Platelet-derived growth factor (PDGF) BB homodimer regulates PDGF A- and PDGF B-chain gene transcription in human mesangial cells

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Mesangial cells express platelet-derived growth factor (PDGF) A- and B-chain mRNA and release PDGF. Several polypeptide growth factors, including PDGF itself, induce PDGF A- and B-chain mRNA abundance. To understand the molecular mechanisms associated with the changes in mRNA abundance, we measured the effects of PDGF BB homodimer on PDGF A- and B-chain gene transcription in cultured mesangial cells. The data demonstrate 2- and 4-fold increases in PDGF A-chain gene transcription in response to PDGF BB homodimer at 5 and 24 h time points respectively. PDGF B-chain gene transcription was also induced approximately 3-fold at 2, 5 and 24 h time points in response to treatment with PDGF BB homodimer. The effect of

PDGF BB on the half-life of PDGF A- as well as PDGF B-chain mRNA was measured directly by the pulse-chase method. There was no effect on PDGF A-chain mRNA half-life whereas PDGF B-chain mRNA half-life was increased 1.5-fold. These studies indicate that, in human mesangial cells, the increase in the levels of PDGF A- and B-chain mRNA in response to PDGF-receptor(s) activation is mediated at the level of gene transcription. In addition, the regulation of PDGF B- but not PDGF A-chain gene involves increased mRNA stability. Mesangial cells are a useful model for studying molecular mechanisms of PDGF-gene regulation in non-transformed human cells.

INTRODUCTION

Platelet-derived growth factor (PDGF) has been implicated in the pathogenesis of diverse biological processes including glomerulonephritis, atherosclerosis, carcinogenesis and wound healing [1]. It is a cationic glycoprotein consisting of two peptides referred to as A- and B-chains which are encoded by two separate genes [1]. It exists in three dimeric forms: AA, AB and BB. All three forms are biologically active and are involved in receptor-mediated induction of several of the biological effects of PDGF including cellular proliferation, ruffling and chemotaxis [1]. Studies by several investigators have revealed the presence of two PDGF receptors termed α and β [1,2]. The α -receptor binds to all of the three isoforms of PDGF whereas the β -receptor has a high affinity for PDGF BB but not for PDGF AB or PDGF AA [1,3].

Mesangial cells are vascular pericytes in the glomerular micro-vascular bed [4]. They proliferate and produce matrix in response to injury and contribute to functional alteration in glomerular disease. The expression of PDGF A- or B-chain mRNAs in mesangial cells can be regulated by a number of agents including PDGF itself acting primarily through a β -receptor [5]. We have recently reported that PDGF purified from human platelets (predominantly BB and AB dimers) stimulates DNA synthesis and PDGF A- and B-chain mRNA abundance [5]. In addition to gene transcription, mRNA stability plays an important role in the regulation of mRNA abundance [6]. mRNA stability similar to transcription can be regulated by growth factors and hormones [6]. The studies reported here were undertaken to elucidate the molecular mechanism(s) by which PDGF BB homodimer regulates PDGF A- and B-chain mRNA levels in cultured mesangial cells. Our data demonstrate that, in human mesangial cells, PDGF BB increases PDGF A- and B-chain mRNA abundance. The increase in the levels of PDGF A- and B-chain mRNA in response to PDGF-receptor(s) activation is mediated at the level

of gene transcription. In addition, regulation of the PDGF B- but not the PDGF A-chain gene involves increased mRNA stability.

MATERIALS AND METHODS

Materials

PDGF BB homodimer was from Amgen. [5,6-³H]Uridine was from New England Nuclear (Boston, MA, U.S.A.) and [³²P]UTP from Amersham Corporation (Arlington Heights, IL, U.S.A.). Other chemicals were of the highest purity available. Plasmid PDGF A-chain cDNA was kindly provided by Dr. Betscholtz, Uppsala, Sweden [7]. Plasmid PDGF B-chain cDNA was obtained from American Type Culture Collection, Rockville, MD, U.S.A. (A.T.C.C. no. 57050, clone pSM-1). Cultures of mesangial cells were obtained from glomeruli isolated from human donor kidneys which were unsuitable for transplantation or normal portions of surgical nephrectomy samples. Cells were maintained in Waymouth medium with fetal calf serum as described previously [5].

PDGF A- and B-chain mRNA abundance

Human mesangial cells were made quiescent in serum-free insulin-free medium for 72 h and then incubated in serum-free medium with or without PDGF BB (10 ng/ml). Cells were harvested and total RNA was isolated by lysis in guanidinium isothiocyanate and centrifugation on caesium chloride gradients. Northern-blot hybridization analyses of 20 μ g of total RNA were performed using ³²P-labelled PDGF A-chain cDNA [7,8]. Because of its low abundance, solution-hybridization analysis of PDGF B-chain mRNA was performed as described previously [5]. Briefly, labelled RNA probe was synthesized and hybridized to total RNA as follows: a 960 bp genomic fragment containing regions of 6th and 7th exons of the *c-sis* gene [9] in the vector

pT7-2 was used for synthesis of labelled probe using [32 P]UTP and T7 RNA polymerase. DNA template was digested with DNAase I and [32 P]RNA was isolated. Labelled RNA (1×10^6 c.p.m.) was hybridized to total RNA from mesangial cells followed by treatment with RNAase A and T1, and products were separated on 7 M urea/6% acrylamide gel.

Nuclear run-on transcription assay

Confluent mesangial cells were plated in serum-free medium for 24 h before treatment with PDGF BB homodimer (10 ng/ml). Nuclei from mesangial cells treated or not with PDGF BB homodimer at the indicated time points were prepared as described previously [10]. After purification through sucrose cushion, PDGF A- and B-chain gene transcription was performed by the nuclear-transcript-elongation protocol of McKnight and Palmiter [11] as described previously [12]. The assay mixture (260 μ l) contained 0.4 mM each of ATP, GTP and CTP, 100 μ Ci of [32 P]UTP (3000 Ci/mmol) and 20 μ g of nuclear DNA. Reaction was carried out for 90 min at 37 °C. Under these conditions, a linear response between total gene transcription and the amount of nuclear DNA was obtained. Maximum total gene transcription was observed during a 60–90 min incubation period irrespective of the amount of nuclear DNA (5–20 μ g) in the reaction mixture. After the addition of 20 units of DNAase I for 10 min, proteinase K was added followed by purification of labelled RNA [13]. DNA excess filter hybridization technique was used to estimate hybridizable radioactivity in PDGF A- and B-chain mRNA as described previously [12,14]. We used plasmid vector PBS to correct for non-specific binding. The PDGF A-chain or B-chain mRNA radioactivity was calculated from the differences between hybridizable radioactivity bound to PDGF A-chain or B-chain and PBS vector. The data are expressed as p.p.m. for either PDGF A-chain or PDGF B-chain gene transcription as described previously [14].

Determination of PDGF A- and B-chain mRNA half-life in response to PDGF BB homodimer

We estimated the half-life of PDGF A-chain and B-chain mRNA directly by using the [3 H]uridine pulse-decay technique as described previously [15–17]. Mesangial cells were incubated with or without PDGF BB homodimer for 4 h with fresh serum-free medium. [3 H]Uridine (90 μ Ci/dish) was added to label the cells for 3 h followed by washing of cultured dishes twice with PBS. Glucosamine (10 mM), cytidine (5 mM) and uridine (5 mM) were added to the culture dishes [16,18]. Culture medium was removed and fresh medium with or without PDGF BB homodimer containing 5 mM each of cytidine and uridine was added. After 0, 1, 2 and 4 h, 'post-chase' total labelled RNA was isolated and purified. The half-life of PDGF A-chain or B-chain mRNA was estimated from the rate of disappearance of 3 H-labelled PDGF A-chain and B-chain transcripts using cDNA excess filter hybridization as described previously [14,17]. Plasmid PBS was used to correct for non-specific binding to filters. As a control, we also measured the α -tubulin mRNA half-life (13 h; results not shown). Half-lives of 6–36 h have previously been reported for α -tubulin mRNA by other investigators [19–21].

RESULTS

Effect of PDGF BB on PDGF A- and B-chain mRNA abundance

We analysed the effects of PDGF BB on PDGF A- and B-chain

mRNA abundance in human mesangial cells. Figure 1(a) demonstrates that PDGF BB increases PDGF A-chain mRNA abundance. The sizes of the PDGF A-chain mRNA transcripts are 2.3, 1.9 and 1.5 kb. The 2.3 and 1.9 kb transcripts do not resolve clearly into two distinct bands. Ethidium bromide staining of total RNA after agarose-gel electrophoresis (Figure 1b) indicates that an equal amount of total RNA was loaded for the Northern-blot analysis. Solution-hybridization analysis (Figure 2) demonstrates two protected fragments of the expected sizes, 150 and 310 bp, on hybridization with PDGF B-chain probe. Our results clearly demonstrate a marked increase in PDGF A- and B-chain mRNA abundance in response to PDGF BB.

PDGF A- and B-chain gene transcription in response to PDGF BB homodimer

We next investigated the effects of PDGF BB homodimer on PDGF A- and B-chain gene transcription in cultured human mesangial cells. Human mesangial cells were made quiescent in serum-free medium for 24 h. Identical cultures of mesangial cells were divided into two treatment groups and incubated with or without PDGF BB (10 ng/ml). The cells were incubated for 2, 5 and 24 h. The results in Table 1 show that at 5 and 24 h PDGF BB stimulated PDGF A-chain gene transcription by approx. 2- and 4-fold respectively. α -Amanitin, an RNA polymerase II inhibitor, significantly blocked PDGF A-chain gene transcription by more than 80%. We have also investigated the effect of PDGF BB homodimer on PDGF B-chain gene transcription. Data in Table 1 indicate that PDGF BB induced PDGF B-chain gene transcription by approximately 3-fold at 2, 5 and 24 h time points. α -Amanitin also inhibited PDGF B-chain gene transcription by more than 80%.

PDGF A- and B-chain mRNA stability

The effects of PDGF BB on the half-life of PDGF A- and B-

Figure 1 Effect of PDGF BB on PDGF A-chain mRNA abundance in cultured human mesangial cells analysed by Northern-blot hybridization

Experimental details were as described in the Materials and methods section. Mesangial cells were incubated in serum-free insulin-free medium for 3 days before incubation with PDGF BB (10 ng/ml) for 3 and 8 h. (a) Total RNA (20 μ g) was subjected to 1% agarose-gel electrophoresis and transferred to gene-screen filter membranes for hybridization with radiolabelled PDGF A-chain cDNA. Ethidium bromide staining of the total RNA after 1% agarose-gel electrophoresis demonstrating the 28 S and 18 S rRNA is shown in (b).

Figure 2 Effect of PDGF BB on PDGF B-chain mRNA abundance in cultured human mesangial cells analysed by solution hybridization analysis

Experimental details were as described in the Materials and methods section. RNA probe was synthesized and hybridized to total RNA. A genomic fragment (960 bp) of the *c-sis* gene in the vector pT7-2 was used for the synthesis of labelled probe. DNA template was digested with DNAase I and [³²P]RNA was isolated. Labelled RNA (10⁶ c.p.m.) was hybridized to total RNA. RNAase A and T1 treatment was performed and products were separated on 7 M urea/6% acrylamide gel and detected by autoradiography. Bands shown of 310 and 150 nucleotides represent those portions of the 6th and 7th exons of *c-sis* probe that were protected from RNAase digestion. Size markers (not shown) were derived from *Hae*III fragments of ϕ X174 DNA, end-labelled with T4 DNA polymerase.

Table 1 Effect of PDGF BB homodimer on PDGF A- and B-chain gene transcription

Confluent human mesangial cells were placed in serum-free medium for 24 h. Fresh serum-free medium was added and cells were kept untreated (control) or treated with PDGF BB homodimer (10 ng/ml) at the indicated time points. Experimental details were as described in the Materials and methods section. Plasmid PBS was used to correct for non-specific binding. The PDGF A- or B-chain mRNA-hybridizable activity was obtained from the difference between radioactivity bound to filters containing plasmid PDGF A or PDGF B and plasmid PBS. α -Amanitin (2 μ g/ml) was used to inhibit RNA polymerase II activity in the reaction mixture. PDGF A- or B-chain gene transcription is calculated as described previously [14] and expressed as p.p.m. Data points are means $\pm$ S.E.M. from three experiments. Total [³²P]RNA counts added per reaction were between 0.70×10^6 and 1.02×10^6 c.p.m. and ³²P hybridized to plasmid PBS was 16.5–18.1 c.p.m. The values for fold change are normalized to control values.

Treatment	PDGF A-chain gene transcription		PDGF B-chain gene transcription	
	p.p.m.	Fold change	p.p.m.	Fold change
Control (2 h)	12.4 $\pm$ 0.14	1.0	15.5 $\pm$ 0.14	1.0
PDGF BB	15.6 $\pm$ 0.14	1.3	50.4 $\pm$ 2.05	3.2
Control plus α -amanitin	2.4 $\pm$ 0.14	0.2	2.3 $\pm$ 0.08	0.15
Control (5 h)	12.7 $\pm$ 0.11	1.0	16.6 $\pm$ 0.08	1.0
PDGF BB	22.9 $\pm$ 0.12	1.8	43.1 $\pm$ 0.05	2.6
Control (24 h)	13.1 $\pm$ 0.18	1.0	17.1 $\pm$ 0.08	1.0
PDGF BB	57.8 $\pm$ 1.48	4.4	45.7 $\pm$ 1.09	2.7

chain mRNAs were determined directly by using the pulse-chase method [15,16]. Mesangial cells were incubated with or without 10 ng/ml PDGF BB homodimer for 4 h, followed by labelling of

Table 2 Effect of PDGF BB homodimer on PDGF A- and B-chain mRNA half-life in cultured human mesangial cells

Confluent human mesangial cells were placed in culture dishes in serum-free medium for 24 h. Untreated cells (control) and cells treated with PDGF BB homodimer were incubated with [³H]uridine (90 μ Ci/dish) for 3 h. Culture dishes were then washed twice with PBS followed by incubation with 10 mM glucosamine and 5 mM each of cytidine and uridine for 10 min as described previously [18]. Fresh medium with or without PDGF BB homodimer and 5 mM each of cytidine and uridine were added to start the chase period as described in the Materials and methods section. Cells were harvested for isolation of total RNA at 0, 1, 2 and 4 h. Incorporation of labelled nucleotides at $t = 0$ (initial) was considered as 100% and half-lives ($t_{1/2}$) were calculated from the best-fit straight lines. The data are also normalized to control values and expressed as fold change.

mRNA	Treatment	mRNA $t_{1/2}$ (min)		Average $t_{1/2}$ (min)	Fold change
		Expt. I	Expt. II		
PDGF A	Control	93	109	101	1.0
	PDGF BB	111	111	111	1.1
PDGF B	Control	105	99	102	1.0
	PDGF BB	162	150	156	1.5

the cells with [³H]uridine for 3 h. Chase was initiated with unlabelled uridine and cytidine as described in the Materials and methods section. Labelled RNA was isolated at 0, 1, 2 and 4 h after the chase. The data show PDGF A-chain and B-chain (Table 2) mRNA degradation from two separate experiments. The half-lives of the PDGF A- and B-chain mRNAs are approximately 100 min (Table 2). The half-life of PDGF A-chain mRNA did not change significantly when mesangial cells were incubated with PDGF BB homodimer, but, in contrast, the PDGF B-chain mRNA half-life increased by 1.5-fold (156 min), indicating that PDGF BB homodimer stabilizes the PDGF B-chain mRNA (Table 2).

DISCUSSION

Human mesangial cells express PDGF A- and B-chain mRNAs and release PDGF. In human mesangial and microvascular endothelial cells, PDGF mRNA is subject to regulation by several peptides as well as phorbol esters [5,22–26]. The induction of PDGF mRNAs in these cells is associated with increased synthesis of the protein [5,22,23]. We have recently demonstrated that PDGF A-chain gene transcription is induced in response to phorbol ester [27]. The data presented here indicate that PDGF BB increases PDGF A-chain as well as PDGF B-chain mRNA levels in human mesangial cells. To understand the molecular mechanisms associated with the changes in mRNA abundance, we measured the effects of PDGF BB homodimer on PDGF gene transcription. Our data demonstrate that PDGF BB homodimer induces transcription of PDGF A- and B-chain genes. These data are in agreement with the increase in mRNA abundance.

In addition to gene transcription, mRNA degradation plays an important role in gene regulation. The rate at which mRNA decays determines the rate of synthesis of translated proteins. We determined the effect of PDGF BB homodimer on PDGF A-chain and PDGF B-chain mRNA half-life measured directly by a pulse-chase method, as other methods that require the use of RNA-synthesis inhibitors have been reported to influence the normal turnover of specific mRNAs [16,28]. The half-lives of PDGF A- and B-chain mRNAs were approx. 100 min in cultured renal mesangial cells. The half-life of PDGF A-chain mRNA in other cells using an indirect approach has been reported to vary from 102 min to 240 min [29,30], whereas the half-life for PDGF

B-chain mRNA varies from 40 to 204 min [29–32]. In the present work, we observed that the PDGF BB homodimer had no effect on PDGF A-chain mRNA stability whereas the B-chain mRNA half-life was increased by 1.5-fold. Thus, although the PDGF BB homodimer induces transcription of both PDGF A- and B-chain mRNAs, its effect on these mRNA stabilities is different.

The data in this paper provide the first demonstration that PDGF BB homodimer induces PDGF A- as well as PDGF B-chain gene transcription. Together with mRNA-stability data, these studies suggest that regulation of PDGF A-chain gene in response to PDGF BB homodimer is mediated at the level of gene transcription. The regulation of PDGF B-chain gene expression, on the other hand, appears to involve a co-ordinated effect at the level of transcription and mRNA stability. Transcription factors and *cis*-acting elements that mediate transcription events and the factors involved in regulating stability of PDGF A- and B-chain mRNA in mesangial cells remain to be identified. Recent studies analysing PDGF A-chain promoter have identified positive and negative regulatory elements [33]. One such positive element in the PDGF A-chain promoter is a functional serum response element that binds serum response factor when human foreskin fibroblasts are activated by PDGF itself [33]. It remains to be determined if mesangial cells express similar transcription factor(s) [33], as there is increasing evidence for tissue-specific distribution of these proteins [34]. Mesangial cells may provide a useful model for studying PDGF gene regulation.

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