardiomyopathy, and alterations of the vasculature, such as dilation of microvessels and vascular leakage, with increased density of smooth muscle-coated vessels in the myocardium (Pontén et al., 2003) (Table 1). Similarly, pdgf-d transgenic mice expressing the core domain of PDGF-D driven by a heart-specific α-MHC promoter showed cardiac fibrosis followed by dilated cardiomyopathy and displayed vascular remodeling, such as dilation of vessels, elevated density of SMC-covered vessels, and proliferation of vascular SMCs, which led to thickening of arterial walls (Pontén et al., 2005). Compared with pdgfc transgenic mice, pdgf-d transgenic mice display generally similar but more severe phenotypes (e.g., thickening of tunica media). However, a recent report demonstrated that mouse hearts injected with PDGF-B-expressing adenovirus display large scars with extensive inflammation, whereas hearts injected with PDGF-D-expressing adenovirus showed decreased inflammation with small and dense scars. In addition, injection of PDGF-A- or PDGF-B-expressing adenovirus reduced the amount of scar tissues and increased the numbers of PDGFR-α positive fibroblasts (Gallini et al., 2016). These findings indicate that different PDGF-induced responses may vary greatly and in a context-dependent manner during development or under pathological conditions.

In addition, PDGF-C stimulates the recruitment of endothelial progenitor cells from the bone marrow (Tang et al., 2013). In vitro studies using rat cardiac fibroblasts treated with PDGF-D have demonstrated that cardiac fibroblast proliferation and myofibroblast differentiation are increased with enhanced expression of MMP-1, MMP-2, MMP-9, tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2 (Zhao et al., 2013). Interestingly, PDGF-D-treated cardiac fibroblasts show increased TGF-β1 expression, and silencing of TGF-β1 abolishes PDGF-D-induced TGF-β1 production and its profibrogenic effects, indicating that PDGF-D has a role in fibrogenesis via the TGF-β1 signaling pathway.

9.2. Myocardial infarction

Ischemic heart disease is a leading cause of morbidity and mortality in the US. During 2012 to 2013, the estimated direct and

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indirect costs of heart diseases was $199.6 billion and myocardial infarction was one of the ten most expensive hospital diagnoses in 2011 (Benjamin et al., 2017). Approximately, 14% of the people who experience myocardial infarction die of it. Myocardial infarction is defined as myocardial cell death due to prolonged ischemia (Anderson and Morrow, 2017). It occurs when blood supply is blocked to a segment of myocardium, which causes the development of necrosis and apoptosis of heart muscle. Myocardial infarction is often caused by rupture of an atherosclerotic lesion in a coronary artery, which leads to the formation of a thrombus that plugs the artery, stopping it from supplying blood to the regions of the heart (Ozaki and Tanaka, 2016; Thygesen et al., 2012). Thus, therapeutic interventions that can decrease necrosis and apoptosis, increase cell survival and revascularization and reduce collagen deposition and inflammation are desired. For these purposes, combinational therapeutic strategy of angiogenic factors may be a promising therapeutic strategy to improve cardiac functions in the infarct heart. Indeed, it has been shown that treatment of PDGF-CC protein enhanced post-ischemic revascularization in mouse hearts with myocardial infarction (Li et al., 2005). Moreover, combined PDGF-B and FGF-2 gene transfer in a rat model of chronic myocardial infarction has an additive effect on angiogenesis and arteriogenesis, which reduced the infarct size and improved cardiac function (Cui et al., 2014; Hao et al., 2004). In addition, combination gene transfer of PDGF-B and VEGF-A_165_ stimulated revascularization at both a capillary and an arteriolar level and transiently counteracted cardiac remodeling after myocardial infarction in rats (Hao et al. 2007). These studies thus demonstrate that PDGFs have cardioprotective and angiogenic roles in myocardial infarction animal models.

Following myocardial infarction, PDGF-A and PDGF-D expression levels are increased in the infarcted area, whereas PDGF-B and PDGF-C expression levels are decreased in the normal heart surrounding the infarction (Zhao et al., 2011). In addition, PDGF-D expression is increased in the late stages of infarction and remained elevated for 6 weeks, with strong staining detected at the border zone and infarcted myocardium. Both PDGFR-α and PDGFR-β expression levels are increased in the early and late stages of myocardial infarction (Zhao et al., 2013). Studies using PDGFR-α and PDGFR-β neutralizing antibodies show that inhibition of these receptors leads to decreased collagen deposition in the infarcted area. PDGFR-β inhibition leads to defective angiogenesis and increased permeability of the newly formed vasculature. By contrast, PDGFR-α inhibition does not have such an effect. Furthermore, 7 days following infarction, mice undergoing PDGFR-β inhibition exhibit hemorrhagic areas containing erythrocytes in the infarcted hearts, suggesting that PDGF-mediated signaling pathways play important but distinct roles in the regulation of post-infarction repair (Zymek et al., 2006). Similarly, in rats with myocardial infarction treated with the PDGFR inhibitor Imatinib (Gleevec®), cardiac fibrogenesis and angiogenesis were observed with ventricular dysfunction, including increased ejection fraction (EF) and fraction shortening (FS) (Liu et al., 2014). On the other hand, studies have shown that PDGF-C and PDGF-D may be required for the post-infarction repair in cardiac ischemia. For example, it has been shown that treatment with PDGF-C protein promotes revascularization in a cardiac ischemic mouse model, leading to increased blood vessel density and SMC coverage in the infarcted hearts (Li et al., 2005).

9.3. Atherosclerosis

Atherosclerosis is a multifactorial disease manifested by focal development of lesions in the arterial wall in response to various insults (Ozaki and Tanaka, 2016; Ross, 1999). Classical epidemiology has identified many risk factors, such as dyslipidemia, hypertension, hyperglycemia, pro-inflammatory cytokines and smoking, all of which contribute to the initiation and progression of arterial lesions (Libby, 2012; Libby et al., 2011). In addition, the inflammatory reaction induced by stimulated T lymphocytes, heat shock proteins, and components of plasma lipoproteins also induces atherosclerotic plaques in the absence of systemic hypercholesterolemia (Libby et al., 2009).

PDGF-A and PDGF-B are known growth factors that regulate the proliferation and migration of monocytes/macrophages, which are abundant and play important roles in the development of atherosclerosis. Macrophages produce PDGF-C and PDGF-D in atherosclerotic lesions, and monocyte differentiation is regulated by PDGF-C and PDGF-D (Wagsater et al., 2009). In addition, PDGF-C and PDGF-D induce monocyte migration and invasion with induction of MPP-2 and MOMP-9 expression, suggesting that PDGF-C and PDGF-D contribute to atherosclerosis by promoting MMP synthesis and monocyte migration. Indeed, it has been shown that PDGF-C and PDGF-D are expressed in the artery wall in different stages of atherosclerosis and that PDGFR-α and PDGFR-β are expressed in the atherosclerotic lesions (Karvinen et al., 2009). In particular, PDGF-C is expressed in endothelial cells in normal arteries and in all types of lesions, whereas PDGF-D is widely expressed in the artery wall, macrophages and SMCs, suggesting that both PDGF-C and PDGF-D may be important players in atherosclerosis. Interestingly, genome-wide association studies have identified PDGF-D as a common variant associated with coronary artery disease (Coronary Artery Disease Genetics, 2011; Ozaki and Tanaka, 2016). Further studies are needed to understand the precise roles of PDGF-C and PDGF-D in atherosclerosis and the underlying mechanisms.

10. Conclusions and perspectives

Numerous studies have shown that PDGF-C and PDGF-D are critical regulators of the cardiovascular system. PDGF-C and PDGF-D are widely expressed in many different types of cells with pleotropic effects. Both in vivo and in vitro studies have demonstrated that PDGF-C and PDGF-D treatment can improve cardiac function and increase angiogenesis, and antagonists of PDGF-C, PDGF-D and PDGFs may be of therapeutic usage in inhibiting fibrosis or atherosclerosis. Future in-depth studies that can better reveal the molecular basis of the functions and regulatory mechanisms of PDGF-C and PDGF-D in the cardiovascular system may facilitate the development of novel therapeutic strategies for the treatment of human diseases.

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