PDGF Mediates Cardiac Microvascular Communication

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Abstract

The diversity of cellular and tissue functions within organs requires that local communication circuits control distinct populations of cells. Recently, we reported that cardiac myocytes regulate the expression of both von Willebrand factor (vWF) and a transgene with elements of the vWF promoter in a subpopulation of cardiac microvascular endothelial cells (J. Cell Biol. 138:1117). The present study explores this communication. Histological examination of the cardiac microvasculature revealed colocalization of the vWF transgene with the PDGF α-receptor. Transcript analysis demonstrated that in vitro cardiac microvascular endothelial cells constitutively express PDGF-A, but not B. Cardiac myocytes induced endothelial expression of PDGF-B, resulting in PDGF-AB. Protein measurement and transcript analysis revealed that PDGF-AB, but not PDGF-AA, induced endothelial expression of vWF and its transgene. Antibody neutralization of PDGF-AB blocked the myocyte-mediated induction. Immunostaining demonstrated that vWF induction is confined to PDGF α-receptor–positive endothelial cells. Similar experiments revealed that the PDGF-AB/α-receptor communication also induces expression of vascular endothelial growth factor and Flk-1, critical components of angiogenesis. The existence of this communication pathway was confirmed in vivo. Injection of PDGF-AB neutralizing antibody into the amniotic fluid surrounding murine embryos extinguished expression of the transgene. In summary, these studies suggest that environmental induction of PDGF-AB/α-receptor interaction is central to the regulation of cardiac microvascular endothelial cell hemostatic and angiogenic activity. (J. Clin. Invest. 1998, 102:837–843.) Key words: cardiac microcirculation • endothelium • heart • angiogenesis • hemostasis

Introduction

It is widely recognized that endothelial cells of the blood vessel wall play an essential role in regulating angiogenesis and modulating hemostasis. The activity of endothelial cells from various vascular beds may differ in these roles, resulting in phenotypic diversity. In turn, this diversity may be involved in the susceptibility of particular regions to vascular diseases such as atherosclerosis, thrombosis, and neovascularization.

Less often appreciated, however, is that endothelial cells of different vascular beds synthesize discrete sets of gene products and are, therefore, able to carry out organ-specific tasks. This diversity of endothelial cell function has been described in relationship to the hemostatic and angiogenic activity of various vascular beds. Previous studies have demonstrated that plasminogen activator inhibitor-1 is expressed at high levels in vascular beds of the murine aorta, heart, and adipose tissue, and low levels in the liver, adrenals, and kidney (1). Heterogeneous gene expression is also found in endothelial cells within a given vascular bed. One example is the endothelial expression of thrombomodulin in the murine aorta, with higher levels in the abdominal than in the thoracic aorta (2). Differences in expression patterns are also found between microvascular and macrovascular endothelial cells. Endothelial cells derived from microvascular beds express 100-fold more tissue-type plasminogen activator as compared with cells from macrovascular beds (3). The converse pattern is revealed in the expression of von Willebrand factor (vWF) (3). The differences in local hemostatic regulation likely represent only a fraction of endothelial cell heterogeneity. Diversity in activity may extend to the growth characteristics of endothelial cells. Endothelial cells derived from arterial, venous, and microcirculatory beds display notable differences in mitotic rates (4). In addition, endothelial cells from macro- versus microvascular beds exhibit differential growth responses to exogenous growth factor or hypoxic stimuli (5). The molecular basis of this endothelial cell diversity is unknown. This diversity may be due to differences in endothelial cell clonality, development, and/or environment.

Recently we described an example of environmentally mediated endothelial diversity with differential regulation of gene expression by the surrounding cells of various vascular beds. These studies demonstrated the presence of a communication between cardiac microvascular endothelial cells and the surrounding cardiac myocytes that regulates endothelial cell gene expression in vivo, ex vivo, and in vitro (6). Myocytes, but not other cell types, specifically induce endothelial expression of vWF, as well as expression of a transgene with the elements of the vWF promoter (vWFLacZ-2). Expression of both vWF and the transgene is restricted to a minority of the cardiac microvascular endothelial cells. This result suggested that there is a subpopulation of endothelial cells with unique receptor(s) and/or signaling pathway(s) that result in the expression of vWF and the transgene.

1. Abbreviations used in this paper: RT-PCR, reverse transcriptase polymerase chain reaction; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor.
Potential insight into this cardiac microvascular communication may be gained from both basic and clinical reports. Previous studies have demonstrated the importance of platelet-derived growth factor to the cardiac vasculature. Deletion or neutralization of either PDGF A (7), B (8, 9), or PDGF α receptor (PDGFRα) (10–12) but not the PDGF β receptor (PDGFRβ) (13), results in embryonic lethality with marked cardiac vascular abnormalities. Moreover, PDGF pathways have also been implicated in cardiovascular pathophysiology. Elevated PDGF isoforms have been associated with myocardial ischemia (14, 15) and allograft rejection (16). This relationship of PDGF A and B, and the PDGFRα to cardiac development and disease suggests that these isoforms and receptor may be involved in governing the activity of the cardiac vasculature.

Overall, the vascular system is an excellent model in which to examine the control of cellular diversity. The basis of the marked endothelial cell diversity is unknown but may result from communication pathways specific to the local beds. In the heart these pathways involve the extracellular tissues surrounding the cardiac microcirculation. This report attempts to understand the signals that govern this communication, and the results may yield insight into the basis of diversity and function, as well as potential local propensity to disease in the vasculature.

Methods

Cell culture. These studies used both wild-type FVB mice and vWF-LacZ-2 transgenic mice previously generated with a 2.1-kb 5' fragment of the human vWF promoter including the first exon fused to LacZ (6). Cardiac microvascular endothelial cells and myocytes were isolated, cultured, and characterized as previously described (17, 18). The endothelial cells were cultured alone or in the presence of the cardiac myocytes grown in 12-mm 0.4-mm transwell plates (Costar, Cambridge, MA). The cells were cultured for 72 h, with a media change at 48 h. The cultures were then washed with PBS 10 times. Rabbit polyclonal antibodies to vWF, VEGF, and Flk-1 by Western dot blotting with the following, 1:1,000. The plates were then developed with 1,2phenylenediamine, 0.67 g/L, and the absorbance at 490 nm. PDGF AA, sc-128 (Santa Cruz Biotechnology, Santa Cruz, CA), and B, 5’CATGAGCCATG-AGGTCCACCA3’; mouse VEGF, (forward): 5’GTGCTGCTGTGC-CTCTTTGGG3’; LacZ, (forward): 5’GCATCGAGGCTT-GGTAATAAGGGTGGACATTTCA3’, (reverse): 5’TCTCAGACACC- AAACAAACAC3’; mouse VEGF, (forward): 5’GGATACGATAG-ACTTCTGCTGCTGTGC3’; (reverse): 5’TCTCGGTCGTTG-CCTGCTTTTTCCTGG3’. Relative antigenic levels of PDGF isoforms were assayed by two-antibody sandwich enzyme–linked immunosassay, respectively described (21). A goat polyclonal antibody to PDGF A, AF-221-NA (R&D Systems), was used as the capture antibody (0.5 μg/mL) in Immunon 2 ELISA strips (Dynatech Laboratories, Chantilly, VA). Secreted samples (50 μl) from endothelial cells cultured alone or in the presence of cardiac myocytes were applied to the antibody-coated strips for 2 h at room temperature. The samples were then washed with PBS 10 times. Rabbit polyclonal antibodies to PDGF A, sc-128 (Santa Cruz Biotechnology, Santa Cruz, CA), and B, 5’-215 (Genzyme Diagnostics, Cambridge, MA) were then used as detection antibodies at a dilution of 1:1,000. A peroxidase-labeled donkey polyclonal antibody to rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) was used as a secondary antibody at a dilution of 1:1,000. The plates were then developed with 1.2 phenylenediamine, 0.67 g/L, and the absorbance at 490 nm. PDGF AA, AB, and BB at 10 μg/mL served as controls. Relative antigenic levels of vWF, VEGF, and Flk-1 by Western dot blotting with the following, respectively described and diluted in: 0.2 μg/mL (Dako, Carpinteria, CA), sc-152, 1:1,000 (Santa Cruz Biotechnology), and sc-315, 1:500 (Santa Cruz Biotechnology). A peroxidase-labeled donkey polyclonal antibody to rabbit IgG (Jackson ImmunoResearch Laboratories) was used as a secondary antibody at a dilution of 1:1,000. The blots were developed with a chemiluminescence system (ECL; Amer sham Corp., Arlington, IL). Relative β-galactosidase activities were determined by cleavage of ONPG (Sigma Chemical Co., St. Louis, MO). All protein analysis was performed in triplicate sets with a minimum of two independent experiments.

Immunostaining. Adult vWF/LacZ-2 hearts were harvested and 8-μm serial cryosections were obtained. Alternate sections were processed for detection of the transgene or the PDGFRα. vWF/LacZ-2 was determined by X-gal staining. Immunostaining for PDGFRα was performed with the primary and secondary antibodies, sc-338 (Santa Cruz Biotechnology) and Cy3-conjugated anti-rabbit IgG (Jackson ImmunoResearch), respectively. Photomicrographs of adjacent serial sections with X-gal and immunostaining, respectively, were superimposed. Cardiac microvascular endothelial cells and myocytes were cultured and fixed as previously described (6). The samples were immunostained for vWF or Flk-1, with above antibodies and contained with a goat antibody to PDGFRα (AF-307-NA; R&D Systems), and secondary antibody staining with FITC- and Cy3-conju-
gated anti–rabbit IgG (Jackson ImmunoResearch). Duel label photomicrographs were superimposed.

**In vivo neutralization.** PDGF AB neutralization in vivo was performed by the embryonic injection of antibody similar to the previously described injection into the murine decidua (7). Pregnant vWF-LacZ-2 mice (day 12.5) were anesthetized with avertin (2.5% vol/vol), and the uterus was exposed and individual amniotic sacs were injected with 2 μL antibody, anti-PDGF AB (AB-20-NA; R&D Systems), (n = 19), or nonimmune IgG (Jackson ImmunoResearch), (n = 21), at 1 μg/mL, through 100-mm diameter glass pipette. The lateral abdominal wall was sutured closed. The following morning, the embryonic hearts were harvested and stained with X-Gal. The hearts were scored for the presence of LacZ staining. The statistical significance was determined by a Student’s t test analysis.

**Results**

**PDGFRα subpopulation of endothelial cells.** The potential for PDGF to be involved in cardiac microvascular endothelial cell communication was tested. Histochemical studies of the cardiac microcirculation revealed that the expression of the transgene and PDGFRα were restricted to a subpopulation of microvascular endothelial cells. Representative X-Gal and immunostaining of alternate serial sections of an adult vWF-mice), (7) were injected with 2 μL antibody, anti-PDGF AB (AB-20-NA; R&D Systems), (n = 19), or nonimmune IgG (Jackson ImmunoResearch), (n = 21), at 1 μg/mL, through 100-mm diameter glass pipette. The lateral abdominal wall was sutured closed. The following morning, the embryonic hearts were harvested and stained with X-Gal. The hearts were scored for the presence of LacZ staining. The statistical significance was determined by a Student’s t test analysis.

**Cardiac myocyte induction of endothelial PDGF B.** The potential for PDGF to be involved in the induction of cardiac microvascular gene expression was explored in vitro. Cardiac microvascular endothelial cells were cultured alone or in the presence of cardiac myocytes grown in transwells. The cultures were then analyzed as outlined in Fig. 2a. To examine ligand production expression of the PDGF, isoforms were assessed in endothelial cells cultured alone as well as in the presence of cardiac myocytes. Fig. 2b reveals that cardiac microvascular endothelial cells constitutively expressed the PDGF A isoform, leading to the PDGF AA homodimer. However, when the endothelial cells were cultured in the presence of cardiac myocytes, they were induced to express PDGF B. Protein analysis of isoform dimer formation by ELISA revealed that PDGF AA is generated by the endothelial cells constitutively (Fig. 2c). However, in the presence of the cardiac myocytes, the PDGF AB heterodimer is generated.

**PDGF AB induction of vWF and vWFLacZ-2.** Cardiac myocytes induced increases in vWF (Fig. 2d) as previously demonstrated by ELISA (6). Similar increases in vWFLacZ-2 were also demonstrated. Both PDGF homodimers, as well as the heterodimer, are ligands for the PDGFRα (22). Addition of the PDGF AB heterodimer repressed the cardiac myocyte-mediated induction of vWF and the transgene (Fig. 2d). In contrast, the AA homodimer failed to reproduce the induction. Furthermore, the BB dimer is less than half as potent as the PDGF AB. Addition of neutralizing antibodies to PDGF AB blocked the myocyte-mediated induction, but had no significant effect on the constitutive levels when added to endothelial cells alone (Fig. 2d). The PDGF AB induction of vWF and vWFLacZ-2 was also revealed by RNA analysis (Fig. 2e). Immunostaining colocalized the PDGFRα with the induced vWF in cocultures of cardiac myocytes and endothelial cells, whereas PDGFRα-negative cells did not stain for vWF (Fig. 2f). Furthermore, inhibition of PDGFRα signaling by the expression of a dominant negative mutant PDGFRα blocked the communication induction of vWF by 95±9%.

**Discussion**

The diversity of cellular and tissue functions within organs requires that local communication circuits control distinct populations of cells. Regional signaling pathways may use relatively unique molecular interactions to regulate gene expression in specific cells, and thereby confer a local specialization of function. Endothelial cells, the ubiquitous cellular lining of the vascular system, exhibit such a marked functional heterogeneity in distinct vascular beds. The present study demonstrates that PDGF signaling mediates the extravascular tissue regulation of a subset of cardiac microvascular endothelial cells to induce the expression of genes involved in hemostasis and angiogenesis. **PDGF signaling mediates cardiac microvascular communication.** The potential role of PDGF signaling in the cardiac microvascular communication was studied by multiple approaches both in vitro as well as in vivo. Immunostaining demonstrated that PDGFRα colocalized with the induced vWF and its transgene in cardiac microvascular endothelial cells. RNA and protein analysis revealed that cardiac myocytes regulated the expression of PDGF isoforms, and the communication resulted in the generation of PDGF AB. These results are
in agreement with previous observations that simultaneous expression of both PDGF A and B isoforms result in the preferential production of the PDGF AB heterodimer (23). The heterodimer, in turn, induced increases in both vWF and its transgene in the PDGFRα-positive cardiac microvascular endothelial cells. Finally, neutralization of PDGF AB blocked the inductive communication both in vitro and in vivo. Reasons for the occasional absence of neutralization in vivo may be due to variations in the injection and diffusion of the antibody. These studies demonstrated that the cardiac microvascular communication is mediated by the interaction of PDGF AB and PDGFRα-positive endothelial cells. The role of the heterodimer mediation of this communication is in agreement with previous demonstrations of similar preferential activity of the heterodimer (24), as well as the requirement of PDGFRα for high-affinity heterodimer signal transduction (25). Overall,
when considered together, these results demonstrate that the PDGF AB–α receptor interaction may regulate the activity of a subset of endothelial cells in the cardiac microcirculation.

**PDGF induces endothelial genes critical to angiogenesis.** The significance of the cardiac microvascular PDGF-signaling pathway extends beyond the induction of vWF. Deficiencies in this protein confer no specific developmental or cardiac vascular abnormalities (26). However, vWF is elevated in microvascular foci of angiogenesis (27, 28). Moreover, the PDGFRα is also regulated in angiogenesis (29), and suggested that the PDGF AB–α receptor communication pathway may be involved in the regulation of gene(s) critical to cardiac vascular development. Previous reports have described the importance of PDGF A and B, and PDGFRα in the cardiac vasculature (7–12). PDGF β receptor knockout mice have no cardiac pathology (13), but they have increases in PDGFRα, which may result in an underestimation of the role of the β receptor in the cardiac vascular PDGF communication (13). Overall, these studies demonstrated that the cardiac vasculature requires intact PDGF signaling components. However, they did not elucidate the downstream pathways regulated by the critical communication.

The present studies link the PDGF AB–α receptor communication to the induction of other critical genes, as well as to the function of a subpopulation of endothelial cells yields a more complete understanding of the regulation of cardiac microvascular gene expression. The potential link of this communication pathway to other critical endothelial signaling pathways was suggested by recent knockout studies, which demonstrated that VEGF and Flk-1 are also critical for angiogenesis and normal cardiac vessel development. Mice having heterozygous deletions of the gene for VEGF die in utero with a failure of cardiac vascular angiogenesis (30, 31). Homozygous deletions of Flk-1 die at approximately the same time in

**Figure 3.** In vivo neutralization of PDGF AB. X-gal stained heart of 12.5d vWFLacZ-2 embryo injected with control antibody. X-gal stained heart of 12.5d vWFLacZ-2 embryo injected with PDGF AB neutralizing antibody. Percentage of hearts positive for X-gal following injection control antibody (n = 21) and PDGF neutralizing antibody (n = 19). P < 0.0001.

**Figure 4.** Cartoon of cardiac microvascular communication. PDGF B expression by cardiac microvascular endothelial cells is induced in the presence of cardiac myocytes. PDGF B dimerizes with PDGF A and in turn induces PDGFRα endothelial cells to express vWF, the vWFLacZ-2 transgene, VEGF, and Flk-1.
development as the VEGF knockout embryos and have similar deficits in cardiac angiogenesis (32). This requirement of both VEGF and Flk-1 for cardiac angiogenesis suggested that their expression may be critically controlled in the vasculature. This study revealed that the cardiac microvascular expression of both VEGF and Flk-1 are regulated by the PDGF AB-α receptor signaling pathway.

Model of cardiac microvascular communication. This present study integrates PDGF signals into a model of cardiac microvascular hemostatic and angiogenic regulation. The proposed model illustrates the components of the communication (Fig. 4). The physiological role of this PDGF AB-α receptor pathway may be due to the enhancement of its various components under conditions affecting cardiac myocytes and endothelial cell function. Recent studies have demonstrated increases in PDGF isoforms in response to, or as a potential component of, cardiac vascular pathophysiological conditions including myocardial ischemia (14) and allograft rejection (16). These states induce a neovascular response by the endothelial cells, which is in part mediated by increases in VEGF and Flk-1. The increases in downstream elements of the PDGF AB-α receptor pathway implicate a potential function of the communication in the adult heart. The PDGFRα colocalization with the transgene suggests that these microvascular endothelial cells constitute a subpopulation of endothelial cells available to respond to disease states.

Local control regulates the diversity of endothelial cell activity. A hallmark of a local communication scheme is the regional control of gene expression in a distinct population of cells. Previous studies have demonstrated significant variation in the endothelial responses to growth factors in different vascular beds (33, 34), including the expression of PDGF isoforms and receptors (35). This heterogeneity suggests that the proposed model of the regulation of endothelial gene expression may be relatively unique to the cardiac microvasculature. Other local regulatory systems, potentially including specific growth factor isoforms and receptors, may be critical to the communication pathways of other vascular beds influencing the expression of the same or other sets of genes.

Regulation of endothelial cell function is the basis of diversity in the vascular system. A subset of cells that are responsive to the signals from the surrounding tissue may constitute a potentially critical population of cells required to allow the endothelium to fulfill specific requirements in local beds. The present description of cardiac microvascular communication regulating genes involved in hemostasis and angiogenesis represents the first molecularly characterized local pathway in which the organ specifically controls gene expression in endothelial cells. This shall serve as a model for similar local regulatory mechanisms in other vascular beds.

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References


