

The human osteosarcoma cell line U-2 OS expresses a 3.8 kilobase mRNA which codes for the sequence of the PDGF-B chain

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A cDNA clone of about 2500 base pairs was prepared from the human osteosarcoma cell line U-2 OS by hybridizing with a *v-sis* probe. Sequence analysis showed that this cDNA contains the coding region for the PDGF-B chain. Here we report that the mitogen secreted by these osteosarcoma cells contains the PDGF-B chain and is probably a homodimer of two B-chains.

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

1. INTRODUCTION

Platelet-derived growth factor (PDGF) is the major mitogen in serum for connective tissue-derived cells in culture (review [1]). The protein has an apparent M_r of 30000 and is a dimer of two chains linked by disulfide bonds. Protein sequence analysis of PDGF from human platelets revealed the presence of two types of sequences [2,3], which are about 60% homologous (type A and B). Recent studies have demonstrated that the sequence of PDGF-B is almost identical with the transforming oncogene product $p28^{v-sis}$ of the simian sarcoma virus [2-7].

It has been proposed that the secretion of mitogenic factors by transformed cells is important in the establishment and maintenance of the transformed state. Human osteosarcoma, fibrosarcoma and glioblastoma cells in culture have been shown to synthesize and release into their medium biologically active PDGF-like polypeptides [8-12]. These human malignant cells contain RNAs that hybridize to *v-sis* probes [10-14]. The *c-sis* locus is furthermore expressed in various endothelial cell cultures, developing human placenta, cultured arterial smooth muscle

cells and a number of T-lymphoid cell lines transformed by HTLV-I or II [15-19].

To establish the identity of the PDGF-like mitogen secreted by the human osteosarcoma cell line U2-OS [14] to the sequence of PDGF-B we have cloned and sequenced part of the cDNA hybridizing with the *v-sis* DNA.

2. MATERIALS AND METHODS

2.1. Materials

Restriction endonucleases, T_4 DNA ligase, 5'-polynucleotide kinase, DNA polymerase I and the large fragment of DNA polymerase I (Klenow fragment) were purchased from Boehringer, Mannheim. RNase H and *E. coli* ligase were obtained from BRL and AMV reverse transcriptase was from Stehelin. Terminal deoxynucleotidyltransferase, dNTPs and ddNTPs were from Pharmacia P-L Biochemicals. Chloramphenicol, ampicillin and tetracyclin were purchased from Sigma. [α - 32 P]dATP and [α - 35 S]dATP were obtained from Amersham. *E. coli* strains 5K and JM 103 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 [14].

2.3. Isolation of RNA and Northern blotting

Total RNA was isolated from cells according to [20] and poly(A)⁺ RNA was selected by two passages over an oligo(dT)-cellulose column.

For Northern blotting RNA was denatured for 5 min at 65°C in 50% formamide, 2.2 M formaldehyde and 10 mM sodium phosphate, pH 7.0, and subsequently electrophoresed in 1.2% agarose slab gels containing 0.5 M formaldehyde. Transfer to nitrocellulose sheets was done as described [21]. RNAs containing *sis*-related sequences were identified by hybridization with 1×10^6 cpm/ml ³²P-nick-translated 1.2 kb *Pst*I fragment (1×10^8 cpm/ μ g) of the retroviral transforming *v-sis* gene [4]. Hybridization was performed for 20 h at 65°C in a mixture containing $2 \times$ SSC ($1 \times$ SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0), $1 \times$ Denhard's solution (0.1% SDS, 0.1%

Na₂P₂O₇, 2 mM EDTA, 5% dextran sulfate and 50 μ g/ml denatured salmon sperm DNA). The nitrocellulose sheets were washed twice for 15 min at 65°C with $0.4 \times$ SSC, 0.1% SDS and 2 mM EDTA. *sis*-related transcripts were visualized by autoradiography using Kodak XAR-5 films and an intensifier screen at -70°C for 5-7 days.

2.4. Construction and screening of a cDNA library

cDNA from poly(A)⁺ RNA from U-2 OS cells was synthesized according to [22,23] using oligo(dT)₁₂₋₁₈ as a primer for the first strand synthesis. Double strand cDNA was size fractionated on a 1.5% low-melting agarose gel, cDNAs > 900 base pairs were isolated as described [24], and tailed with (dG) residues. The (dG)-tailed cDNA was annealed to (dC)-tailed *Eco*RV cut pBR322 and was used to transform *E. coli* strain 5K according to [25,26]. About 160 000 transformants were obtained from 23 ng cDNA. Transformants containing *sis*-related sequences were identified by colony hybridization [22]. For hybridization 2×10^6 cpm (1×10^8 cpm/ μ g) nick-translated *v-sis* probe was applied per 132 mm diameter nylon filter in $2 \times$ SSC at 65°C for 11 h. Filters were washed at 65°C with a final wash in 0.2% SSC. Autoradiography was performed at -70°C with an intensifier screen for 1 day.

2.5. DNA sequencing

The 1.9 kb *Bam*HI fragment from clone pMVW-2 containing the *v-sis* homologous region was subcloned into *Bam*HI-digested M13mp18 [27]. After digestion with *Pst*I/*Sal*I the RFDNA was shortened with exonuclease III as described [28]. RFDNA was then sequenced in one direction from multiple overlapping subclones [29,30] using specific oligonucleotide primers.

3. RESULTS

A prominent *v-sis* hybridizing band of about 3.8 kb was detected in polyadenylated RNA from cultured human osteosarcoma cells U-2 OS (fig. 1). This band is of similar size to RNA species isolated from various other cell lines including cultured endothelial cells [10-15]. In contrast to previous reports [10] we could detect only a single band in Northern blots. Smaller species of about 2.7 kb were missing.

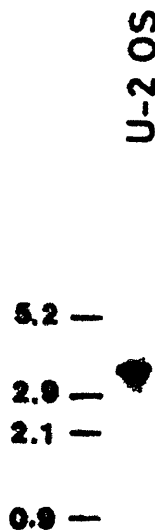


Fig. 1. Northern blot analysis of RNA from U2-OS cells. 10 μ g RNA were separated by electrophoresis in agarose (cf. section 2), transferred to nitrocellulose sheets and hybridized with a ³²P-labelled 1.2 kb *Pst*I *v-sis* cDNA fragment. Autoradiography was done for 7 days. On the left the molecular masses of RNA standards are given in kb.

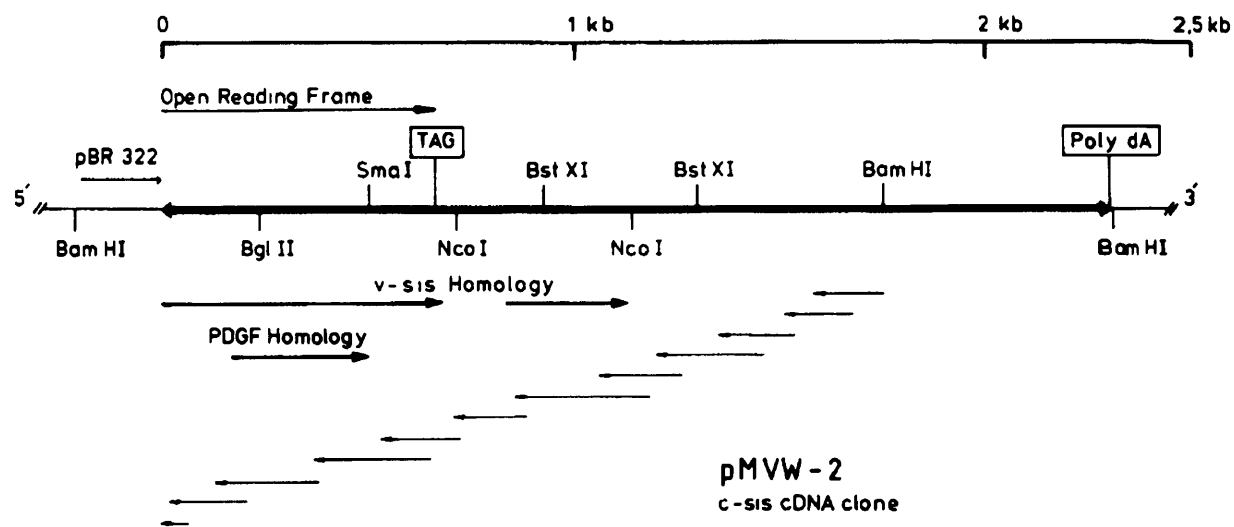


Fig.2. Restriction map of the c-sis cDNA clone pMVW-2. The thick, double-ended arrow represents the c-sis cDNA insert in pMVW-2. The insert is flanked on the left and right by restriction sites from the vector pBR322. The v-sis and PDGF-B homologous regions are indicated by bold arrows. The small arrows running from right to left indicate the fragments used for M13 sequencing.

A cDNA library was constructed with the vector pBR322 using polyadenylated RNA from the cell line U-2 OS. Clone pMVW-2 was isolated using a v-sis probe [13]. The organization and restriction map of this clone is shown in fig.2. In this clone a 2.5 kb polyadenylated cDNA fragment is inserted into the *EcoRV* site of pBR322.

The sequence of the large *Bam*HI fragment is shown in fig.3. A v-sis homologous region extends from nucleotide 1 to 1034. In this region a stretch from nucleotide 682 to 828 is present which is not homologous to v-sis. This is most likely due to the utilization of a different splice acceptor site in the human cells from which the cDNA was obtained compared to the woolly monkey cells from which simian sarcoma virus was originally isolated. A long uninterrupted reading frame is present from nucleotide 1 to 648 including the stop codon TAG. Within this reading frame a stretch is located whose predicted amino acid sequence is identical with that of the PDGF-B chain with the exception of two positions. These two positions differing from the protein sequence of PDGF-B have been interpreted to reflect variants [7].

The sequence coding for the PDGF-B chain was identical to that of normal human c-sis gene [5-7,15] and to the corresponding region in the plasmid pSM-1 from HUT 102 cells [16,17]. We

found some differences in the 3'-noncoding region. The reason for these minor alterations is unclear and might be due to small differences between species.

4. DISCUSSION

We have cloned a 2.5 kb fragment of the 3.8 kb mRNA hybridizing with the v-sis probe. Our studies establish that this 3.8 kb mRNA specifically expressed in various virally or nonvirally transformed tumor cells (e.g. osteosarcoma, fibrosarcoma, glioblastoma cells) contains the coding region for the precursor of the PDGF-B chain. Since our clone does not extend far enough to the 5'-region to cover a possible start codon we can make neither judgements about the full length of the PDGF-B precursor nor any statements as to whether PDGF-A is also encoded on this long mRNA. On the other hand, the DNA sequences from HUT 102 cells and from human endothelial cells are almost identical with the sequence from U-2 OS cells and it is thus reasonable to assume that translation will start from the identical position yielding a preproPDGF-B of 27281 Da.

There are no coding regions for PDGF-A in the 3'-flanking regions of pMVW-2 or in pSM-1 (HUT 102 cells), nor are there any coding regions

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1- 60 | PRO GLU GLU LEU TYR GLU MET LEU SER ASP HIS SER ILE ARG SER PHE ASP ASP LEU GLN
| c c c g a g g a g c t t t a t g a g a t i g c t g a g t g a c c a c c t c g a t c c | g c t c c t t t g a t t g a t c t c c a a |
61- 120 | ARG LEU HIS GLY ASP PRO GLY GLU GLU ASP GLY ALA GLU LEU ASP LEU ASH MET THR
| c g c c t g c t g c i a c g g a g a c c c c | c g g a g g a g c a l g a t g g g g c c g | a g t t g g a c c t i g a a c a t g a c c |
121- 180 | ARG SER HIS SER GLY GLY GLU LEU GLU SER LEU ALA ARG GLY ARG ARG SER LEU GLY SER
| c g c t c c a c t i c t g g a g g c a i g c t g g a g a g c | t t g g c t c g t g | g a a g a a g g a g | c c t g g g t t c c |
181- 240 | LEU THR ILE ALA GLU PRO ALA MET ILE ALA GLU CYS LYS THR ARG THR GLU VAL PHE GLU
| c t g a c c a t t g i c t g a g c c g g c | a t g a t c g c c | g a g t g c a a g a | c g c g c a c c g a | g g t g t t c g a g |
241- 300 | ILE SER ARG ARG LEU ILE ASP ARG THR ASH ALA ASH PHE LEU VAL TRP PRO PRO CYS VAL
| a t c t c c c g g c | g c t c a t a g a | c g c a c c a a c | g c c a a c t t c c | t g g t g t g g c c | g c c c t g t g t g |
301- 360 | GLU VAL GLN ARG CYS SER GLY CYS CYS ASH ASH ARG ASH VAL GLN CYS ARG PRO THR GLN
| g a g g t g c a g c i g c t g c t c c g g | c t g c t g c a a c | a a c c g c a a c g | t t g c a g t g c g | c c c a c c c a g |
361- 420 | VAL GLN LEU ARG PRO VAL GLN VAL ARG LYS ILE GLU ILE VAL ARG LYS LYS PRO ILE PHE
| g t g c a g c t g c i g a c c t g t c c a | g g t g a g a a g i a t c g a g a t t g | t g c g g a a g a a | g c c a a t c t t t |
421- 480 | LYS LYS ALA THR VAL THR LEU GLU ASP HIS LEU ALA CYS LYS CYS GLU THR VAL ALA ALA
| a a g a a g g c c a i c g g t g a c g c t | g g a a g a c c a c | c t g g c a t g c a | a g t g t g a g a c | a g t g g c a g c t |
481- 540 | ALA ARG PRO VAL THR ARG SER PRO GLY GLY SER GLN GLU GLN ARG ALA LYS THR PRO GLN
| g c a a g g c c t g i t g a c c | c g a a g i c c g g a g | c t g c t c c a a c | c c a a g g a g c | a g c a a g c c a a | a a c c c c a a g |
541- 600 | THR ARG VAL THR ILE ARG THR VAL ARG VAL ARG ARG PRO PRO LYS GLY LYS HIS ARG LYS
| a c t c g g g t g a i c a a t t c g g a c | g t g c g a g t c | c g c c g g c c c | c a a g g c a a | g c a c c g g a a a |
601- 660 | PHE LYS HIS THR HIS ASP LYS THR ALA LEU LYS GLU THR LEU GLY ALA
| t t c a a g c a c a i c g c a t g a c a a | g a f g g c a c t e g | a a g g a g a c c c | t t g g a g c c t a | g g g g c a t c g g |
661- 720 | c a g g a g a g t g i t g t g g g c a g g | g t t a t t a a t | a t g g t a t t t g | c t g t a t t g c c | c c a t g g g g c |
721- 780 | c t t c g g a g t . . . | a t a a t a t t g t | t c c c t c g t c | c g t c t y t c t c | g a t g c c t g a t t | c g g a c g g c c |
781- 840 | a a t g g t g c t t | c c c c a c c c c | t c c a c g t g t c | c g t c c a c c c t | t c c a t c a g c g | g g t c t c c t c c |
841- 900 | c a g g g g c c t c | c g g g c t c t t g | c c a g c a g c t | c a g a a g a a a | a a g a a g g a c t | g a a c t c c a t c |
901- 960 | g c c a t c t t c t | t c c c t a a c t | c a a g a a c t t | g g g a t a a g a g | t g t g a g a g a g | a c t g a t g g g g |
961-1020 | t c g c t c t t t g | g g g a a a c g g | g t c c t t c c c | t g c a c c t g g c | c t g g g c c a c a | c c t g a g c g c t |
1021-1080 | g t g g a c t g t c | t g a g g a g c | c t g a g a c c t | c t a g c a t a g | c c t g c c t g a t | c c c t g a c c c |
1081-1140 | c t g g c c a g c | t c t g a g g g a g | g c a c c t c c a g | g c a g g c c a g g | c a g g c t g c c | t c g g a c t c c a |
1141-1200 | t g g c t a a g a c | c a c a g a c g g g | c a c a c a g a c t | g a g a a a a c | c c t c c c a c g g | t g c c c a a a c a |
1201-1260 | c c a g t c a c a c | c g t c t c c c t g | g t g c c t c t g t | g c a a g t g g c | t t c t t t c g t | t t c g t t t t g |
1261-1320 | a a g a c g t g g a | c t c c t c t t g g | t g g g t g t g g c | c a g c a c a c c a | a t g g c t g g g t | g c c c t c t c a g |
1321-1380 | g t g g g t a g a | g a t g g a g t t | g c t g t t g a g g | t g g c t g t a g a | t g g t g a c c t g | g t a t c c c c t |
1381-1440 | g c c c c t g c c | a c c c t t c c t | c c c a c a c t c | c a c t c g a t t | c a c c t c t c c | t c t g g t t c c t |
1441-1500 | t t c a t c t c t c | t a c c t c c a c c | c t g c a t t t t c | c t c t t g t c c t | g g c c c t t c a g | t c t g c t c c a c |
1501-1560 | c a a g g g g c t c | t t g a a c c c t | t a t t a a g g c c | c c a g a t g a t c | c c a g t e a c t c | c t c t c t a g g g |
1561-1620 | c a g a a g a c t a | g a g g c c a g g g | c a g c a a g g g a | c c t g c t c a t c | a t a t t c c a a c | c c a g c c a c g a |
1621-1680 | c t g c c a t g t a | a g g t t g t g c a | g g t g t g t a c t | g c a c a a g g a c | a t t g t a t g c a | g g g a g c a c t g |
1681-1740 | t t c a c a t c a t | a g a t a a a g c t | g a t t t g t a t | t t a t t a t g a | c a a t t t c t g g | c a g a t g t a g g |
1741-1798 | t a a a g a g g a a | a a g g a t c c

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Fig.3. Sequence of the large *Bam*HI fragment of the clone pMVW-2. The predicted amino acid sequence of the largest open reading frame is shown and the amino acid residues identical to those determined by protein sequence analysis for PDGF-B are underlined. Alterations in the DNA sequence from that of pSM-1 are indicated by dotted lines.

in the 5'-flanking regions of pSM-1 or pB2-1 (human endothelial cells). It thus appears that the 3.8 kb mRNA detected by v-sis hybridization encodes only the PDGF-B chain.

PDGF purified from human platelets contains a second polypeptide (PDGF-A) that is less homologous to p28^{v-sis} [2,3], and it has been proposed that human PDGF is predominantly a 1:1 disulfide-linked heterodimer of A and B chains [31,32]. On the other hand, porcine PDGF seems to be a homodimer of two B-chains [33]. Our data

favour the model that the mitogen secreted by the U-2 OS cells is a homodimer composed of B-chains. But so far the genetic locus for the A-chains has not been identified and it is unknown whether this locus is coexpressed with that for the B-chains. Assuming such a mechanism, the formation of heterodimers would be possible.

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