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Pro-fibrogenic potential of PDGF-D in liver fibrosis☆

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***Background/Aims*: We analyzed the expression of platelet-derived growth factor D (PDGF-D) in an experimental bile duct-ligated (BDL) rat model and assessed its biological function in cultured hepatic stellate cells (HSC) and myofibroblasts (MFB).**

***Methods*: The mRNA for PDGF-A, -B, -C, -D and for PDGF receptor-α and -β chains (PDGFRα and PDGFRβ) in normal and fibrotic rat livers was assessed quantitatively. Protein levels of PDGF-D were quantified by immunoblotting and immunohistochemistry.**

***Results*: The relative mRNA expression of all PDGF isoforms and receptors upregulated upon BDL and PDGF-A, -B and -D expression was significantly higher than that of PDGF-C. PDGF-D and PDGFRβ protein also increased markedly. Immunostaining revealed that PDGF-D is localized along the fibrotic septa of the periportal- and perisinusoidal areas. Besides PDGF-B, PDGF-D is the second most potent PDGF isoform in PDGFRβ signaling within HSC/MFB, evidenced by PDGFRβ autophosphorylation and activation of the downstream signaling molecules ERK1/2-, JNK-, p38 MAPK, and PKB/Akt while PDGF-C effects were minimal. PDGF-D exerted mitogenic and fibrogenic effects in both cultured HSC and MFB comparable to PDGF-B but PDGF-A and -C showed only marginal fibrogenic effects.**

***Conclusions*: PDGF-D possesses potential pathogenetic properties for HSC activation and matrix remodeling in liver fibrosis.**

***Keywords*: BDL; Liver fibrosis; Hepatic stellate cell; Myofibroblast; PDGF; PDGF-B; PDGF-D; PDGFR; PDGFRα; PDGFRβ; sPDGFRβ**

1. Introduction

Platelet-derived growth factor (PDGF) represents a family of growth regulatory molecules consisting of PDGF-A and -B and the newly discovered PDGF-C and -D [1–4]. They signal through the cell membrane receptors PDGF receptor α (PDGFRα) and receptor β (PDGFRβ). Original members of the PDGF family are secreted as disulfide-bonded homo- or heterodimers (PDGF-AA, -AB, and -BB), whereas PDGF-C and -D

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Abbreviations: BDL, bile duct ligation; CUB, Complement subcomponents C1r/C1s, Urchin EGF-like protein and Bone morphogenic protein-1; ECM, extracellular matrix; ERK, extracellular signal-regulated kinase; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; GFD, growth factor domain; HSC, hepatic stellate cell(s); MAPK, mitogen-activated protein kinases; MFB, myofibroblast(s); PCR, polymerase chain reaction; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; PI3K, phosphatidylinositol-3-kinase; PKB/Akt, protein kinase B; sPDGFRβ, soluble PDGF receptor type β; TGF-β1, transforming growth factor-β1; TIMP, tissue inhibitors of metalloproteinases.

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are secreted as homodimers in latent forms consisting of an N-terminal Complement subcomponents C1r/C1s, Urchin EGF-like protein and Bone morphogenic protein-1 (CUB) domain before the conserved growth factor domain (GFD) and require extracellular proteolytic cleavage to release the active GFD [1–3]. The amino acid sequence of PDGF-D is closely related to that of PDGF-C (~50%) and to those of PDGF-A and PDGF-B (~25%). Functional differences between PDGF-C and -D are based on their binding properties to PDGF receptors. A recent report shows that PDGF-C binds to both PDGFRαα and PDGFRαβ [5], while PDGF-D binds to and activates PDGFRβ but not PDGFRα in cells expressing individual PDGFR [2,3]. In cells expressing both receptors, PDGF-D activates both, indicating that PDGFRα activation may result from PDGFRα/β heterodimerization [2].

Besides transforming growth factor type β (TGF-β), PDGF-B is considered a second major fibrotic cytokine involved in liver fibrogenesis and hepatic stellate cell (HSC) activation. PDGF-B, the most potent mitogen in culture-activated HSC [6], mediates early proliferative responses through HSC activation in animal models following bile duct ligation (BDL) [7]. PDGFRβ expression also upregulates in injured livers of CCl_4-treated animals, while the PDGF-α receptor remains unchanged [8]. Selective overexpression of PDGF-B in liver induces HSC proliferation and liver fibrosis in transgenic mice [9]. In clinical liver fibrosis, expression of PDGF-B and its receptor subunits shows strict correlation in the extent of necrotic inflammation and fibrotic damage [10]. Recent reports show significant upregulation of PDGF-C and -D in culture-activated HSC and, surprisingly, a rapid down-regulation of PDGF-B [11].

PDGF-D stimulates angiogenesis and extracellular matrix (ECM) deposition, thus playing a part in wound healing and tumorigenesis [12–14]. After induction of interstitial kidney fibrosis in mice, *de novo* expression of PDGF-B, -D and PDGFRβ was detected in interstitial cells. Upregulated PDGFRβ protein expression in tubulointerstitial fibrosis showed close spatial association with overexpressed PDGF-D, but lesser within PDGF-B. Similar results were observed in humans suffering from chronic renal nephropathy [15]. Of the PDGF ligand/receptor systems, PDGF-B, signaling through PDGFRβ, is an important mediator in initiation and progression of liver fibrosis [6,10]. Newly discovered isoforms PDGF-C and -D are reported to induce liver fibrosis [16,17], but these data are largely derived from forced transgenic or virally mediated overexpression. We here examined the endogenous expression of PDGF ligands and their receptors with emphasis on PDGF-C and -D in bile duct-ligated rats.

2. Materials and methods

2.1. Experimental in vivo liver fibrogenetic model

Utilized were 6–8-week-old male Sprague–Dawley rats in groups of five animals. Common bile ducts were double ligated and excised under anesthesia [18,19] and one group each was sacrificed after 2 days, 1 week and 2 weeks, respectively, while sham-operated rats served as controls. Liver specimens were fixed in 4% paraformaldehyde for histological examination or snap-frozen and stored at −80 °C for protein and RNA isolation. This experiment was approved by the local Review Board according to prevailing guidelines for scientific animal experimentation.

2.2. RNA isolation and RT-PCR

Liver total RNA was isolated by Guanidine Thiocyanate/CsCl method, digested with DNAase I and reverse transcribed (2 μg each) in a 20 μl volume using Superscript II reverse transcriptase (Invitrogen) and random hexamer primers. For integrity verification, 2 μl aliquots of cDNA samples were subjected to standard PCR for *GAPDH* (Acc. No. M32599).

2.3. Real-time quantitative PCR

cDNA derived from 25 ng RNA was amplified in 25 μl volume using qPCR Core Kits (Eurogentec). PCR conditions were 50 °C for 2 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Taqman primers and probes (Table 1) for amplification of the different PDGF isoforms and receptors [11,20] were designed from sequences deposited in the

Table 1
Primers and probes

Gene	Primer	Taqman probe
PDGF-A	for 5′-TTCTTGATCTGGCCCCCAT-3′ rev 5′-TTGACGCTGCTGGTGTTACAG-3′	5′-CAGTGCAGCGCTTCACCTCCACA-3′
PDGF-B	for 5′-GCAAGACGCGTACAGAGG TG-3′ rev 5′-GAAGTTGGCATTGGTGCGA-3′	5′-TCCAGATCTCGCGGAACCTCATCG-3′
PDGF-C	for 5′-CAGCAAGTTGCAGCTCTCCA-3′ rev 5′-GACAACTCTCTCATGCCGGG-3′	5′-CGACAAGGAGCAGAACGGAGTGCAA-3′
PDGF-D	for 5′-ATCGGGACACTTTTGCGACT-3′ rev 5′-GTGCCTGTCACCCGAATGTT-3′	5′-TTGCGCAATGCCAACCTCAGGAG-3′
PDGFRα	for 5′-GCCACGAAAGAGGTCAAGGA-3′ rev 5′-GCCTGATCTGGACGAAGCC-3′	5′-TGAAGACAGTCACCATTTCTGTTCACGAGAA-3′
PDGFRβ	for 5′-AATGACCACGGCGATGAGA-3′ rev 5′-TCTTCCAGTGTTTCCAGCAGC-3′	5′-CATCAACGTTACTGTGATCGAAAATGGCTATG-3′