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# Alternative Splicing of the Platelet-Derived Growth Factor A-Chain Transcript Occurs in Normal as Well as Tumor Cells and Is Conserved among Mammalian Species

BRONA MATOSKOVA,† FREDRIK RORSMAN, VIVEKA SVENSSON, AND CHRISTER BETSHOLTZ*

*Department of Pathology, University Hospital, S-751 85 Uppsala, Sweden*

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**Using a polymerase chain reaction approach, we have analyzed the alternative usage of the platelet-derived growth factor A-chain exon 6 in mRNA from various cell types. The results show that this sequence is utilized in a small fraction of the mRNA molecules in normal as well as transformed cells and that this phenomenon is conserved among mammalian species.**

Platelet-derived growth factor (PDGF) is a family of dimeric molecules made up of two different gene products, the A and B chains. All three possible configurations (PDGF-AA, PDGF-BB, and PDGF-AB) have been purified from natural sources and shown to be biologically active (3, 8, 11, 17). To date, two different PDGF receptors have been characterized. Both possess tyrosine kinase domains and form a subfamily of receptor tyrosine kinases together with the colony-stimulating factor 1 receptor and the c-*kit* proto-oncogene (15, 26). The three PDGF isoforms differ in their biological activities as well as in their binding to the two receptor types. PDGF-AA, which binds to only one of the two receptors (type A), has a lower mitogenic activity and lacks chemotactic as well as actin-reorganizing properties on human fibroblasts (9, 10, 17).

The genes for the two PDGF chains reside on different chromosomes; the B-chain gene resides on chromosome 22 (7), and the A-chain gene resides on chromosome 7 (3, 23). The genes are differentially expressed in tumor cell lines (3, 25) as well as in normal cells (13, 18, 21) and are therefore probably independently regulated.

Two forms of the A-chain transcript, differing by the presence or absence of a 69-base-pair (bp) segment, have been identified in a human glioma cell line (3, 19). The coding sequence is affected so that the longer transcript will encode an A-chain precursor with a 15-amino-acid carboxy-terminal extension with highly basic and hydrophilic properties. Characterization of the human A-chain gene showed that the 69-bp segment on the gene level constitutes a separate exon, number 6 (4, 19). The functional implications of the alternative usage of exon 6 in glioma cells is not known. Expression of cDNA clones corresponding to the two A-chain versions has yielded conflicting results regarding synthesis and secretion of the two forms (2, 5, 6). Furthermore, two reports have pointed out the absence of the longer A-chain mRNA in normal human endothelial cells and suggested that the alternative splicing is tumor specific (6, 24).

To investigate the occurrence of the alternative usage of exon 6 in PDGF A-chain transcripts, we have applied the enzymatic amplification technique known as polymerase chain reaction (PCR) (20) on cDNA generated from various cultured cells and tissues. We chose primers corresponding to sequences in exons 4 and 7 of the human PDGF A-chain gene. The outline of the primers and the expected amplification products are shown in Fig. 1. mRNA was prepared (1) and converted to single-stranded cDNA as described elsewhere (12). PCR performed with a thermostable *Thermus aquaticus* (*Taq*) DNA polymerase (Perkin-Elmer-Cetus Corp., Norwalk, Conn.; protocol according to specifications of the manufacturer) revealed two amplification products with sizes corresponding to the expected region of the A-chain transcript, including or excluding exon 6 (Fig. 2). Blotting to nitrocellulose membranes followed by sequential hybridization with an exon 6-specific DNA probe (an excised 500-bp genomic *Pst*I fragment [19], including exon 6 as the only coding sequence) and with a complete cDNA probe (3) confirmed that only the larger amplification product included exon 6-derived sequences, whereas both products hybridized with the cDNA probe (Fig. 3).

Since the large number of PCR cycles allows the amount of amplified product to plateau, this method is not informative regarding the total levels of PDGF-A mRNA in the tested cells; instead, the method gives an estimate of the relative abundance of the two alternatively spliced transcripts. The smaller amplification product was at least 10-fold more abundant than the larger one in all tested samples, as judged from the ethidium bromide stainings. We believe

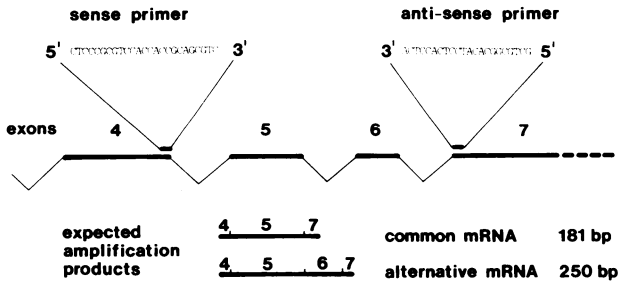


FIG. 1. Schematical outline of 3′ end of human PDGF A-chain gene and oligonucleotide primers used for amplification. Data on the gene and cDNA structure are from references 3 and 19. Depending on the presence or absence of the exon 6-encoded 69 bp in the mRNA molecules, two amplification products with sizes of 181 and 250 bp are expected when the amplification is performed with single-stranded cDNA as template.

* Corresponding author.
† Present address: Cancer Research Institute, Slovak Academy of Sciences, 812 32 Bratislava, Czechoslovakia.

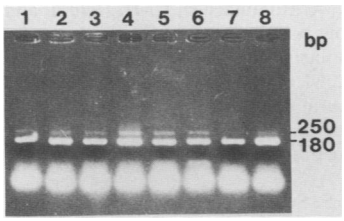


FIG. 2. Analysis of PCR amplification products by agarose gel electrophoresis and ethidium bromide staining. Two bands with approximate sizes of 180 and 250 bp appear following amplification. The relative amounts of the smaller and larger bands vary slightly from 5:1 to 20:1. RNA was prepared from human macrophages (lane 1), PDGF-stimulated human skin fibroblasts (lane 2), simian sarcoma virus-transformed human fibroblasts (lane 3), human osteosarcoma cell line U-2 OS (lane 4), human malignant melanoma cell line WM 266-4 (lane 5), human large-cell lung cancer cell line U-1810 (lane 6), transforming growth factor β-stimulated human skin fibroblasts (lane 7), and human glioma cell line U-343 MGa clone 2:6 (lane 8).

that this illustrates the proportions of the alternatively spliced mRNAs. Even if a bias in favor of a shorter fragment would be expected when two fragments are coamplified, the figures obtained agree with what has been indicated by

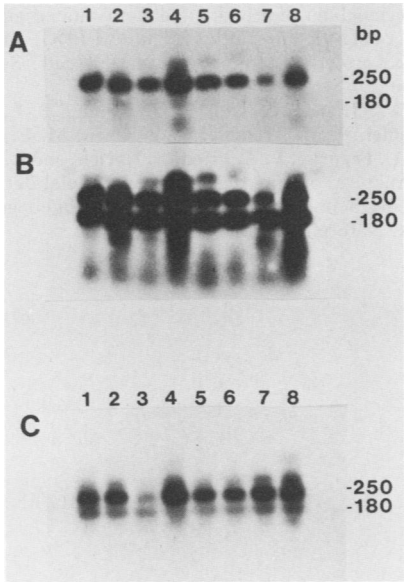


FIG. 3. Southern blot hybridization analysis of PCR amplification products. (A) Hybridization against an exon 6-specific probe. Lane 1, Human macrophages; lane 2, PDGF-stimulated human skin fibroblasts; lane 3, simian sarcoma virus-transformed human fibroblasts; lane 4, human osteosarcoma cell line U-2 OS; lane 5, human melanoma cell line WM 266-4; lane 6, human lung cancer cell line U-1810; lane 7, transforming growth factor β-stimulated human skin fibroblasts; lane 8, human glioma cell line U-343 MGa clone 2:6. (B) Rehybridization of the same blot as in panel A with a full-length PDGF A-chain cDNA probe. (C) Hybridization against an exon 6-specific probe. Apart from the exon 6-specific 250-bp band, some residual radioactivity from a previous hybridization against a full-length cDNA probe is seen in the 181-bp band. Lane 1, Human osteosarcoma cell line U-2 OS; lane 2, human glioma cell line U-343 MGa clone 2:6; lane 3, human umbilical-vein endothelial cells; lane 4, porcine endothelial cells; lane 5, porcine thyroid tissue; lane 6, rat lung tissue; lane 7, rat brain tissue; lane 8, simian virus 40-transformed hamster fibroblast cell line SV28.

cDNA cloning (3, 6, 24), i.e., that the A-chain transcript variant lacking the exon 6-derived sequence is most abundant. The novel finding is that this type of alternative splicing occurs in all types of PDGF A-chain mRNA-expressing cells and is not tumor specific, as has previously been suggested (6, 24). Human umbilical-vein endothelial cells, which have been suggested to express the shorter version of the PDGF A-chain transcript only (6, 24), were found in this study to express the longer transcript as well, but at relatively lower levels than did the other tested cell types (Fig. 3). However, this does not seem to be a specific endothelial trait, since porcine endothelial cells expressed long-PDGF mRNA in as high proportions as did other tested cells (Fig. 2 and 3).

In addition to endothelial cells, other normal human cells (such as fibroblasts and macrophages) which have previously been shown to express PDGF A-chain mRNA following mitogenic and functional activation (14, 18, 22) were also shown here to express both types of A-chain transcripts (Fig. 2 and 3).

The biological relevance of the alternative usage of exon 6 in the A-chain mRNA is not known. The presence and alternative usage of this sequence in such other mammalian species as rats, hamsters, and pigs (Fig. 2 and 3) indicate an evolutionarily conserved function. During the preparation of this article, Mercola and collaborators reported the structure of *Xenopus laevis* PDGF A chain (16). The primary structure is closely similar to that of the human PDGF A chain; interestingly, sequences closely homologous to human exon 6 were also identified in a proportion of the mRNA molecules (16). Thus, the exon 6 sequence appears to be conserved in amphibians as well.

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