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Regulation of the Human Platelet-derived Growth Factor B-chain Gene

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ABSTRACT

Human platelet-derived growth factor (PDGF) B chain is a cellular homologue of v-sis, closely involved in cell growth and oncogenicity. Analysis in this study showed the importance of not only the region near the TATAA box but also the fragment -355 bases or more upstream in the expression of the PDGF-B chain gene. However, tissue specificity was not clear in the 5' upstream region alone, and regulation by gene methylation or by elements other than in the 5' region seemed to be necessary. In A172 cells, the half-life of mRNA was much shorter in the PDGF-B chain (<2 hours) than in the PDGF-A chain (7 hours). Regulation of the PDGF-B chain gene may markedly differ from that of the PDGF-A chain gene not only at the transcriptional level but also at the post-transcriptional level.

***Key words**: PDGF-B chain gene, Transcription, Half life*

Human platelet-derived growth factor (PDGF), a major mitogen of mesenchymal cells, is a heterodimeric glycoprotein composed of A and B chains[5)]. The expression of each chain is regulated by different mechanisms[4,10,13-16,20,26,28)]. The PDGF-B chain gene is encoded by the c-sis gene, the normal cellular homologue of the v-sis oncogene of the simian sarcoma virus (SSV)[6,35)], and has been mapped to human chromosome 22q11[8)]. The PDGF-B chain is expressed in a large proportion of sarcoma and glioblastoma cell lines or tumor tissues[7)], and its oncogenicity has attracted attention. Recently, the genomic structure of the PDGF-B chain and the initiation site of mRNA have been clarified[22,24,33,34)]. However, little is known about the mechanism of the regulation of its gene. We analyzed the 5' regulatory region of the PDGF-B chain and determined the half-life of the mRNA of this chain.

MATERIALS AND METHODS

Cells and cell culture: Human embryonal rhabdomyosarcoma (RD), human glioblastoma (A172) and human epithelioid carcinoma (HeLa) cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (FCS). Human umbilical vein endothelial cells (HUVE) were grown in Hanks' modified medium with 10% fetal bovine serum and endothelial cell growth supplements. Human chronic myeloid leukemia cells (K562) were cultured in RPMI 1640 medium containing 10% FCS. Serum starvation was induced by culturing the cells in the presence of 0.2% FCS for 5 days or more. When indicated, the cells were incubated with the addition of 10% or 20% FCS (serum stimulation), 15 μg/ml cycloheximide (CHX), 1.6×10^{-8}M 12-O-tetradecanoyl phorbol-13-acetate (TPA), and 10 μg/ml Actinomycin D (AcD).

Plasmid constructions: 5'-PDGF-B CAT contains the 5' region of PDGF-B chain gene, corresponding to nucleotides -1361 - +90 (relative to the RNA initiation site) of the pSIS-1[24)]. 5'-PDGF-B, PstI-5 was constructed by partial digestion with PstI. CAT, Xh-S contains the 5' region of PDGF-A chain gene, corresponding to nucleotides -254 - +387 (relative to the RNA initiation site)[29)]. Each constructed fragment was inserted into the site upstream of the chloramphenicol acetyl transferase (CAT) gene in the 5'-3' orientation. PSV2CAT contains both the SV40 early promoter and the SV40 enhancer region. PA10CAT contains the SV40 early promoter and PSVOCAT contains no promoter region.

DNA transfection and CAT assay: About 24 hours before transfection, RD cells, A172 cells, HeLa cells and HUVE cells were seeded at 5.5×10^5 cells per 100-mm Petri dish. Twenty micrograms of each plasmid was transfected by the $CaPO_4$ method[12)]. After 3 hours, the cells were

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treated with 15% (v/v) glycerol in 20 mM Hepes buffer for 3 minutes, washed, and incubated for 48 hours with 0.2%, 10%, or 20% FCS. In K562 cells 1×10^7 cells in late log-phase growth were transfected with 20 μg plasmid and 125 μg carrier DNA by electroporation[32] at 225 volts and 600 microfarads. The cells were incubated for 24 hours with or without 1.6×10^{-8} M TPA. They were harvested, and 100 μg of lysates was assayed for CAT activity[11].

Gel retardation assay: Nuclear protein was extracted by the method of Shapiro et al[27]. After hypotonic treatment the suspended cells were destroyed using a Dounce homogenizer. The nucleus was obtained by centrifugation, and nuclear protein was extracted with ammonium sulfate. For the gel retardation assay, ^{32}P-labeled DNA fragment (25 K cpm), 5 μg of nuclear protein, and 5 μg of poly (dI-dC) were mixed in 25 μl of binding buffer (10 mM Hepes, pH 7.9; 60 mM KCl; 7.5 mM $MgCl_2$; 0.1 mM EDTA; 1 mM DTT; and 10% glycerol), incubated at room temperature for 30 minutes, and subjected to electrophoresis (10 V/cm) with 5% polyacrylamide gel.

RNA extraction and SI nuclease protection assays: Total cellular RNA was isolated by the acid phenol method[2] and coprecipitated in ethanol with the end-labeled DNA fragment (10^5 cpm), suspended in 15 ul of hybridization buffer (80% formamide/40 mM Pipes, pH 6.4/400 mM NaCl/1 mM EDTA), heated for 15 min at 90°C and hybridized overnight at 65°C. The DNA-RNA hybrid was diluted into 300 μl of SI nuclease buffer [280 mM NaCl/30 mM NaOAc, pH 4.4/4.5 mM Zn $(OAc)_2$ with sonicated salmon sperm DNA at 20 μg/ml] containing 200 units of SI nuclease and was incubated at 37°C for 30 min. The digestion was terminated with 75 μl of 2.5 M NH_4OAC/50 mM EDTA, and the protected fragments were recovered by ethanol precipitation, denatured and analyzed by electrophoresis in a 6% polyacrylamide sequencing gel. For A chain mRNA, the end-labeled Xho-Sau3AI fragment (-254 - +387) was used for the SI nuclease protection assay[29]. If A chain mRNA is synthesized at the initiation site and splicing site previously reported[29], 400 base protected fragment should be detected. For B chain mRNA, the end-labeled PstI-Bgl II fragment of the pSM-1[23] was used. If B chain mRNA is synthesized at the splicing site previously reported[22], 440 base protected fragment should be detected.

RESULTS

CAT assay of the 5' region of the PDGF-B chain gene (Fig. 1): In A172 cells expressing PDGF-A and -B chains, similar CAT activity was observed in 5'-PDGF-B CAT containing the B chain 5' re-

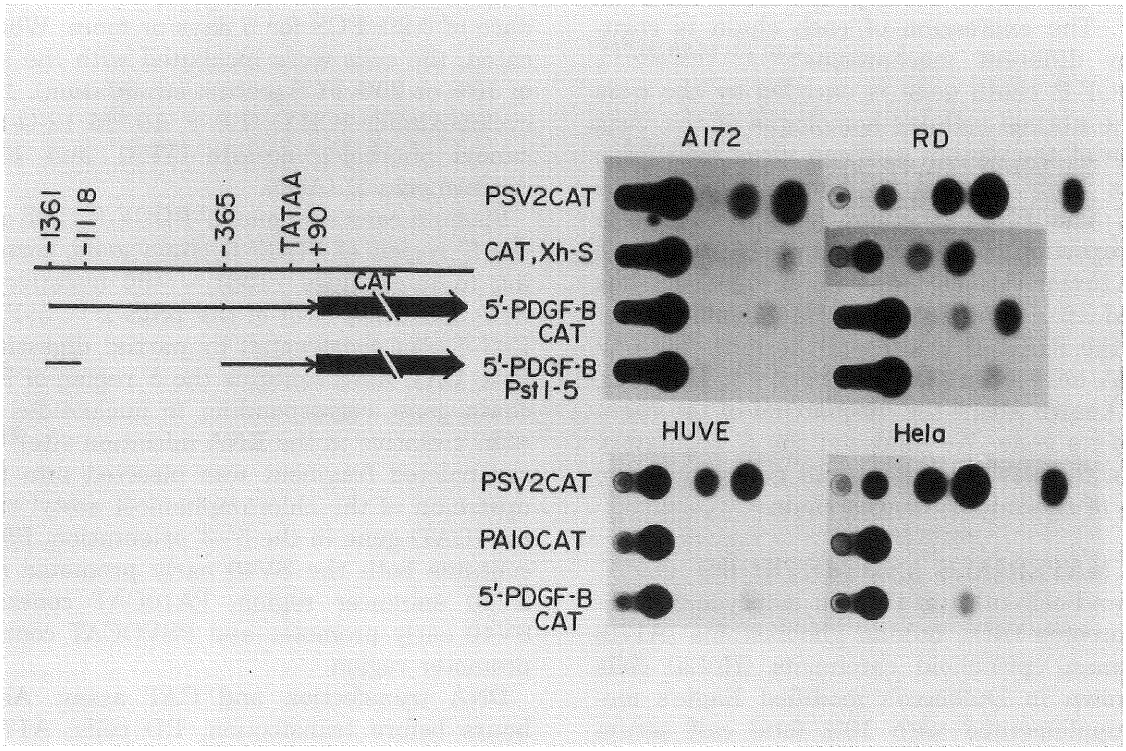

Fig. 1. Plasmids constraction and CAT assay
Human glioblastoma (A172) and human umbilical vein endothelial (HUVE) cells expressed both A and B chains. Human embryonal rhabdomyosarcoma (RD) and human epithelioid carcinoma (HeLa) cells expressed only A chain.
The nucleotide number is the relative position from the RNA initiation site.

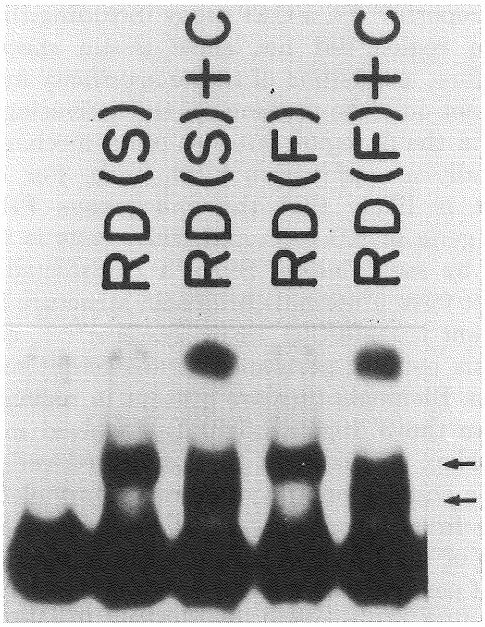

Fig. 2. Gel retardation assay
As the probe, 5'-PDGF-B fragment was used. Nuclear protein was extracted from RD cells at the state of serum starvation (S) and after treatment with FCS and CHX (F). A 15 volume of the competitor was used.

gion and CAT, Xh-S containing the A chain 5' region. In HUVE cells expressing B chain, the 5'-PDGF-B CAT was also positive. CAT activity was markedly decreased in 5'-PDGF-B, PstI-5 in which -365 - -1,118 from the initiation site were deleted. This finding suggests the presence of a potent transcription activator in the deleted fragment.

Tissue specificity of 5' regulatory region of the PDGF-B chain gene: Contrary to expectation, 5'-PDGF-B CAT was also positive in RD cells and HeLa cells in which PDGF-B chain RNA was not detected. CAT activity was markedly decreased in 5'-PDGF-B, PstI-5 in these cells as in A 172 cells. A gel retardation assay using the 5'-PDGF-B fragment and the nuclear protein of RD cells (Fig. 2) showed inhibition of band formation after addition of a competitor, and the appearance of a band with a low molecular weight. Thus, transcription was also activated by a nuclear protein that specifically binds to the 5' upstream region of the PDGF-B chain in RD cells in which PDGF-B chain was not expressed.

Responses of 5'-PDGF-B CAT to serum and TPA (Fig. 3): There was no element in the 5' upstream region that responded to serum in RD cells or to TPA in K562 cells.

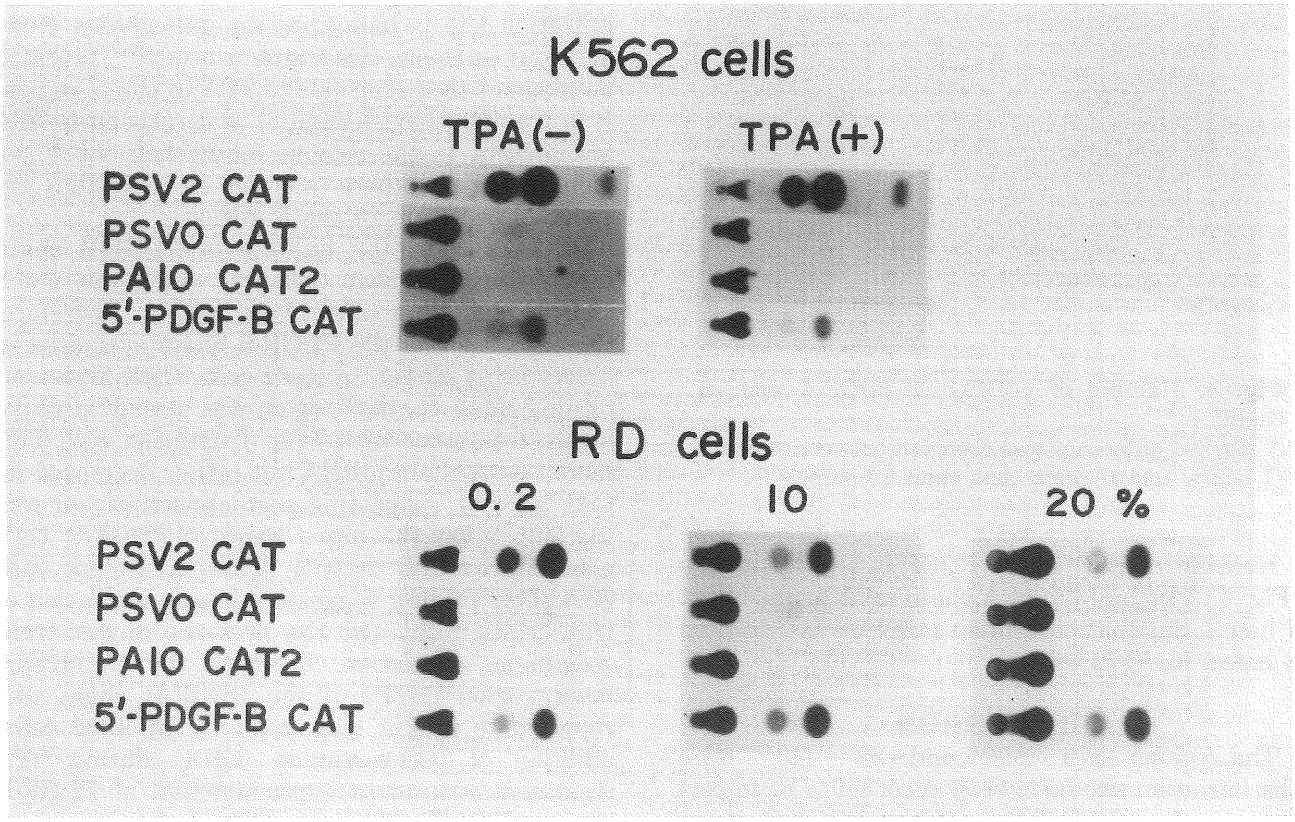

Fig. 3. CAT assay at the time of TPA stimulation (K562 cells) and serum stimulation (RD cells)
CAT activity was not affected by either stimulation.

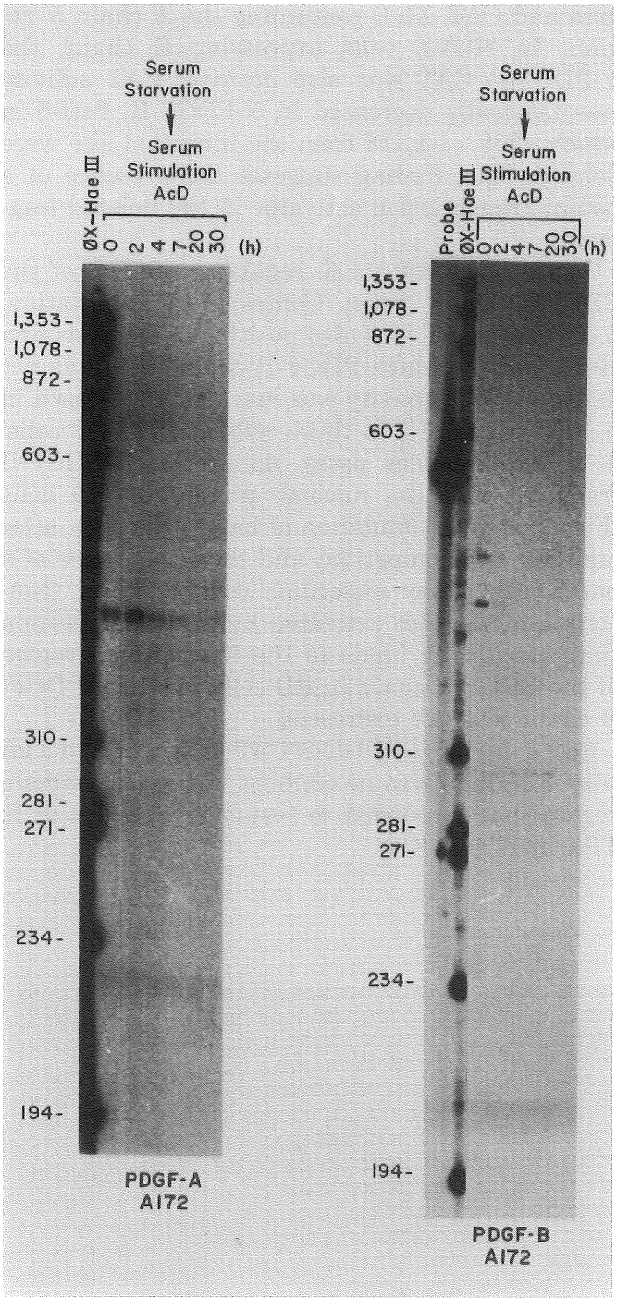

Fig. 4. Half-life of mRNAs of PDGF-A and -B chains.
A marked difference was observed between A chain (7 hours) and B chain (less than 2 hours).

Half-life of mRNAs of PDGF-A and -B chains (Fig. 4): The half-life of A chain mRNA was about 7 hours, but that of B chain mRNA was less than 2 hours in A172 cells.

DISCUSSION

The regions -375 - 99[25] and -83 - -40[21] from the initiation site have been reported to be important in the transcription of the PDGF-B chain gene. Our analysis showed the presence of more potent transcription activating element(s) in the fragment further upstream. However, as in previous reports[21,25], a CAT assay including this upstream region did not show tissue specificity. Therefore, acquisition of tissue specificity may require not only the presence of the activating fragment in the 5' region but also other mechanisms, especially a suppression mechanism. For example, it is likely that the endogenous PDGF-B chain gene in RD cells and HeLa cells is inactivated by methylation. Since a transfected gene can not form a normal chromatin structure in the transient transfection assay system[17,18], careful analysis using a permanent transfectant may be needed. Elements that are present in regions other than the 5' region might be involved in gene regulation. Franklin et al[9] identified cell type-specific positive and negative regulatory elements in the first intron of the PDGF-B chain. The 5' region of the PDGF-A chain shows a mosaic pattern of positive and negative regulatory elements, suggesting complicated regulation. Recently, we detected a fragment in the first intron of the PDGF-A chain that also suppresses the expression of the B chain[30].

K562 cells treated with TPA differentiate into megakaryocytes, and PDGF-A and -B chain mRNAs markedly increase[1,3,16]. A chain mRNA is increased primarily by a post-transcriptional mechanism[13,31], while B chain mRNA is increased by activation of transcription[4,13,21]. Pech et al[21] reported that the CCTCCTGGCGC sequence in the region -83 - -40 bases from the initiation site is important for inducibility. However, our analysis, like Ratner's study[25], revealed no element that responds to TPA in the 5' region. It is possible that element(s) that respond to TPA are present in the regions other than the 5' region. Similarly, there was no element that responded to serum in the 5' region.

In A172 cells, the half-life of PDGF-B chain mRNA was less than 2 hours, which was much shorter than that of A chain mRNA. Therefore, the expression of the PDGF-B chain is limited to a very short period. In K562 cells, CAT, Xh-S containing the A chain 5' region was already positive before treatment with TPA. Treatment with TPA increased A chain mRNA but rather decreased its CAT activity, suggesting post-transcriptional regulation[31]. On the other hand, 5'-PDGF-B CAT also showed CAT activity before treatment with TPA. This finding suggests not only activation of transcription but also the presence of post-transcriptional regulation. Recently, the 5'-untranslated region (UTR) of the PDGF-B chain was reported to inhibit translation[24]. In K562 cells, addition of cycloheximide (CHX) during TPA treatment results in superinduction of PDGF-B chain mRNA[19]. This was suggested to be due to a prolongation in the half-life of B chain mRNA[15,26]. Thus, the regulation of the PDGF-B chain markedly differed from that of the PDGF-A

chain not only at the transcriptional level but also at the post-transcriptional level.

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