

The long 3'-untranslated regions of the PDGF-A and -B mRNAs are only distantly related

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A cDNA clone of about 2300 base pairs was prepared from the human osteosarcoma cell line U-2 OS by hybridization with a 22-mer oligonucleotide complementary to the NH₂-terminus of PDGF-A. Restriction and sequence analysis showed that this clone contains the entire coding region for PDGF-A and a long 3'-untranslated region which is only distantly related to that in the mRNA of PDGF-B.

Platelet-derived growth factor; cDNA; Oncogene; (Tumor cell)

1. INTRODUCTION

Human platelet-derived growth factor (PDGF) is composed of dimers of homologous polypeptide chains, termed A and B [1-3]. It is unknown, whether human PDGF is a heterodimer or a mixture of homodimers, but it has been shown that homodimers of both types (A-A or B-B) express the full biological activity [4-6].

The PDGF-B gene (c-cis) has been mapped on the long arm of chromosome 22, the PDGF-A gene on chromosome 7 [7]. The different location of the two genes has suggested a different regulation of the transcription. Indeed in numerous tumor cells the two genes are expressed to quite a different extent [7-9]. Interestingly enough only those cells that express the PDGF-A gene secrete a PDGF-like mitogen into the medium which has been identified as PDGF-A homodimer. There is some evidence that untranslated 3'-regions might have some function in expression and/or stability of the mRNA [10-12]. To investigate a possible role of the unusually long 3'-moieties of the PDGF

mRNAs we have cloned and sequenced the mRNA of PDGF-A.

2. MATERIALS AND METHODS

2.1. Materials

Restriction endonucleases, T₄ ligase, T₄ DNA polymerase, DNA polymerase I and the large fragment of DNA polymerase I (Klenow fragment) were purchased from Boehringer. Sequenase was from USB. Nucleotides were from Pharmacia Biöchemicals. Antibiotics were purchased from Sigma. Radioactive nucleotides were from Amersham. *E. coli* strains 5K or JM103 were used for transformation by pBR322 or M13 vectors, respectively.

2.2. Cell culture

Osteosarcoma cells (U-2 OS) were kindly provided by Dr B. Westermark (Uppsala, Sweden) and were grown as described [13].

2.3. Construction and screening of a cDNA library

The same cDNA library was used that was constructed for the isolation of the PDGF-B gene [14]. This library was screened by standard techniques [15] using a synthetic 22-mer oligonucleotide complementary to the amino-terminus of the PDGF-A chain.

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3. RESULTS

Screening of 80 000 colonies of the U-2 OS cDNA library with a synthetic 22-mer oligonucleotide primer resulted in the isolation of two plasmids pPGF-1 and pPGF-2 which contained inserts coding for PDGF-A. pPGF-1 is regarding its length of 1.2 kb very similar to clone D1 [7] and contains the entire coding region for the prepro PDGF-A (not shown). Plasmid pPGF-2 has an insert of ≈ 2.3 kb. Restriction analysis (fig.1) revealed that the additional 1000 bases are located at the 3'-end of the coding region. Thus like in the PDGF-B mRNA there is a long untranslated 3'-region.

Fig.2 shows the DNA-sequence of the insert of plasmid pPGF-2. The sequence extends 15 bases further at the 5'-end compared to that of the reported clone D1 [7]. Some alterations have been found that may reflect small differences between the two cell lines from which the cDNA clones were isolated. Both clones (pPGF-1 and pPGF-2) exhibit a deletion of 69 bases starting from position 984, predicting an A chain precursor 15 residues smaller and lacking the basic C-terminal region. The d(GA)_n stretch behind the coding region was considerably longer [7]. The following A-rich region differed markedly from that in clone D1.

Two extremely T-rich regions are located be-

A)	AGAGAGAGAGAGAGA	PDGF-A
	AGAGTGTGAGAGAGA	PDGF-B
	↓ ↓ ↓	
B)	TTTTTTTGTGTTTTGTTTTG	PDGF-A
	TTCTTTTCGTTTTCGTTTTG	PDGF-B
C)	TTTGTTTTTT GTAAA	PDGF-A
	TTTATTTTTTAAATGTAAAA	PDGF-B

Fig.3. Possible homologous region in the mRNAs of PDGF-A and PDGF-B. (A) region 1, ≈ 250 bases from the end of coding region; (B) region 2, ≈ 600 bases from the end of the coding region; (C) region 3, ≈ 80 bases from the 3'-end.

tween position 1560 and 1700. The first stretch comprises about 60 T-residues interrupted by four G-residues. The second one contains 31 uninterrupted T residues. The appearance of the polyadenylation consensus sequence ATTAAA at the 3'-end indicates that the entire 3'-portion has been cloned.

4. DISCUSSION

Multiple PDGF-A mRNA transcripts in the range of 2.8–1.7 kb are visualized after Northern Blot analysis of several cell lines [7–9]. Also, the splicing pattern differs between the investigated cell lines [8] as indicated by the different intensities of the bands. In human cells the most abundant species exhibit a size of 2.3 kb. This length agrees well with the cDNA insert of 2.3 kb found in clone pPGF-2.

The most intriguing features of the PDGF-A mRNA sequence are the extremely long T-stretches in the untranslated 3'-region. Especially notable is the uninterrupted stretch of 31 T residues starting from position 1668 (fig.2). This sequence might form a stable secondary structure with the poly(A) tail preventing the first strand synthesis from the 3'-end. Thus priming might occur internally from the A-rich region shortly behind the translated region as observed previously [7] and with clone pPGF-1.

PDGF-A and PDGF-B are regulated differently in a variety of tumor cells. Very recent findings have shown, that the 3'-noncoding sequences may play an important role in controlling gene expression [12]. We therefore compared the sequences of the untranslated 3'-regions of the PDGF mRNAs. There are, if any, only distantly related sequences in the two mRNA species (fig.3). The first region (≈ 250 to 300 nucleotides 3' from the coding region) comprises the repeat of d(GA). The second homologous region (≈ 600 nucleotides from the coding region) contains the first T-rich region in the PDGF-A sequence. The similar spacing of the G residues in both sequences might indicate a homology and a common function.

It has been discussed that certain sequences in proximity to the 3'-end of a mRNA are conserved amongst different proteins including PDGF-B, nerve growth factor, interferon- β , and

interleukin-2 [16]. Such sequence are largely absent in the PDGF-A mRNA. Possibly a homologous sequence \approx 80 residues from the 3'-end may be present present (fig.3). Furthermore, the two mRNA species differ markedly in their A-T content in the 3'-proximal region. Based on the highly conserved amino acid sequence of the mature PDGF-A and PDGF-B proteins one has to assume that the two forms originate from a gene duplication. Interestingly, the A- and B-chain gene have acquired different chromosomal localizations and considerable alterations in the noncoding regions have occurred, which might have some influence on the stability of the mRNAs and/or on the regulation of transcription. It remains to be determined if these alteration have any significance regarding regulation and biological properties of the two PDGF species.

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