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Review

Signals controlling the expression of PDGF

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Abstract

PDGF is an important polypeptide growth factor that plays an essential role during early vertebrate development and is associated with tissue repair and wound healing in the adult vertebrate. Moreover, PDGF is thought to play a role in a variety of pathological phenomena, such as cancer, fibrosis and atherosclerosis. PDGF is expressed as a dimer of A and/or B chains, the precursors of which are encoded by two single copy genes. Although the PDGF genes are expressed coordinately in a number of cell types, they are independently expressed in a majority of cell types. The expression of either PDGF gene can be affected by very diverse extracellular stimuli and the type of response is dependent on the cell type that is exposed to the stimulus. Expression of the PDGF chains can be modulated at every imaginable level: by regulating accessibility of the transcription start site, by varying the transcription initiation rate, by using alternative transcription start sites, by alternative splicing, by using alternative polyadenylation signals, by varying mRNA decay rates, by regulating efficiency of translation, by protein modification, and by regulating secretion. Even upon secretion, the activity of PDGF can be modulated by non-specific or specific PDGF- binding proteins. This review provides an overview of the cell types in which the PDGF genes are expressed, of the factors that are known to affect the expression of PDGF, and of the various levels at which the expression of PDGF genes can be regulated.

Abbreviations: aFGF – acidic fibroblast growth factor; BAECs – bovine aortic endothelial cells; bFGF – basic fibroblast growth factor; BHK – baby hamster kidney; DAG – diacylglycerol, DH – DNase-I-hypersensitive; ECGF – endothelial cells growth factor; EGF – epidermal growth factor; ER – endoplasmic reticulum; HKMECs – human kidney microvascular endothelial cells; HMECs – human mammary epithelial cells; HTLV – human T-cell leukemia virus; HUVECs – human umbilical vein endothelial cells; IFN – interferon; IL – interleukin; IPF – ideopathic pulmonary fibrosis; LDL – low density lipoprotein; LPS – lipopolysaccharides; PDGF – platelet-derived growth factor; PE – phenylephrine; PKC – protein kinase C; PNS – peripheral nervous system; RPE – cells, retinal pigment epithelial cells; SHS – nuclease S1 hypersensitive site; SMCs – smooth muscle cells; SPARC – secreted protein, acidic and rich in cystein; SRE – serum response element; SRF – serum response factor; SSV – simian sarcoma virus; TGF – transforming growth factor; TNF – tumor necrosis factor; TPA – 12-*O*-tetradecanoylphorbol 13-actetate.

Introduction

Platelet-derived growth factor (PDGF) is an important polypeptide growth factor to which a variety of physiological and pathological roles have been attributed. Expression of PDGF is regulated in a cell type- and developmental-stage-specific manner and is subject to modulation by diverse extracellular stimuli. In a given cell type, regulation of PDGF expression may occur at the transcriptional level, the mRNA processing level,

the translational level and/or the posttranslational level. This review intends to give an overview of the cell types that express PDGF, the factors that affect PDGF expression and the mechanisms that are involved in regulation of PDGF expression.

Platelet-derived growth factor

PDGF is first of all known for its activity as a serum mitogen for cultured smooth muscle cells (SMCs) [1], fibroblasts [2] and glial cells [3] and, secondly, as a chemo-attractant for SMCs [4] and fibroblasts [5]. In addition, it acts as a potent vasoconstrictor on rat aortic strips [6] and as a survival factor for cultured oligodendrocytes and their precursors [7]. Whereas these activities seem to primarily serve normal physiological processes, such as early development and wound healing, aberrant expression of PDGF may also play a role in the etiology of atherosclerosis (reviewed in [8]), fibrosis, neoplasia (reviewed in [9]) and desmoplasia (reviewed in [10]).

PDGF is a collective term for the three dimeric disulfide bond-linked proteins that can be constituted out of type A and/or type B polypeptide chains (reviewed in [11]). The responsiveness of cells to PDGF is mediated by two specific receptors: the α receptor binds PDGF AA, AB and BB with high affinity, whereas the β receptor binds BB with high affinity and AB with low affinity and has negligible affinity for AA homodimers [12, 13]. The receptor specificity of the PDGF A and B chains is determined by very subtle structural differences. The change of only a few amino acids is sufficient to confer PDGF β receptor binding specificity to the PDGF A chain [14, 15]. Binding of PDGF to its receptor induces dimerization and tyrosine-specific phosphorylation of the receptor [16–21], which initiates a signal transduction cascade and ultimately confers specific phenotypical changes to the responsive cell (reviewed in [10, 22]) . PDGF α and β receptor cDNA clones have been isolated [23–26] and are currently being used to unravel the mechanistic pathways that are triggered by PDGF (reviewed in [22, 27, 28]). Whereas all three dimeric forms of PDGF are mitogenic and chemotactic [29, 30], only B chain homodimers transform NIH 3T3 fibroblasts with high efficiency [31–34]. This seems to be caused by differences in the signal transduction cascade that is initiated by α and β receptors, as activation of the catalytic domain of the β receptor in NIH 3T3 cells leads to 17-fold higher transforming activity than is achieved by activation of the corresponding α receptor domain [35].

PDGF genes and proteins

PDGF was originally purified from human platelets and characterized as a cationic 30-kDa protein, consisting of 14-kDa and 17-kDa disulfide-linked polypeptide chains [36, 37]. Elucidation of their amino acid sequence revealed that it consists of two homologous polypeptide chains, the A and B chains [38]. The discovery that the PDGF B chain is the human homologue of the transforming protein $p28^{v\text{-}sis}$ of simian sarcoma virus (SSV) [39–41] enabled the rapid isolation of the human c-*sis*/PDGF B gene, a single copy gene located on chromosome 22 [42–46]. The complete structure of the human PDGF B gene transcription unit was determined by cDNA cloning, nuclease S1 mapping and primer extension. Seven exons spanning approximately 22 kb give rise to a 3.5-kb mRNA, a large part of which consists of nontranslated sequences. A 1022-bases long leader and a 1625-bases long trailer flank a 723-bases long open reading frame. The location of the transcriptional promoter was confirmed by the presence of a consensus TATA box sequence at 30 bp upstream of the transcription start site [47, 48] and by demonstrating its activity in a transient reporter gene assay [49, 50]. The first human PDGF A cDNA clone was isolated much later than its B chain counterpart [51] and shortly thereafter the PDGF A chain gene was isolated and characterized. It is a single copy gene located on chromosome 7 [51] and, similar to the B chain gene, contains seven exons spanning approximately 22 kb [52, 53]. The transcription start site was localized at approximately 36 bp downstream of a TATA box by nuclease S1 and primer extension mapping [53, 54]. The promoter was shown to be active in an *in vitro* transcription assay and a transient reporter gene assay [54].

The availability of PDGF A and B cDNA clones enabled a detailed analysis of the structure/function relationship and of the processing of the corresponding polypeptides (reviewed in [11, 55]). Whereas the B chain has higher transforming activity, the A chain is much more efficiently secreted [34]. PDGF A/B chimeras were used to map a membrane retention signal to amino acids 212–226 of the B chain. An analogous region is present in the A chain, but normally subject to proteolytic cleavage [56, 57]. PDGF B residues 105–144 are responsible for β receptor inter-

action [56]. Newly synthesized PDGF A and B chain precursors dimerize and undergo N-glycosylation and proteolytic processing in the endoplasmic reticulum (ER) and Golgi system. 30-kDa PDGF AA, AB and BB dimers are secreted via exocytosis, but the major part of PDGF BB is generated by additional proteolysis in the Golgi system and is expressed as a 24-kDa BB homodimer that remains membrane associated or is degraded in lysosomes [58–62]. As PDGF was also detected in the cell nucleus, it may act via hitherto unknown mechanisms [63, 64].

Sites of expression of PDGF

PDGF is expressed in a broad but specific subset of cell types and in a developmental stage-specific manner. The current idea is that its major function is in wound healing (reviewed in [65, 66]) and early development. Upon deregulation it may play a role in the etiology of certain cancers and of atherosclerosis. Several normal cell types express PDGF *in culture and/or in situ* (most of them are listed in Table 1). In nearly every cell type the expression of PDGF can be modulated by extracellular stimuli, which allows the cell to adapt the production of PDGF to changes in its environment.

Endothelial cells

Cultured vascular endothelial cells were among the first normal cells found to secrete PDGF [67] and express PDGF A and B mRNA [87–89]. As they lack PDGF receptors, expression of PDGF has been thought to act in a paracrine way on connective tissue cells during development, maintenance and repair of the vasculature. The discovery that endothelial cells from microvessels express PDGF receptors in culture and *in situ* led to the hypothesis that PDGF may also have an angiogenic function [90–92]. Co-expression of PDGF B and PDGF β receptor was also observed in proliferating vascular endothelial cells of hyperplastic capillaries within gliomas *in situ*, which suggests an autocrine role in vascularization of tumors [93–95]. Recently, PDGF A and B and PDGF α receptor were also detected in endothelial cells of injured large blood vessels *in situ* [96]. Thus, the capacity of autocrine growth stimulation via PDGF receptors may be a general property of vascular endothelial cells.

Several factors have been shown to affect expression of PDGF in endothelial cells (summarized in Table 2). Freshly isolated human umbilical vein endothelial cells (HUVECs) and bovine aortic endothelial cells (BAECs) express PDGF B mRNA, but the expression level increases strongly upon proliferation *in vitro* [87]. Vice versa, withdrawal of endothelial cell growth factor (ECGF) from the culture medium of HUVECs inhibits their proliferation and triggers their organization into tubular structures, which is accompanied by a strong decrease in PDGF B mRNA content [97]. PDGF A and B mRNA levels

Table 1. Normal human cell types that are capable of PDGF expression

Cell type	PDGF	References
Vascular endothelial cells	A, B	[67]
Vascular smooth muscle cells	A, B	[68]
Megakaryocytes/platelets	A, B	[69, 70]
Monocytes, macrophages	A, B	[71, 72]
Placental cytotrophoblasts	A, B	[73]
Oocyte, blastocyst	A	[74, 75]
Kidney mesangial cells	A, B	[76]
Fibroblasts	A, B	[77]
Neurons	A, B	[78]
Astrocytes	A	[79–81]
Skeletal myoblasts	A	[82]
Uterus endometrium/myometrium	A, B	[83, 84]
Mammary epithelial cells	A, B	[85]
Retinal pigment epithelial cells	A, B	[86]

Table 2. Overview of factors that modulate expression of PDGF in vascular endothelial cells

Factor	Effect	References
Culturing	PDGF B ↑	[87]
Differentiation	PDGF B ↓	[97]
ECGF	PDGF A/B ↑	[98]
Thrombin	PDGF B ↑	[99–103]
TGFβ	PDGF A/B ↑	[99–103]
Forskolin (cAMP ↑)	PDGF A/B ↓	[100, 101, 103]
LDL	PDGF ↓	[104–106]
TNF	PDGF ↑	[107, 108]
Bacterial endotoxin	PDGF ↑	[109]
LPS	PDGF ↑	[110]
IL-1, IL-6	PDGF ↑	[108, 110, 111]
bFGF	PDGF ↓	[112]
aFGF	PDGF A ↑	[111]
IFN	PDGF ↓	[108, 110]
Hypoxia	PDGF B ↑	[113]
H_2O_2	PDGF B ↑	[114]
Shear stress	PDGF A/B ↑	[115, 116]

are differentially affected by ECGF [98]. Thrombin and transforming growth factor-β (TGF-β) stimulate expression of PDGF by vascular endothelial cells, but apparently through different mechanisms. Thrombin may stimulate PDGF secretion at the posttranslational level [99]. In addition, both factors increase the transcription rate of the PDGF B gene, but do not affect the PDGF B mRNA half-life (70–90 min in human kidney microvascular endothelial cells (HKMECs)) [100–103]. TGF-β also stimulates transcription of the PDGF A gene [100, 101, 103]. The effect of thrombin is mimicked by phorbol ester suggesting that it acts through protein kinase C (PKC) [117], whereas TGF-β acts independently of PKC [102]. Elevated cAMP levels (induced by forskolin) block the expression of PDGF A and B mRNA and inhibit the effect of thrombin and TGF-β on PDGF B mRNA expression, but not of TGF-β on PDGF A mRNA induction [100, 101, 103]. The thrombin-mediated increase in PDGF production by human aortic endothelial cells was shown to be dependent on Na^+–H^+-exchange and on G proteins, whereas the thrombin-mediated increase in PDGF A and B mRNA levels appeared to be independent of Na^+-H^+-exchange [118]. The thrombin stimulated production of PDGF is inhibited at the transcriptional level by 3-deazaadenosine, which possibly acts via inhibition of S-adenosylhomocysteine hydrolase. This may result in accumulation of S-adenosyl homocysteine, a potent inhibitor of certain transmethylation reactions [119]. Oxidized low density lipoprotein (LDL) and fish oil suppress the production of PDGF-like proteins by vascular endothelial cells, probably at the posttranscriptional level [104–106].

Several other factors were reported to stimulate PDGF mRNA and/or protein expression, such as tumor necrosis factor (TNF) [107, 108], bacterial endotoxin [109], lipopolysaccharides (LPS) [110], interleukin-1 (IL–1) [108, 110] and IL–6 [108, 111]. Basic fibroblast growth factor (bFGF) reversibly downregulates expression of PDGF B mRNA and secretion of PDGF by HUVECs [112]. Acidic fibroblast growth factor (aFGF) transiently stimulates PDGF A gene transcription and PDGF secretion, but does not affect the B gene [111]. The half-life of PDGF A and B mRNA in quiescent HUVECs (2.4 and 1.8 h, respectively) is not affected by aFGF [120]. Interferon-gamma (IFN-γ) suppresses PDGF A and B mRNA levels and release of PDGF-like proteins in vascular endothelial cells [108, 110]. Hypoxic conditions (0–3% oxygen environment) reversibly stimulate PDGF B gene transcription in cultured HUVECs [113]. H_2O_2 induces fourfold increased PDGF B mRNA levels in cultured bovine pulmonary artery endothelial cells [114]. Finally, physiological shear stress was reported to stimulate PDGF A and B mRNA levels in cultured endothelial cells, possibly through PKC [115, 116]. In contrast, Mitsumata et al. [121] found that the PDGF B mRNA level was induced by shear stress via a PKC independent mechanism, whereas the PDGF A mRNA level was not affected. Stimulation of PDGF B by shear stress is at the transcriptional level and a shear-stress-responsive element was mapped in the PDGF B gene promoter by reporter gene analysis and gel shift assays [122]. Conflicting data were published by other investigators, which showed that in BAECs shear stress reduced PDGF B mRNA levels by 4-fold [123].

Smooth muscle cells

Expression of PDGF by vascular smooth muscle cells (SMCs) is developmentally regulated. Cultured aortic SMCs from rat pups secrete at least 60-fold more PDGF than SMCs from adult rats [68, 124]. Prolonged culture triggers the adult rat cells to change from a contractile to a synthetic phenotype, which is accompanied by the expression of PDGF [125]. SMCs derived from injured rat arteries also show a synthetic phenotype [126]. PDGF A and B mRNA are expressed at similar low levels in intact aortas from newborn and adult rats. Upon culturing, PDGF B mRNA accumulates in passaged newborn but not adult rat SMCs, whereas PDGF A mRNA is found in comparable amounts in cells from both groups. PDGF B mRNA in newborn rat cells is shortlived, whereas PDGF A mRNA is more stable [82, 124, 127]. Cultured adult rat SMCs also express PDGF receptors and secrete PDGF upon exposure to PDGF itself [127]. Thus, PDGF may act both in an autocrine and a paracrine way on SMCs. Normal blood vessel walls have only a low cell turnover. Therefore, it was suggested that PDGF A and B mRNA, which are present *in situ* in the tunica media and the tunica adventitia, respectively, play a nonmitogenic maintenance role in the normal vessel wall [128].

Expression of PDGF in SMCs is also associated with pathological conditions. PDGF and PDGF receptor are expressed at specific times and places in injured carotid artery, which suggests a role in regulating arterial wound repair [129]. PDGF expression levels were found to be elevated in atheroma and atherosclerotic plaques compared with normal arteries, which suggests that PDGF may play a paracrine or autocrine

Table 3. Overview of factors that modulate expression of PDGF in vascular smooth muscle cells

Factor	Effect	References
Age	PDGF ↓	[68, 124]
Culturing	PDGF B ↑	[82, 124, 127]
PDGF	PDGF ↑	[127]
Angiotensin II	PDGF A ↑	[135]
TGF-β	PDGF A ↑	[136]
Uric acid	PDGF A ↑	[137]
Thrombin-α	PDGF A ↑	[138, 139]
Dexamethasone	PDGF A ↓	[140]
Serum	PDGF A ↑	[141]
Phorbol ester	PDGF A ↑	[141]
aFGF	PDGF A ↑	[141]
TNFα	PDGF A ↑	[141]
LDL	PDGF A ↑	[142, 143]
Phenylephrine	PDGF A/B ↑	[144]

role in the etiology of atherosclerosis [130–132]. However, a direct causal role of PDGF in atherosclerosis has thusfar never been proven. Within the plaques, PDGF A mRNA expression correlates with SMCs, whereas PDGF B mRNA and protein are predominantly expressed in macrophages [128, 133]. Elevated levels of PDGF A mRNA and of the transcription factor Sp1, a putative activator of the PDGF A gene, were found in SMC's of spontaneously hypertensive rats compared with normotensive control rats, suggesting that high blood pressure-induced hypertensive vascular hypertrophy is mediated by an autocrine PDGF loop [134].

Analogous to vascular endothelial cells, PDGF expression in SMC's is also affected by diverse factors (summarized in Table 3). Angiotensin II induces PDGF A mRNA and PDGF protein expression in cultured quiescent rat aortic SMC's [135]. Angiotensin II was demonstrated to specifically induce expression of an alternatively spliced PDGF A mRNA species in cultured rabbit aortic SMC's [145]. TGF-β, uric acid and thrombin-α promote the proliferation of vascular SMCs via induction of PDGF A mRNA and protein expression, thus creating an autocrine loop [136–139]. Dexamethasone inhibits the thrombin-induced induction of PDGF A secretion and the constitutive expression of PDGF A at the transcriptional level in SMCs [140]. PDGF A mRNA levels in cultured human vascular SMC's are also stimulated by serum, phorbol ester, aFGF, TNFα [141], oxidized LDL [142] and the α-adrenergic agonist phenylephrine (PE) [143]. PE also induces a transient 10-fold increase in PDGF B mRNA content in rat thoracic aorta [144]. Similar to vascular endothelial cells, vascular SMCs respond to mechanical strain by production of growth factors among which PDGF AA and BB. In addition, the strain induces increased proliferation of the SMCs, which can be partly neutralized by anti-PDGF antibodies. Thus strain-induced proliferation may act via an autocrine PDGF loop [144].

Blood cells

Except in the cells of the vascular walls, PDGF is also present in several types of blood cells, in accordance with its putative role in wound healing. PDGF is stored within α-granules of platelets, from which it is released during the clotting process [69]. PDGF from human platelets consists of AA, AB and BB dimers [146–148], whereas PDGF from porcine platelets consists solely of BB homodimers [149]. Blood platelets are small cytoplasmic particles that lack a transcriptional machinery. The PDGF that they contain is synthesized in bone marrow megakaryocytes, from which the platelets are derived. Indeed, PDGF was detected by immunofluorescence in cultured human megakaryocytic colonies [70] and PDGF B mRNA in isolated human bone marrow megakaryocytes [150]. Megakaryocytes form only a minor population of the bone marrow cells and therefore have been difficult to study. Phorbol ester treatment of the human hematopoietic stem cell line K562 was found to induce megakaryoblastic differentiation, which is accompanied by PDGF A and B mRNA expression and secretion of PDGF. The induction of PDGF B gene expression by phorbol ester is regulated at the transcriptional level [151]. At present, phorbol ester treated K562 cells are generally accepted as a model for bone marrow megakaryocytes [152]. PDGF B but not PDGF A mRNA expression is also induced by sodium butyrate mediated erythroid differentiation of K562 cells [153] and by phorbol ester mediated megakaryocytic differentiation of human erythroleukemia cell line HEL [154].

PDGF A and B mRNA and PDGF protein are present in cultured human alveolar and peritoneal macrophages and in *in vitro* maturated or activated human monocytes [71, 72, 155, 156]. Human blood monocytes were found to secrete a PDGF-like mitogen resembling a monomer [157]. Human wound fluid contains PDGF-like peptides of 16–17 kDa and 34–36 kDa [158], which may thus correspond with monomer-

ic and dimeric forms of PDGF. Oxidized LDL inhibits constitutive expression of PDGF B mRNA and protein by adherent cultures of human blood monocyte-derived macrophages. Oxidized LDL also attenuates LPS-induced PDGF B mRNA expression [159]. Enhanced expression of PDGF by macrophages was also found in association with pathological conditions. E.g., alveolar macrophages from patients with idiopathic pulmonary fibrosis (IPF) release four times more PDGF [160] and express threefold higher PDGF A and B mRNA levels than alveolar macrophages derived from healthy persons [161]. Expression of PDGF B mRNA and of PDGF was also detected *in situ* in epithelial cells and macrophages of lungs from patients with IPF, where it may play a role in the abnormal fibroblast proliferation and collagen production [162]. Transcription of the PDGF B gene in bronchoalveolar mononuclear cells from patients with pulmonary sarcoidosis [163] or with autoimmune diseases with lung involvement is enhanced compared with cells derived from healthy persons [164]. *In vitro* maturation of monocytes to macrophages is accompanied by increased transcription rates of the PDGF A and B genes. This results in increased PDGF B mRNA and protein but does not affect PDGF A mRNA levels, suggesting that PDGF A expression is regulated at the posttranscriptional level. Similarly, alveolar macrophages transcribe the PDGF A and B genes at similar rates while the steady state PDGF B mRNA and protein levels are 5-fold higher than those of the PDGF A chain. Whereas resting monocytes express only the short PDGF A mRNA that lacks the exon 6-derived sequences, *in vitro* maturated monocytes and alveolar macrophages express both the short and the long PDGF A mRNA species [165].

Analogous to the megakaryocytic differentiation of K562 cells, some leukemia cell lines can be induced to differentiate along the monocyte/macrophage pathway. In addition, various factors have been shown to affect PDGF mRNA and protein expression in these cell lines (summarized in Table 4). Phorbol ester induces monocytic differentiation of human myeloid leukemia cell lines HL-60 and U-937, which is accompanied by expression of PDGF A and B mRNA and synthesis and secretion of PDGF-like proteins. In contrast, PDGF A and B mRNA are not expressed in untreated HL-60 cells or in HL-60 cells induced to differentiate along the granulocyte pathway [153, 166, 167]. Phorbol ester treatment of THP-1 monocytic leukemia cells induces only PDGF A mRNA expression. Cycloheximide induces PDGF B mRNA expression in monocytes, but whether the PDGF B gene is transcriptionally active in resting monocytes was not examined [167]. IFN-γ mediated monocytic differentiation of HL-60 cells is not accompanied by PDGF B mRNA expression [173]. IFN-γ suppresses PDGF mRNA and protein production by phorbol ester activated THP-1 cells, but does not affect PDGF mRNA stability. IFN-γ also inhibits PDGF mRNA expression in monocyte-derived macrophages cultured in the presence of GM-CSF [174]. TNF-α and dexamethasone induce expression of, respectively, PDGF A and B mRNA in HL-60 cells [153, 168]. TGF-β stimulates PDGF A mRNA expression in U-937 and T-cell leukemia MOLT-4 cells and also induces PDGF A mRNA expression in HL-60 and Jurkat T-cell leukemia cells. TGF-β mediated induction is stable in U-937 and transient in HL-60 cells and is accompanied by secretion of PDGF-like protein [169]. Hypoxic macrophages were recently shown to synthesize and secrete PDGF, which could subsequently stimulate the proliferation of hypoxic endothelial cells, thus contributing to angiogenesis under hypoxic conditions [170].

Although PDGF is not expressed in normal lymphocytes, HTLV-infected lymphocytes were shown to express PDGF A and B mRNA [171, 172]. Recently, undifferentiated murine erythroleukemia cells were found to express PDGF protein and induction of erythroid differentiation was accompanied by more than 15-fold increased PDGF expression levels. A similar increase of PDGF expression levels was found in the mouse spleen *in vivo* upon stimulation of normal erythropoiesis by phenylhydrazine or erythropoietin [175].

Early development

In the adult vertebrate, expression of PDGF is closely associated with tissue maintenance and repair. Another major role for PDGF is in early vertebrate development, already from the earliest stages onwards. PDGF A mRNA and protein are expressed in mouse blastocysts and the mRNA is expressed as a maternal transcript in unfertilized ovulated oocytes [74] and in Xenopus eggs [75]. PDGF A mRNA is also detectable in bovine preimplantation embryos [176], where PDGF stimulates development of bovine embryos during the fourth cell cycle [177]. By using inhibitors of the PDGF signalling pathway, it was recently shown that PDGF plays a direct role in gastrulation of sea urchin embryos [178] and Xenopus lae-

Table 4. Factors that modulate expression of PDGF in blood cells

Cell type	Factor	Effect	References
K562 erythroleukemia	TPA	PDGF A/B ↑	[151]
K562 erythroleukemia	sodium butyrate	PDGF B ↑	[153]
HEL erythroleukemia	TPA	PDGF B ↑	[154]
Macrophages	LDL	PDGF B ↓	[159]
Monocytes	maturation	PDGF A/B ↑	[165]
HL-60 myeloid leukemia	phorbol ester	PDGF A/B ↑	[153, 166, 167]
U-937 myeloid leukemia	phorbol ester	PDGF A/B ↑	[153, 166, 167]
THP-1 monocytic leukemia	phorbol ester	PDGF A↑	[167]
Monocytes	cycloheximide	PDGF B ↑	[167]
HL-60 myeloid leukemia	TNF-α	PDGF A ↑	[153]
HL-60 myeloid leukemia	dexamethasone	PDGF B ↑	[168]
U-937 myeloid leukemia	TGFβ	PDGF A ↑	[169]
MOLT-4 T-cell leukemia	TGFβ	PDGF A ↑	[169]
HL-60 myeloid leukemia	TGFβ	PDGF A ↑	[169]
Jurkat T-cell leukemia	TGFβ	PDGF A ↑	[169]
Macrophages	hypoxia	PDGF ↑	[170]
Lymphocytes	HTLV	PDGF A/B ↑	[171, 172]

vis embryos [179]. Additional evidence for a role for PDGF in early Xenopus development was provided by *in situ* hybridization studies, which showed expression of PDGF A mRNA in neural ectoderm, otic vesicle and pharyngeal endoderm, and of PDGF α receptor mRNA in neural crest cells [180]. PDGF A and α receptors are coexpressed in preimplantation mouse embryos and, thus, may play an autocrine role [181]. Following implantation, PDGF A is mainly expressed in the epithelium, whereas the receptor is expressed in the mesenchyme, which suggests a paracrine activity across cell layers [181, 182]. PDGF is also expressed at later stages of vertebrate development. Early studies already demonstrated expression of PDGF B mRNA during embryonic and fetal development of the mouse [183]. PDGF A but not B mRNA is expressed in 6.5–8.5 day mouse embryonic and postembryonic tissues and both α and β receptor mRNA are expressed in early mouse embryos [184]. In day 16 embryonic rat, PDGF and its receptor can be detected in central and peripheral nervous system, gastrointestinal and gastrourinary system, respiratory tract, muscle and cartilage. In day 18 embryonic rat, the expression peaks and is detectable in almost all organs, whereas after day 18 expression is especially seen in stomach, kidney, liver, pancreas and sex cords [185]. Recently, expression of the PDGF β receptor was examined *in situ* during organogenesis in the mouse embryo. The PDGF β receptor was first detected in the periaortic mesenchyme and, later, particularly in derivatives of the primitive gut. Expression was exceptionally high in mesenchyme directly supporting epithelia of many organs, such as trachea and intestine. High expression levels were also detected in endothelium of small blood vessels and in vascular structures, such as hyaloid plexus and choroid plexus. In large blood vessels, the receptor was expressed in mesenchyme surrounding the epithelium [186]. The importance of PDGF B and the PDGF β receptor in mouse embryonic development has recently been proven by the analysis of null-mutant mice [187, 188]. PDGF B null mutants die perinatally from fatal bleedings and suffer from erythoblastosis, macrocytic anemia and thrombocytopenia. The bleedings may result from deficiencies in blood clotting, reduced platelet function or lack of function and integrity of the vascular wall. The mutant mice lack kidney mesangial cells and glomerular tufts. The heart and some large arteries are dilated, possibly due to the lack of vasoconstrictive activity of PDGF [187]. The PDGF β receptor null mutant mice exhibit the same blood and kidney deficiencies as were found in the PDGF B null mutants and also die perinatally from fatal bleedings. Their major blood vessels and heart appear normal, possibly because the role of the PDGF β receptor can be partially compensated by the α receptor. Since the clotting capacity of the blood was not affected, the fatal bleeding may result from defects in the microvasculature [188].

PDGF is also expressed during early development in humans. PDGF B and β receptor are detectable immunocytochemically during the development of glomeruli in human kidneys of 54 to 105 days gestational age. During early glomerulogenesis, PDGF B expressed in the epithelium may act in a paracrine way, whereas later PDGF B and the β receptor are coexpressed in mesangial cells and, thus, may play an autocrine role [189]. Normal human fetal kidney cells secrete PDGF and express PDGF A mRNA [190].

A role for PDGF in embryonic development has also been studied in tumor cell models. Murine and human embryonal carcinoma cell lines were found to express PDGF A mRNA and secrete PDGF [184, 191–193]. TGF-β induces PDGF B mRNA and PDGF secretion in mouse embryo-derived AKR-2B cells, which may play a role in an autocrine loop [194]. Human teratocarcinoma stem cell line Tera-2 expresses high levels of PDGF A but not B mRNA and the PDGF A mRNA level is markedly reduced upon retinoic acid mediated endodermal or neuroectodermal differentiation [195]. Undifferentiated murine teratocarcinoma cell line F9 also expresses PDGF A mRNA and protein and much lower levels of PDGF B mRNA. Exposure of F9 cells to retinoic acid induces differentiation to endoderm-like cells, which is accompanied by cessation of PDGF A production, but not of PDGF A mRNA expression. Whereas in undifferentiated F9 cells the PDGF A mRNA is present in polyribosomes, in differentiated cells the mRNA colocalizes with the free ribosome fraction, which indicates that the PDGF A mRNA is not translated in differentiated cells [196].

A role for PDGF in placental development was proposed upon the discovery that PDGF B mRNA is expressed in human placental cytotrophoblasts *in situ*. As cytotrophoblasts also express PDGF receptors, an autocrine mechanism in placental development was suggested [73, 197]. Expression of PDGF by the placenta is under apparent developmental control, with a peak during the second trimester [73, 198]. Coexpression of PDGF B and β type receptor was also observed in microcapillary endothelial cells of first trimester human placentae, suggesting that an autocrine loop may play a role in placental angiogenesis as well [199].

Kidney mesangial cells

Cultured human glomerular mesangial cells release PDGF and express PDGF A and B mRNA. They also respond mitogenically to PDGF suggesting an autocrine role in mesangial cell proliferation [76, 200, 201]. Moreover, PDGF AB and BB induce synthesis of PDGF A and B mRNA in mesangial cells [202]. A normal monkey kidney cell line secretes PDGF and expresses PDGF B mRNA, which is stimulated by TGF-β and ADP [203]. PDGF B mRNA is also expressed in baby hamster kidney (BHK) cells upon transfection with foreign DNA [204]. Thrombin [205], phosphatidic acid [206] and endothelin [207] stimulate expression of PDGF mRNA and phorbol ester stimulates PDGF A gene transcription in human mesangial cells [208]. The thrombin-mediated induction of PDGF B gene expression in mesangial cells was recently shown to be dependent on PKC α [209]. Several factors that are mitogenic for mesangial cells, such as serum, PDGF, EGF, TGF-α, bFGF and TNF-α stimulate PDGF mRNA expression. TGF-β also induces PDGF mRNA expression, but inhibits proliferation of mesangial cells [210, 211]. In a rat model of mesangial proliferative glomerulonephritis, PDGF B mRNA and protein are expressed *in situ*. Moreover, PDGF A and B mRNA levels are increased in glomerular RNA, which correlates with mesangial cell proliferation. In addition, PDGF β receptor mRNA and protein are increased in glomerular nephritis [212, 213]. Recently, rat visceral glomerular epithelial cells were also shown to express PDGF B mRNA and protein in culture and *in situ*, but PDGF β receptor mRNA or protein could not be detected [214].

Fibroblasts

Normally, PDGF mRNA and protein are not expressed in cultured human dermal fibroblasts [87, 89]. However, transient expression of PDGF A mRNA and protein occurs when cultured dermal fibroblasts are exposed to PDGF, EGF [77], TGF-β [215, 216], TNF [217] or IL-1 [218]. It was suggested that these factors stimulate proliferation of fibroblasts through autocrine production of PDGF A. Recently it was shown that the PDGF B induced PDGF A production is reduced in fibroblasts from old donors compared with fibroblasts derived from embryonic and neonatal donors, suggesting developmental stage-specific regulation of PDGF or PDGF receptor expression [219]. Following cutaneous injury in pigs, PDGF B and PDGF β receptor mRNA and protein are reversibly coexpressed *in situ* in skin epithelial cells and in fibroblasts, whereas PDGF is not detectably expressed in unwounded

skin *in situ* [220]. This suggests that expression of PDGF in fibroblasts plays a role in wound healing *in vivo*. PDGF A mRNA and/or protein expression in fibroblasts was also observed under certain pathological conditions, e.g. in a strain of Hutchinson- Gilford (progeria) syndrome fibroblasts [221] and in primary cultures of breast fibroblasts derived from benign and malignant lesions of breast tumors [222]. PDGF A is also expressed in cultured rat lung fibroblasts [223], and, recently, expression of PDGF A and B and of the PDGF β receptor was detected in fetal rat lung fibroblasts. The expression of PDGF A was constantly low during late gestation and did not significantly change subsequently, whereas the expression of the PDGF B chain was maximal at embryonic day 19 and decreased sharply thereafter [224]. The expression level of PDGF A mRNA and protein increases upon exposure of cultured rat lung fibroblasts to asbestos, which may cause autocrine growth stimulation. Thus, PDGF A production may play a role in asbestos-induced pulmonary fibrosis [225].

Neural tissues

Rat brain expresses PDGF mRNA throughout gliogenesis [81] and rat brain neurons express PDGF β receptors *in vitro* and *in vivo*. Moreover, primary rat brain cells can be mitogenically stimulated by PDGF [226]. Whereas the transcription rates of the A and B chain genes in the rat central nervous system are similar, the mRNA level of the B chain is 10-fold lower than that of the A chain, suggesting differences in mRNA half-life [227]. PDGF may play a role in regeneration of nerves, as PDGF A and B mRNA and PDGF protein are expressed in facial neurons upon facial nerve axotomy in rats [228]. PDGF A mRNA and protein are also expressed at high levels *in situ* in neurons of embryonic and adult mouse, where they may regulate proliferation and differentiation of glial cells [78, 229]. Specific immunostaining for PDGF B chains was found in primate neurons, principal dendrites, some axons and terminals throughout the brain; in the dorsal horn of the spinal chord and in the posterior pituitary. PDGF A and B and PDGF receptor mRNA are expressed throughout the brain and posterior pituitary; a transgenic model in which the chloramphenicol acetyltransferase (CAT) reporter gene was driven by the PDGF B promoter revealed preferential expression within neuronal cell bodies in the cortex, hypocampus and cerebellum. Thus, PDGF may act as a neuronal regulatory agent [230]. Recently, PDGF B-related mitogenic activity and 2.6 kb and 3.5 kb PDGF B mRNA species were detected in ubiquitous neurons of mature rat brain *in situ*, especially in hippocampal pyramidal cells. Since these neurons do also express PDGF receptors, PDGF may act as an autocrine and/or paracrine neuronal regulatory or trophic agent [231]. In human brain tissue, PDGF A and PDGF β receptor mRNA are detectable, but PDGF B mRNA is not detectably expressed [95].

PDGF is also expressed in neonatal rat peripheral nervous system (PNS), Schwann cells and neurons. In adult rat, myelinated nerve fibers express low PDGF levels, whereas unmyelinated nerve fibers express high levels. PDGF α and β receptors are also expressed in neonatal PNS. In adult rat, both receptors genes are expressed in unmyelinated nerves [232]. In cultures of rat neurons and astroglial cells PDGF and its receptors are coexpressed, suggesting an autocrine role in neuronal differentiation [233].

PDGF A is secreted by type-1 astrocytes present in the developing rat optic nerve and stimulates *in vitro* proliferation of O-2A progenitor cells, which express predominantly PDGF α receptors [79–81, 234, 235]. Axonal electrical activity may control production and/or release of PDGF [236]. After rat embryonic day 16, PDGF α receptor mRNA is expressed in a subset of glial cells, predominantly cells of the O-2A lineage, but not by neurons [237]. Recently, v-*erb*A, a mutated thyroid hormone receptor, was shown to induce PDGF B gene expression in glial cells, which may play a role in the development of gliomas [238].

Other cell types

Most studies on PDGF have described its expression in the vasculature, in blood cells, during early development, in kidney and in neural tissues. However, there have been several reports of PDGF expression in other tissues, which are summmarized in this section. PDGF A expression was detected in myoblast cell lines, primary rat skeletal myoblasts and developing rat crude muscle tissue [82, 239]. PDGF B (two mRNA species) is expressed in human uterus endometrium and myometrium samples [83]. PDGF A mRNA is also expressed in the uterus and the expression level increases in myometrial SMC *in situ* during pregnancy and diminishes again during the puerperium. This implicates a role for PDGF in uterine expansion during pregnancy [84].

Cultured normal human mammary epithelial cells (HMECs) express PDGF A mRNA and protein but little or no PDGF B mRNA. TGF-β, which inhibits growth of HMECs, induces a 20–40-fold increase of PDGF B mRNA, a 2-fold increase of PDGF A mRNA and a 2–3-fold increase of PDGF secretion. Sodium butyrate and phorbol ester, which also inhibit growth of HMECs, do not affect PDGF expression [85].

PDGF A and B mRNA are expressed in rat lung and, upon exposure to hyperoxia (87% oxygen), the PDGF A mRNA level increases and transcription of the PDGF B gene increases by up to 10-fold. Elevated PDGF expression preceeds a proliferative response of microvascular adventitial fibroblasts, precursor SMCs and epithelial cells [240, 241].

Cultured human retinal pigment epithelial (RPE) cells secrete PDGF [86] and express PDGF A and B and PDGF α and β receptor mRNA. Phorbol ester and thrombin induce a 16–20-fold increase of the PDGF B mRNA level in RPE cells [242]. PDGF A mRNA was also detected *in situ* in rat RPE cells as well as in retinal neurons and ganglion cells. PDGF A protein was detectable in ganglion neurons of the mouse retina and PDGF α receptor mRNA in retinal astrocytes. Therefore, a short range paracrine role for PDGF A in the retina was suggested [243]. Recent studies indicated that the PDGF receptors on RPE cells cultured in serum-free medium are autophosphorylated and that suramin blocks the autophosphorylation. Moreover, anti-PDGF antibodies inhibit the growth of RPE cells in serum-free medium. Together, these studies strongly suggest that RPE cells use an autocrine PDGF loop for serum-independent growth [244]. PDGF B and β receptor mRNA were detected in the vascular systems of the eye, probably in the capillary endothelial cells [243]. As PDGF BB also induces proliferation of rabbit retina capillary endothelial cells, an autocrine PDGF pathway may play a significant role in angiogenesis [245]. PDGF B and the PDGF β receptor are coexpressed in many cells of proliferative human retinal membranes, where they could contribute to pathogenesis of different proliferative retinopathies by paracrine or autocrine stimulation of cell migration and growth [246]. Recently, PDGF α receptors were identified in the peripheral epithelium of the chicken lens, where PDGF may control lens growth [247].

Human primary osteoblastic bone cells and cultured fetal osteoblast cells express PDGF A [248, 249] and the human cells also express PDGF α and β receptors, raising the possibility of autocrine growth stimulation [248]. TGF-β1 increases PDGF A mRNA and protein expression by 3–6-fold in the fetal osteoblast cells [249]. IL-1β stimulates proliferation of rabbit articular chondrocytes and induces synthesis and release of PDGF. The IL-1β effect is specific for PDGF AA and acts at the transcriptional level. TGF-β inhibits IL-1β induction of PDGF A [250].

Cultured human keratinocytes express PDGF A and trace amounts of PDGF B mRNA and secrete PDGF AA protein. IL-1α induces PDGF B expression, whereas TGF-β induces both PDGF A and B mRNA expression. PDGF AA and BB protein are also present in normal human epidermis *in situ*. PDGF receptor is not detectable in cultured keratinocytes or epidermis, which suggests that PDGF is not part of an autocrine loop in these cells [251].

Recently, PDGF B mRNA was detected in Leydig and Sertoli cells of the rat testis, and also PDGF A mRNA was found in the Sertoli cells. As these cells do also express α and β PDGF receptors, both paracrine and autocrine roles for PDGF in the testis were suggested [252].

Expression in transformed cells

Shortly after the identification of PDGF it was discovered that certain human osteosarcoma and glioma cell lines secrete PDGF-like proteins, which led to the hypothesis that expression of PDGF may be associated with the etiology of these cancers [253, 254]. The current idea is that PDGF may play an autocrine and/or paracrine role in tumorigenesis dependent on whether a tumor cell coexpresses PDGF and its receptor or whether it stimulates adjacent receptor expressing cells; through paracrine stimulation of connective tissue and blood vessels, PDGF secreted by tumor cells may induce desmoplasia and angiogenesis. Indeed, stable transfection and expression of PDGF B in an originally PDGF B negative melanoma cell line induces the development of a vascular connective tissue stroma in subsequent xenografts [255].

The list of tumor species that express PDGF is still increasing (see e.g. Table 5) and in some cases the expression level was shown to be affected by extracellular factors. The expression level of PDGF may be an important parameter for its putative role in tumorigenesis: e.g. the level of PDGF B mRNA expression in 3T3 cells determines the acquisition of features of transformation in a dose-dependent manner [311]. Glucocorticoids suppress the expression of PDGF A mRNA and protein by a rat hepatoma cell

Table 5. Expression of PDGF mRNA and protein and of PDGF receptor in transformed cells in culture (C), *in situ* (S) or in tumor biopsies (Bi). '–', not determined

Tumor species	PDGF mRNA	PDGF protein	PDGF receptor	References
Sarcoma (C)	A,B	–	yes	[256, 257]
Osteosarcoma (C)	A,B	yes	yes	[258–261]
Mesenchymal (C)	–	yes	yes	[262]
Glioma (C,S)	A,B	yes	yes	[94, 261, 263–265]
Mouse neuroblastoma (C)	B	yes	–	[266]
Prostate carcinoma (C,S)	A,B	yes	yes	[267–269]
Glioblastoma (C,Bi,S)	B	yes	–	[93, 256, 270, 271]
Fibrosarcoma (C,S)	B	yes	–	[270, 272]
Melanoma (C)	A,B	yes	yes	[261, 273–275]
Giant cell of bone (Bi)	B	–	–	[276]
Mesothelioma (C)	A,B	yes	–	[277–281]
Nephroblastoma (C)	A	yes	yes	[190]
Breast carcinoma (C,S)	A,B	yes	no	[282–286]
Lung carcinoma (C,S)	A,B	yes	yes	[285, 287–290]
Gastric carcinoma (C,Bi,S)	A,B	yes	yes	[285, 291–293
Ovarian carcinoma (C)	A,B	yes	no	[285]
Hepatoma (C)	yes	–	yes	[294]
Medulloblastoma (C)	–	yes	–	[295]
Colon adenocarcinoma (C)	–	yes	–	[296]
Astrocytoma (Bi,S)	A,B	yes	yes	[95, 297]
Meningioma (S,Bi)	B	yes	yes	[298–301]
Kaposi sarcoma (S)	A,B	–	yes	[302]
Midgut carcinoid (S)	yes	yes	yes	[303]
Endocrine pancreatic (C,S)	A,B	yes	yes	[303, 304]
Neurinoma (Bi)	B	–	–	[301]
Choriocarcinoma (C,S)	B	–	yes	[305, 306]
Pancreatic adenocarcinoma (C,B)	A,B	yes	yes	[307]
SV40-transformed BHK (C)	B	–	–	[308, 309]
HSV-transformed 3T3 (C)	A	–	–	[310]
MoSV-transformed NRK (C)	A	–	–	[310]

line [312], whereas expression of PDGF by MCF-7 cells is induced by 17β-estradiol [283]. PDGF A and B mRNA levels in cultured human pancreatic adenocarcinoma cells are stimulated by TNF-α and TNF-β. Both inhibition of PKC by staurosporin and stimulation of PKC by phorbol ester increases PDGF B mRNA levels [304]. Phorbol ester, TGF-β and diacylglycerol (DAG) induce a transient increase of the transcription rate of the PDGF B gene in A172 cells, but phorbol ester and TGF-β use different signal transduction pathways [313]. An increase of the cellular cAMP level blocks PDGF B mRNA expression in some human glioma and osteosarcoma cell lines without affecting stability of PDGF B mRNA [314]. PDGF stimulates PDGF A mRNA expression in cultured Kaposi's sarcoma derived cells [315]. Sodium butyrate inhibits growth and induces differentiation of F-98 rat glioma cells. The differentiation is accompanied by a significant and irreversible increase of the PDGF B mRNA level, which is at least partly caused by an increase of the mRNA half-life from 40 to 100 min [316].

Regulation of PDGF expression

From the overview above one could get the impression that PDGF can be expressed in any cell type. However, the fact that aberrant expression of PDGF B in e.g. fibroblasts may lead to oncogenic transformation already implicates that the expression must be tightly

regulated. Thusfar, the majority of research on regulation of expression of PDGF has been descriptive. The molecular mechanisms that are involved in regulation of expression of the PDGF genes are only beginning to be understood. Since there are numerous manners in which the expression of the PDGF genes can be modulated, it is not surprising that these mechanisms have evolved into a highly complicated network.

Regulation of transcription rate

Transcription of the PDGF genes can be modulated by very diverse extracellular stimuli, such as mitogens, cytokines, tumor promoters, shear stress, uric acid and oxygen concentration (see above; reviewed in [317]). The response of the PDGF genes to these stimuli and the cell type- and developmental-stage-dependent transcription of the genes are mediated by the interaction between specific DNA elements in the PDGF genes and regulatory transcription factors, which trigger the transcriptional machinery. These regulatory DNA elements have been mapped in only a few cell types, whereas the transcription factors that interact with the elements and their link to second messenger pathways are largely unknown.

Given the fact that the PDGF A and B genes are often independently expressed, it is not surprising that their promoter sequences are not homologous. Actually, one of the few similarities is the presence of a TATA box at approximately 30 bp upstream of either gene. Fig. 1 gives an overview of regulatory DNA elements that have thus far been identified in the promoters of the PDGF A and B chain genes.

Regulation of PDGF B gene transcription

Immediately upon the cloning of PDGF B exon 1, it was shown that the first 386 bp upstream of the transcription start site are sufficient for promoter activity in HeLa cells [49]. The first cell type in which the PDGF B promoter was analysed in more detail was the megakaryocytic cell line K562. Upon treatment with phorbol ester these cells differentiate towards megakaryocytes. This is accompanied by a more than 200-fold increase in PDGF B mRNA level, which is dependent on protein synthesis. By reporter gene analysis it was shown that the first 72 bp upstream of the transcription start site are essential for minimal promoter activity in uninduced K562 cells. A region between −101 bp and −58 bp appeared to be essential for TPA inducibility. Deletion of the region between −105 bp and −85 bp led to a three-fold decrease of TPA inducibility, and deletion of the region between −105 bp and −41 bp reduced promoter activity to background levels. By means of gel retardation experiments nuclear factors from K562 cells were shown to bind specifically to the region between −103 bp and −60 bp and the binding activity increased upon TPA treatment [322]. In a recent linker scanning analysis, the region between −64 bp and −39 bp appeared to be essential for TPA inducibility of the PDGF B promoter [323]. This is supported by combined *in vivo* footprint and deletion mutant analysis of the PDGF B promoter in phorbol ester-treated megakaryocytic K562 cells, which showed that the region between −61 bp and −54 bp is bound by a potent transcriptional activator. In a similar way it was shown that the region between −64 bp and −60 bp is bound by a weak transcriptional activator required for basal promoter activity in uninduced K562 cells and in HeLa, PC3 and JEG-3 cells [324]. In the nucleus, sites of DNA-protein interactions are usually located in nucleosome-free regions, which can be identified by their hypersensitivity to DNase I. The DNase-I-hypersensitive (DH) site at the PDGF B promoter in TPA-treated K562 cells extends from −90 bp to +60 bp, indicating that only one nucleosome is displaced. The first 112 bp upstream of the PDGF B transcription start site are sufficient for a 15–30-fold increase in PDGF B promoter activity during phorbol ester mediated megakaryocytic differentiation of K562 cells [328], whereas DH sites far upstream of the gene coincide with transcriptional enhancers that together with the promoter could result in a 100–1000-fold increase in promoter activity [329].

By reporter gene analysis of deletion mutants a region located between −292 bp and −278 bp was shown to contain a positive transcription regulatory element that is required for full promoter activity in HOS osteosarcoma cells and A172 glioblastoma cells. Further deletion up to −99 bp reduced promoter activity to background levels in these cells. In the same study it was demonstrated that the first 1.4 kb upstream of PDGF B gene harbours a binding site for the HTLV-I and HTLV-II derived transcription activating tat proteins, but the exact location of this binding site has thusfar not been described [325].

Transcription regulatory elements were also mapped in PDGF B intron 1. In cultured primary placental cytotrophoblasts, DH sites were localized at 1.6 kb, 3.8 kb, and 4.3 kb downstream of the transcrip-

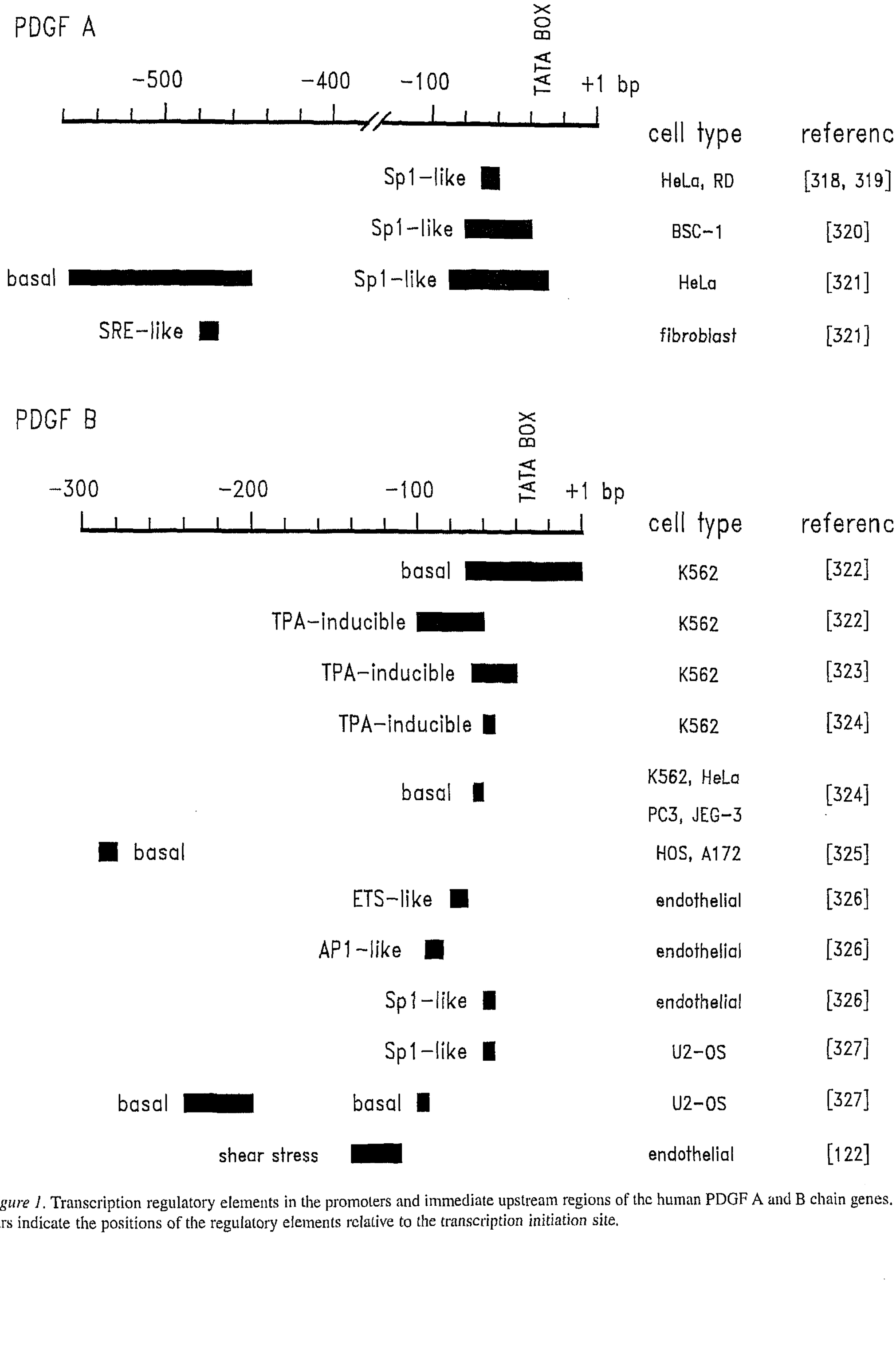

Figure 1. Transcription regulatory elements in the promoters and immediate upstream regions of the human PDGF A and B chain genes. Black bars indicate the positions of the regulatory elements relative to the transcription initiation site.

tion start site, whereas these sites were not found in placental fibroblasts. In JEG-3 choriocarcinoma cells, DH sites were found at 1.6 kb and 3.8 kb downstream of the start site, and in U2-OS osteosarcoma cells a single site at +6.3 kb was found. Fragments that contained either the hypersensitive site at +1.6 kb or at +3.8 kb enhanced promoter activity respectively by 4-fold and 8-fold in JEG-3 cells, whereas the entire PDGF B intron 1 was found to enhance activity of the PDGF B promoter by 26-fold in JEG-3 cells [305]. This is in contrast with the observation that the DH sites at +1.6 kb and +3.8 kb act as potent silencers of the PDGF B promoter in JEG-3 cells and also in K562 cells [328]. These conflicting results can be explained by the recent discovery that the DH site at +3.8 kb coincides with an alternative transcription initiation site [330]. Thus, in the reporter gene constructs described by Franklin et al. [305] and Dirks et al. [328] two promoters were present in one single plasmid, which may have affected the activity of either promoter. A fragment that contained the DH sites at +6.3 kb silenced the PDGF B promoter activity by 2–3-fold in U2-OS cells [305]. The relative activity of the PDGF B promoter in HeLa cells and PC3 prostate carcinoma cells is not significantly different, although the PC3 cells express a much higher level of PDGF B mRNA than the HeLa cells. DH sites at −9.9 kb and +25 kb contain enhancers that may explain the increased activity of the PDGF B promoter in PC3 cells compared with HeLa cells [328, 329].

By reporter gene analysis of deletion mutants and site directed mutants it was shown that an ETS-like sequence between −80 bp and −70 bp and an AP-1-like sequence between −92 bp and −85 bp relative to the transcription start site are important for activity of the PDGF B promoter in vascular endothelial cells. Synthetic oligonucleotides containing either the ETS-like element or the AP-1-like element interact with proteins present in endothelial cell nuclear extracts, but recombinant AP-1 and ETS-like protein GABPα do not interact with the PDGF B promoter *in vitro*. Mutagenesis of the Sp1-like CCACCC sequence between −61 bp and −56 bp reduced activity of the PDGF B promoter in endothelial cells by twofold. The −61/−56 sequence interacts with a nuclear protein from endothelial cell and with purified Sp1, suggesting that an Sp1-like protein is involved in regulation of transcription of the PDGF B gene in vascular endothelial cells [326]. By linker scanning analysis of the PDGF B promoter in U2-OS osteosarcoma cells, regions at −63/−44 bp, −102/−95 bp and −244/−203 bp were identified as important positive regulatory elements. The −63/−44 element increased activity of a heterologous TATA box by 5-fold. By site directed mutagenesis the CCACCC sequence at −61/−56 bp was shown to be essential for the activity of the element and found to interact with an Sp1-like factor present in U2-OS nuclear extracts [327]. Recently, a shear-stress response element that mediates the increase in PDGF B gene expression upon exposure of vascular endothelial cells to fluid shear stress [122] was shown to interact *in vitro* with NF-κB p50-p65 heterodimers. Moreover, p50-p65 was shown to activate PDGF B shear-stress response element-dependent reporter gene activity in cotransfection experiments, suggesting that NF-κB may play a direct role in fluid shear stress-induced activity of the PDGF B gene in vascular endothelial cells [331].

The PDGF B gene is also subject to negative transcription regulation, as e.g. cultured dermal fibroblasts contain a transcriptional activator directed toward the PDGF B promoter, but do not express the endogenous PDGF B gene. The PDGF B promoter is unmethylated and not located within a DH site in fibroblasts [324, 328]. No DNA-protein interactions could be detected by *in vivo* footprint analysis of the PDGF B promoter in fibroblasts [324]. In addition, a silencer could not be found within a region that extends from −12 kb to +25 kb relative to the transcription start site in fibroblasts [328, 329]. We must therefore conclude that the inhibition of transcription of the PDGF B gene in fibroblasts is mediated by a mechanism that has not yet been unraveled. One of the few possibilities that remain is that the accessibility of the PDGF B promoter for the transcriptional activator is blocked by histones, as a result of specific nucleosome phasing. A DH site that is located immediately downstream of the PDGF B promoter in fibroblasts might play a direct role in the phasing of nucleosomes [328]. By analysis of somatic cell hybrids between PDGF B expressing melanoma cells and hamster fibroblasts it was shown that the negative regulation of the PDGF B chain is probably mediated by a *trans*-acting factor [332]. It would be interesting to test whether this factor acts by introducing changes in the chromatin structure at the PDGF B gene promoter.

Regulation of PDGF A gene transcription

In contrast to the PDGF B promoter, the PDGF A promoter is exceptionally G/C-rich [52, 53]. Eight consen-

sus binding sites for transcription factor Sp1 are located within the first kb upstream of the transcription start site. The region between −447 bp and +387 bp has promoter activity in an *in vitro* transcription assay using HeLa nuclear extract and in a reporter gene assay using RD rhabdomyosarcoma cells [54]. A region between −69 bp and −60 bp, immediately downstream of a 13-bp oligo(dG) stretch exhibits supercoiling dependent nuclease-S1-hypersensitivity *in vitro*. It contains three Sp1 consensus binding sites and is required for full activity of the PDGF A promoter in HeLa cells. A synthetic oligonucleotide complementary to the nuclease S1 hypersensitive site (SHS) of the PDGF A promoter protects this site against nuclease S1 and reduces promoter activity in a transient reporter gene assay [318, 333]. Another supercoiling dependent SHS is located immediately upstream of the 13-bp oligo(dG) stretch between −96 bp and −90 bp. By gel shift analysis the −96/−90 and −69/−60 regions were shown to interact specifically with proteins present in nuclear extracts from RD cells. Reporter gene analysis of deletion mutants demonstrated that the first 72 bp upstream of the transcription start site are already sufficient for promoter activity in RD cells and that the −69/−60 region is important for activity [333]. Sp1-like elements between −82 bp and −40 bp are also required for full activity of the PDGF A promoter in African green monkey (BSC-1) cells [320]. The first 600 bp of the PDGF A promoter contain at least six consensus binding sites for transcription factors EGR-1 and the Wilms' tumor associated WT1. Coexpression of EGR-1 and a PDGF A promoter/reporter gene construct indicated that EGR-1 can either activate or repress activity of the PDGF A promoter dependent on the cell type examined. WT1 was shown to interact *in vitro* with the PDGF A promoter and abolishes its activity in a reporter gene assay. As PDGF A is overexpressed in certain Wilms' tumors, this raises the possibility that deregulation of WT1 or PDGF A may contribute to the etiology of this tumor [319, 334]. The repression of PDGF A promoter activity by WT1 requires the presence of WT1-binding sites both upstream and downstream of the transcription start site. The presence of either an upstream or a downstream binding site results in WT1 mediated transcriptional activation. Different domains of WT1 are involved in transcriptional repression and activation [335]. Deletion mutant analysis in HeLa cells demonstrated that a negative transcription regulatory element is located upstream of −618 bp, probably between −1.9 kb and −0.9 kb [321]. A similar reporter gene analysis in African green monkey (BSC-1) cells identified two negative elements between −1029 bp and −880 bp and between −1800 bp and −1029 bp [320]. Positive elements were mapped in HeLa cells between −558 bp and −447 bp and downstream of −150 bp. By DNase I footprint analysis the region between −89 bp and −33 bp was found to interact *in vitro* with purified Sp1. A serum response element (SRE) located between −477 bp and −468 bp appeared to be required for growth factor mediated induction of PDGF A promoter activity in human foreskin fibroblasts. The SRE interacts *in vitro* with a serum response factor- (SRF-) like protein present in HeLa nuclear ectract [321]. The SRE also coincides with a supercoiling dependent SHS. The noncoding strand of the SRE was found to interact specifically with a single-stranded DNA-binding protein present in a HeLa cell nuclear extract [336]. Serum response elements that stimulate transcription of the PDGF A gene were also found between −135 and −223 bp, whereas elements that down-regulate expression of the gene during serum starvation were localized between −223 and −416 bp [337]. Recently, a protein present in nuclear extracts of human mesangial cells was found to bind *in vitro* to region −102/−82 of the PDGF A gene promoter. The abundance of the protein increases during phorbol ester-mediated increase of PDGF A gene transcription, but its nature is still unknown [338]. A supercoiling dependent SHS located between −495 bp and −484 bp relative to the transcription start site of the PDGF A gene interacts specifically with proteins present in nuclear extracts from HeLa and A172 cells and slightly increases the activity of a heterologous promoter [339]. A supercoiling dependent SHS located between +50 bp and +67 bp relative to the transcription start site interacts *in vitro* with purified transcription factors Sp1 and EGR-1 [340]. Finally, a supercoiling dependent SHS was detected in a 147-nucleotides long region in the first intron of the PDGF A gene. By reporter gene analysis the intron element was shown to silence the PDGF A promoter and a heterologous promoter in HeLa cells. The element specifically interacts with a protein present in nuclear extracts from HeLa cells [341]. *In vitro* methylation of the PDGF A gene promoter resulted in strongly reduced activity in a transient reporter gene assay in HeLa cells. DNA methylation might thus cause the low PDGF A mRNA level in these cells. Unfortunately, the methylation state of the endogenous PDGF A promoter in HeLa cells was not examined. Purified Sp1, which is thought to play a role in regulation of transcription

of the PDGF A gene, binds to the PDGF A promoter irrespective of its methylation state [342].

Alternative transcription initiation sites

Small PDGF B mRNA species are induced by treatment of vascular endothelial cells with cycloheximide and TGF-β1. A 2.8-kb transcript initiates 15 nucleotides upstream of the translation start site, whereas a 3.0-kb transcript initiates 200 nucleotides further upstream. The expression of truncated PDGF B mRNA species may provide a way to escape from the potent translation inhibitory activity of the exon 1-derived 5′ untranslated sequence [343]. The expression level of a 2.5-kb PDGF B mRNA expressed in melanoma cell line WM115 also highly increases by treatment with cycloheximide [332], whereas the expression level of a 2.7-kb PDGF B mRNA in rat lung increases upon exposure to hypoxic conditions [344]. Whereas the 2.8-kb and 3.0-kb PDGF B mRNA species expressed in endothelial cells seem to have an intact PDGF B open reading frame, JEG-3 choriocarcinoma cells express a 2.6-kb PDGF B mRNA species that initiates at an alternative promoter located within intron 1 and lacks the code for the signal peptide of the PDGF B chain precursor. The resulting protein product may be targeted to the nucleus due to the presence of a nuclear localization signal encoded by PDGF B exon 6 [330]. A 2.6 kb PDGF B mRNA species was also found in normal mature rat brain, but the exact nature of this transcript was not described [231].

Nuclease S1 and reporter gene analysis demonstrated that the PDGF A gene uses two promoters. The regular transcription start site is located approximately 35 bp downstream of the TATA box, whereas the alternative initiation site is located 470 bp downstream of the first site, but still 380 bp upstream of the translation start site [345]. A novel PDGF A cDNA clone isolated from a rat macrophage cDNA library lacks exon 6-derived sequences and has a 588-nucleotides coding region that is 91% identical with the human PDGF A code. The 79 nucleotides immediately upstream of the PDGF A open reading frame are 92% identical in the human and the rat mRNA, whereas the similarity in the remaining part of the 5′ untranslated sequence is less than 30%. It was hypothesized that the rat PDGF A mRNA could be initiated at an alternative promoter. The 5′ untranslated sequence of the rat cDNA clone contains an ATG codon that is not in frame with the PDGF A open reading frame. Translation initiation from this upstream ATG would yield a polypeptide of 118 amino acids. Except in macrophages, the alternative PDGF A transcript is also expressed in fetal, newborn and adult brain and kidney [346].

Regulation of mRNA stability

In general, the widely varying PDGF B mRNA levels result from differences in transcription rate of the gene, rather than from differences in mRNA stability [103, 151]. The PDGF B mRNA half-life varies roughly from 1–3.5 h in rat smooth muscle cells [124], vascular endothelial cells [102, 120], bladder carcinoma cell line T24 [271], glioblastoma cells [271, 313, 314], PC3 prostate carcinoma cells and phorbol ester treated K562 cells [328].

Regulation of translation

The 1-kb long leader of the 3.5-kb PDGF B transcript can inhibit translation *in vitro* and in reporter gene assays up to 40-fold [50, 325, 347]. The leader is highly GC-rich and contains three small open reading frames. The open reading frames are not important for the translation inhibitory capacity of the leader, as mutation of the AUGs does not affect the production of PDGF B chains [348]. Regions 1–651 and 475–1022 are equally potent inhibitors of translation as the entire PDGF B leader sequence. Either region contains a C-rich element and sequences that are capable of forming strong stem-loop structures [348]. The highly GC-rich 140-nucleotides long sequence immediately upstream of the PDGF B open reading frame is nearly as effective an inhibitor as the entire leader. The inhibitory activity is partly relieved by deletion of nucleotides 154–378 or 398–475. The PDGF B leader also significantly reduces the transforming efficiency of the PDGF B gene in NIH 3T3 cells [50, 325, 347]. The translation inhibitory activity of the PDGF B leader is temporarily relieved during megakaryocytic differentiation of K562 cells, when transcription of the PDGF B gene is also strongly induced. The 179-nucleotides long sequence immediately upstream of the PDGF B open reading frame was shown to be important for relief of translation repression [349]. Cultured primate aortic SMCs express PDGF A and B mRNA but do not express or secrete PDGF-like protein, suggesting negative regulation at the translational level [350].

The PDGF A 5′ untranslated sequence contains three ATG codons [53], the function of which is unknown. In a recent study the region between +99 bp and +184 bp relative to the PDGF A transcription start site, which lacks an ATG codon, was shown to inhibit translation in RD rhabdomyosarcoma cells, but the inhibitory effect could be overcome by sequences located between +184 bp and +338 bp [351].

Alternative polyadenylation sites

The PDGF A gene uses three alternative polyadenylation sites. In combination with the regular transcription start site these yield mRNA species of 2.3 kb, 2.8 kb and 3.6 kb. When combined with the alternative transcription start site, transcripts of 1.8 kb, 2.4 kb and 3.2 kb are generated. Since these PDGF A mRNA species can also be subjected to alternative splicing of exon-6, the theoretical number of different PDGF A mRNA species increases to twelve [345]. Human papillary carcinoma cell line NPA expresses a monomeric PDGF-like protein and a 1.4-kb PDGF B mRNA that hybridizes with 5′ cDNA probes but not with 3′ probes, which could result from alternative polyadenylation [352].

Alternative splicing

Alternative splicing of PDGF mRNA (reviewed in [353]) was first discovered when PDGF A cDNA clones were isolated from a human glioma cell line that represent two alternatively spliced transcript species differing by exon 6-encoded 69 bases at the 3′ part of the open reading frame; normal HUVECs express a short PDGF A chain precursor that lacks the 15 C-terminal highly basic amino acids encoded by the larger exon 6-containing glioma cDNA [53, 98, 354]. Both the short and the long PDGF A mRNA species are expressed in a variety of normal tissues and transformed cells and in diverse mammalian species [281, 355, 356]. In contrast with the short PDGF A chain, which is efficiently secreted, the long version of the A chain remains predominantly cell associated. Several lines of evidence suggest that the exon 6-encoded basic C-terminus associates with heparan-sulphate proteoglycans within the cell membrane and the cellular matrix. The PDGF B chain contains a homologous region in its C-terminus that determines its association with the cell membrane and cellular matrix. Alternative splicing of exon 6 may thus be a mechanism to regulate the formation of secreted or cell-associated forms of PDGF A [357–360]. A synthetic peptide that corresponds with the 18-amino-acids-long hydrophilic C-terminus inhibits the specific binding of growth factors to cells bearing their respective receptors and inhibits serum induced mitosis in cultured fibroblasts [361, 362]. The alternatively spliced exon 6 of PDGF A also contains a nuclear targeting signal that is capable of targeting a non-secreted form of the PDGF A chain to the nucleus [363]. By PCR analysis two transformed human cell lines were shown to express PDGF A transcripts lacking exon 2-derived sequences, which generates truncated and non-functional polypeptides [364]. Aberrant splicing was found to occur between a cryptic splice donor site in the short PDGF A chain cDNA and a viral splice acceptor site present in a eukaryotic expression vector, which resulted in the synthesis of a non- secreted PDGF A chain [365]. A novel PDGF A chain cDNA clone (A3), which was generated by alternative splicing of a 110 bp segment, was isolated from a rabbit embryonic aorta cDNA library [145].

Alternative splicing of PDGF B mRNA was observed in a recombinant retrovirus that expresses the wildtype PDGF B chain and induces fibrosarcomas in mice. Proviruses derived from the tumors had lost a 149-bases sequence derived from exon 7, due to alternative splicing. Interestingly, an identical splice event is visible in the SSV genome, which suggests that these 149 bases are detrimental for retroviral expression or replication [366]. The mouse PDGF B gene has the same exon-intron structure as the human gene. Their coding regions are 89% identical and the first 120 nucleotides of the untranslated exon 7 are even 94% identical, but are followed by a highly divergent sequence. It was hypothesized that this sequence could be involved in alternative splicing of exon 6, as is also observed for the PDGF A gene. An alternative PDGF B transcript that lacks exon 6 derived sequences has not yet been described [367].

Posttranslational regulation

It has become clear that a given cell type can qualitatively or quantitatively affect the expression of the PDGF chains at the transcriptional, mRNA processing and translational level. However, even after efficient transcription of a PDGF gene and translation of its mRNA, the amount and type of the PDGF protein to be expressed can still be determined at the posttranslational level. PDGF purified from platelets consists as two polypeptides, PDGF-I and PDGF-II [368], which differ in carbohydrate composition [369]. The role of

glycosylation in PDGF activity, processing and transport is not clear, and glycosylation is probably not essential for one of these processes. Activity of PDGF released *in vivo* may be regulated by association with α2-macroglobulin, to which a large part of PDGF binds reversibly [370–372].

Another protein that may regulate activity of PDGF is the extracellular glycoprotein SPARC (secreted protein, acidic and rich in cysteine). It binds specifically to PDGF AB and BB, but not to AA, and inhibits binding of PDGF AB and BB to the PDGF receptor. Therefore, it may regulate activity of specific dimeric forms of PDGF [373]. Recent studies on vascular endothelial cells that had been stably transfected with a PDGF B expression vector provided evidence for yet another level of regulation. A major part of the PDGF B expressed by the transfectants remained associated with the extracellular matrix. Thrombin induced a rapid release of PDGF B by selective proteolytic cleavage of the membrane associated PDGF precursor protein. In this way a cell can quickly respond to a sudden need for growth factor, even in the absence of mRNA and protein synthesis [374].

Conclusions and future aspects

Since its purification, some fifteen years ago, it has become clear that PDGF is a highly important polypeptide growth factor that plays an essential role during early vertebrate development and is associated with wound healing in the adult. PDGF acts via the binding to and the activation of specific high affinity cell surface receptors. Both the PDGF A and B chain genes and the PDGF α and β receptor genes have been isolated and characterized, which has enabled the detailed structural and functional analysis of their protein products. A broad range of cell types has been described that are capable of expressing either or both of the two PDGF genes, sometimes in a developmental stage-specific manner. Moreover, many factors have been identified that can modulate the expression (level) of PDGF in a given cell type. Finally, several pathological phenomena have been found associated with increased expression levels of PDGF.

Now that the place and time of expression of PDGF have largely been settled, research has focused more on the mechanistic aspects of PDGF expression. The signal transduction cascade that is initiated by activation of the PDGF receptors is rapidly being elucidated. Knowledge of the molecular mechanisms that are involved in regulation of expression of the PDGF genes is still limited, but rapidly accumulating. In principle, the expression of PDGF genes can be modulated by regulating the initiation, the initiation rate or the initiation site of transcription, by alternative splicing, by alternative polyadenylation, by varying the mRNA stability, by regulation of translation efficiency and by regulation at the posttranslational level. In general, regulation at the transcription level has been found to play a decisive role in regulation of expression of PDGF per se. In a number of cell types the DNA elements that mediate transcription of PDGF genes have now been characterized, which will soon be followed by the isolation and characterization of the transcription factors that bind to them. Identification of these transcription factors will enable one to elucidate the mechanisms that link the changes in the cells' environment or developmental stage with the changes in the transcription rate of the PDGF genes. This is not only important for extending the fundamental insight in regulation of the PDGF genes in normal cells, but will also lead to a better understanding of the changes in PDGF expression under pathological conditions. Ultimately, this may lead to the development of novel strategies in the ongoing fight against disease.

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