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Splicing of the platelet-derived-growth-factor A-chain mRNA in human malignant mesothelioma cell lines and regulation of its expression

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Platelet-derived-growth-factor (PDGF) A-chain transcripts differing in the presence or absence of an alternative exon-derived sequence have been described. In some publications, the presence of PDGF A-chain transcripts with this exon-6-derived sequence was suggested to be tumour specific. However, in this paper it was shown by reverse-transcription polymerase-chain-reaction (PCR) analysis that both normal mesothelial cells and malignant mesothelioma cell lines predominantly express the PDGF A-chain transcript without the exon-6-derived sequence. This sequence encodes a cell-retention signal, which means that the PDGF A-chain protein is most likely to be secreted by both cell types. In cultured normal mesothelial cells, the secreted PDGF A-chain protein might be involved in autocrine growth stimulation via PDGF α receptors. However, human malignant mesothelioma cell lines only possess PDGF β receptors. If this also holds true *in vivo*, the PDGF A-chain protein produced and secreted by malignant mesothelial cells might have a paracrine function.

In a previous paper, we described elevated expression of the PDGF A-chain transcript in human malignant mesothelioma cell lines, compared to normal mesothelial cells. In this paper, the possible reason for this elevation was studied. First, alterations at the genomic level were considered, but cytogenetic and Southern-blot analysis revealed neither consistent chromosomal aberrations, amplification nor structural rearrangement of the PDGF A-chain gene in the malignant cells. Possible differences in transcription rate of the PDGF A-chain gene, and stability of the transcript between normal and malignant cells, were therefore studied. The presence of a protein-synthesis inhibitor, cycloheximide, in the culture medium did not significantly influence the PDGF A-chain mRNA level in normal mesothelial and malignant mesothelioma cell lines. Furthermore, nuclear run-off analysis showed that nuclear PDGF A-chain mRNA levels varied in both cell types to the same extent as the levels observed in Northern blots. Taken together, this suggests that increased transcription is the most probable mechanism for the elevated mRNA level of the PDGF A-chain gene in human malignant mesothelioma cell lines.

Platelet-derived growth factor (PDGF) is composed of homodimers or heterodimers of two polypeptide chains, denoted A and B. These polypeptides are encoded by two distinct genes, which show a high degree of similarity (Betsholtz et al., 1986). All three dimeric combinations (AA, AB and BB) have been identified (Stroobant and Waterfield, 1984; Heldin et al., 1986; Hammacher et al., 1988). Based on ligand-binding and cross-competition analysis, two different PDGF receptors, α and β, have been described (Hart et al., 1988; Heldin et al., 1988). The structure of these receptors is quite similar, showing most variation in the extracellular binding domain (Claesson-Welsh et al., 1988, 1989; Matsui et al., 1989). The two receptor subtypes show different affinities for the dimeric PDGF isoforms. The PDGF α receptor binds all three forms with high affinity, whereas the β-receptor subtype only binds PDGF BB with high affinity (Claesson-Welsh et al., 1988, 1989; Hammacher et al., 1989; Seifert et al., 1989). The reports on the ability of PDGF AB to bind to PDGF β receptors are conflicting (Hammacher et al., 1989; Seifert et al., 1989; Grotendorst et al., 1991; Heidaran et al., 1991; Drozdoff and Pledger, 1991).

Although originally isolated from blood platelets, PDGF seems also to be produced by several other cell types, e.g. endothelial cells and smooth muscle cells. Furthermore, expression of one or both of the PDGF chains has been reported for a variety of tumour cell types, such as osteosarcoma and glioblastoma (reviewed in Heldin and Westermark, 1990; Raines et al., 1990).

Human malignant mesothelioma is a tumour of mesodermal origin and is predominantly found in the pleura. The incidence of malignant mesothelioma is strongly associated with asbestos exposure (Wagner et al., 1960). Malignant mesothelioma is thought to develop from cells of mesothelial

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Abbreviations. PDGF, platelet-derived growth factor; PCR, polymerase chain reaction.

*Enzymes. Hin*dIII (EC 3.1.23.20); *Xba*I restriction endonuclease (EC 3.1.23.41); RNAse A (EC 3.1.27.5); RNase-free DNAse I (EC 3.1.21.1); avian myoblastoma virus reverse transcriptase (EC 2.7.7.49); *Taq* polymerase (EC 2.7.7.7); T4 polynucleotide kinase (EC 2.7.1.78).

origin. Expression of PDGF B-chain and PDGF β-receptor transcripts in a panel of human malignant mesothelioma cell lines was reported earlier, whereas normal mesothelial cell lines express no PDGF B-chain mRNA and little or no PDGF β-receptor mRNA (Gerwin et al., 1987; Versnel et al., 1988, 1991). Normal mesothelial cell lines, in contrast, were found to express PDGF α-receptor mRNA, which could not be detected in mesothelioma cell lines (Versnel et al., 1991). PDGF A-chain mRNA was detected both in normal and malignant mesothelial cell lines, but the latter clearly showed an elevated expression of this gene (Gerwin et al., 1987; Versnel et al., 1988).

In several reports, alternative splicing of exon 6, resulting in an extra 69-bp internal region, was shown for the PDGF A-chain mRNA (Collins et al., 1987; Rorsman et al., 1988; Bonthron et al., 1988; Matoskova et al., 1989). Since in some publications expression of the transcript with the exon-6-derived sequence was suggested to be tumour specific (Collins et al, 1987; Rorsman et al., 1988), the presence of this extra exon in the A-chain mRNA was studied in both normal and malignant mesothelial cell lines. We found no increased use of the exon-6 element in transcripts produced by malignant versus normal mesothelial cell lines. Furthermore, in this paper, the possible cause of the observed elevated expression of the PDGF A-chain gene in malignant mesothelioma cell lines was also studied. Increased transcription was found to be the most probable mechanism for this elevation.

MATERIALS AND METHODS

Cell lines, conditions of growth and cytogenetic analysis

Experiments were performed using the human malignant mesothelioma cell lines Mero-14, Mero-25, Mero-41, Mero-48b, Mero-48c, Mero-72, Mero-82, Mero-83, Mero-84, Mero-95 and Mero-96 (Versnel et al., 1989) and the normal mesothelial cell lines NM-1, NM-2, NM-5, NM-6, NM-7, NM-9, NM-10, NM-11, NM-12 and NM-13. Cell lines were routinely cultured as described by Versnel et al. (1989). When indicated, cycloheximide (Sigma, St. Louis, MO) was added to the medium at a concentration of 10 μg/ml for 2 h, and actinomycin D (Sigma) at 5 μg/ml for 1 – 4 h. Cytogenetic analysis was performed as described by Versnel et al. (1988).

Reverse-transcription PCR analysis and oligonucleotide hybridization

RNA was isolated as described by Versnel et al. (1988). Chromosomal DNA was removed by treating 10 μg total RNA for 1 h at 37 °C with 10 μg RNAse-free DNAse I (BRL, Gaithersburg, MD). After ethanol precipitation, 1 μg DNAse-I-treated RNA was used in a reverse transcriptase reaction, modified from Krug and Berger (1987). First 0.01 U $(dT)_{15}$ (Pharmacia, Uppsala, Sweden) was added to the RNA to give a final volume of 14 μl, and the mixture was heated for 3 min at 85 °C. The oligo(dT)-primed RNA was then added to a mixture containing 50 mM Tris/HCl, pH 8.3, 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM EDTA, 1 μg/ml bovine serum albumin, 1 mM dNTP, 4 mM sodium pyrophosphate, 40 U RNAsin (Promega, Madison, WI) and 5 U avian-myoblastoma-virus reverse transcriptase (Boehringer Mannheim, FRG). This mixture was incubated for 1 h at 39 °C. Of this cDNA mixture, 25% was used in a PCR reaction. cDNA was mixed with 10 mM Tris/HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (mass/vol.) gelatin, 0.2 mM dNTP, PDGF A-chain sense and antisense primers (A_{260} = 0.2) and 1 U *Taq* polymerase (Perkin-Elmer Cetus, Norwalk, CT). The primers used were the same as in Matoskova et al. (1989). 35 cycles (1 min at 94 °C for denaturation, 2 min at 55 °C for annealing and 3 min at 72 °C for primer extension) of amplification were performed, using the Perkin-Elmer Cetus DNA thermal cycler.

The PCR reaction mixture was analysed on a 1.5% (mass/vol.) agarose gel. After 10 min depurination (0.25 M HCl), two 15-min periods of denaturation (1.5 M NaCl, 0.5 M NaOH) and two 20-min periods of neutralization (1.5 M NaCl, 0.5 M Tris/HCl, pH 7), PCR fragments were blotted onto Hybond-N (Amersham, UK) in 1.5 M NaCl, 150 mM trisodium citrate, pH 7, and were immobilized by ultraviolet cross-linking. A 53-bp oligonucleotide primer complementary to a part of the PDGF A-chain exon-6 sequence, [5′-d(GGTGGGTTTTAACCTTTTTCTTTTCCGTTTTTTAC-CTGACTCCCTAGGCCTTC)-3′; Fig. 1A] was used for oligonucleotide hybridization of the filter. The oligomer (12.5 pmol) was end-labeled with 40 μCi [γ-^{32}P]ATP in a reaction mixture containing 50 mM Tris/HCl, pH 7.6, 10 mM $MgCl_2$, 5 mM dithiothreitol, 0.1 mM spermidine, 0.1 mM EDTA and 10 U T4 polynucleotide kinase (Pharmacia) for 30 min at 37 °C. Oligonucleotide hybridization was performed at 65 °C for 2 h and the filter was washed twice for 30 min in 0.1% SDS, 0.9 M NaCl, 50 mM NaH_2PO_4 and 5 mM EDTA at 65 °C. The filter was rehybridized with the 1.3-kb *Eco*RI PDGF A-chain fragment (Figs 1A and 4A), which was labeled with ^{32}P by random-primer labeling. Hybridization conditions with this probe were the same as described for Southern-blot analysis (Versnel et al., 1988). Autoradiography was performed with Fuji-RX films at room temperature for 10 – 20 min.

Northern-blot and Southern-blot analyses

RNA isolation and Northern blotting were performed as described by Versnel et al. (1988). Filters were washed in 45 mM NaCl and 4.5 mM trisodiun citrate, pH 7.0, at 42 °C. DNA isolation and Southern blotting were also described by Versnel et al. (1988). Filters were exposed to Fuji-RX films.

Nuclear run-off assay

Cells were cultured as described. Nuclei were isolated from 10^8 cells, essentially according to Zenke et al. (1988) except for the presence of 0.5% Nonidet P-40 in lysis buffer. From each cell line, 1 – 2 × 10^7 nuclei were used for a nuclear run-off assay, adapted from Linial et al. (1985). The ultimate concentration of Tris/HCl, pH 8.0, in the run-off buffer was 6 mM, and 140 μCi [α-^{32}P]UTP was added. Transcripts were synthesized at 30 °C for 20 min, followed by a 5-min DNAse I (10 μg) digestion at 30 °C. After centrifugation, the nuclear RNA pellet was resuspended in hybridization buffer containing 45% (by vol.) formamide, 0.2 M sodium phosphate, pH 7.2, 1 mM EDTA, 7% SDS and 250 μg/ml yeast RNA.

Plasmid DNA was spotted on to nitrocellulose filters with a slot-blot apparatus (Schleicher and Schuell, Dassel, FRG) and immobilized for 2 h at 80 °C. Subsequently, the filters were hybridized with the ^{32}P-labeled RNA for 2 days at 45 °C. After hybridization, filters were washed for 3 – 4 h in 40 mM sodium phosphate, pH 7.2, and 1% SDS at 65 °C, interrupted by a washing step of 30 min at 37 °C in 0.3 M NaCl and 30 mM trisodium citrate, pH 7.0, containing 5 μg/ml RNAse

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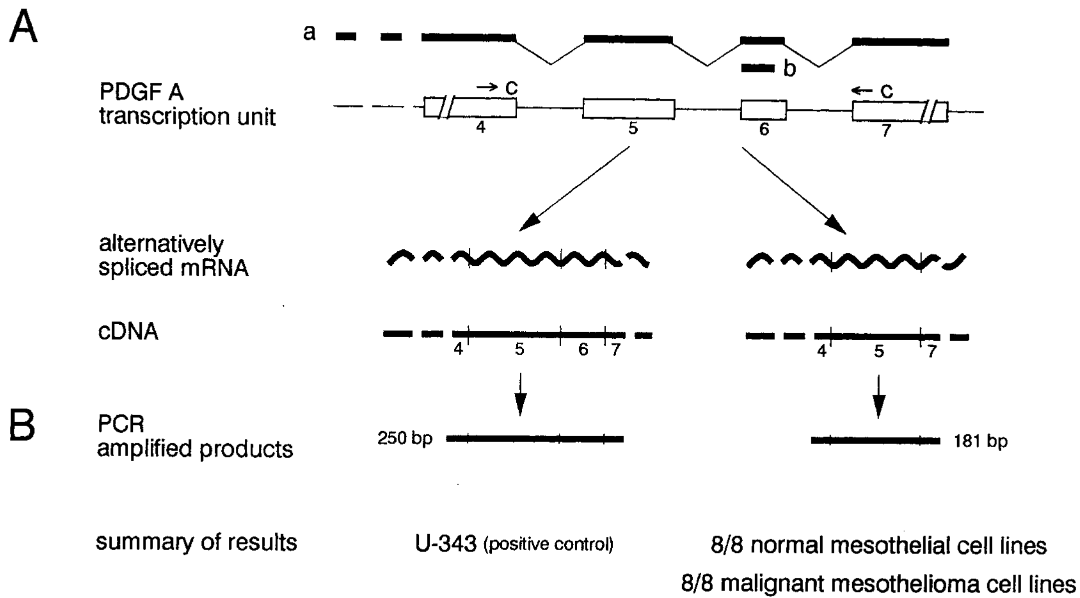

Fig. 1. Alternative splicing of the PDGF A-chain gene in normal and malignant mesothelial cell lines. (A) Part of the PDGF A-chain transcription unit with positions of PDGF A-chain probe (a), the exon-6-specific oligomer (b) and the primers used for PCR (c). The two alternatively spliced mRNA (including exon 6 or not) can be reverse-transcribed to cDNA. (B) PCR-mediated amplification of cDNA results in fragments of 250 bp, as found in the control cell line U-343, or 181 bp, observed in eight normal and eight malignant mesothelial cell lines.

A. Autoradiography was performed at −80°C with Kodak XAR films.

Probes

The PDGF A-chain probe (Figs 1A and 4A) was an *Eco*RI fragment of 1.3 kb (Betsholtz et al., 1986). The glyceraldehyde-3-phosphate-dehydrogenase probe was a 0.7-kb *Eco*RI – *Pst*I fragment (Benham et al., 1984). The T-cell-receptor Cγ probe was a 0.4-kb *Bam*HI fragment (van Dongen and Wolvers-Tettero, 1991). For the run-off analysis, the above-mentioned PDGF A-chain fragment was used, subcloned in pUC. pAct (Dodemont et al., 1982) was used to detect actin expression in this assay.

RESULTS

PDGF A-chain exon-6-derived sequences are not expressed in normal and malignant mesothelial cell lines

For reverse-transcription PCR analysis of the PDGF A-chain mRNA, total RNA of several malignant mesothelioma (Mero-14, Mero-25, Mero-48c, Mero-72, Mero-83, Mero-84, Mero-95 and Mero-96) and normal mesothelial (NM-1, NM-2, NM-6, NM-7, NM-10, NM-11, NM-12 and NM-13) cell lines was reverse-transcribed. cDNA was amplified by PCR. Using the PDGF-A-chain-specific oligonucleotide primers, which were described by Matoskova et al. (1989), fragments of 250 bp (including the exon-6-derived sequence) or 181 bp (without exon-6-derived cDNA) were amplified (Fig. 1). The PDGF A-chain cDNA from glioma cell line U-343 was used as a positive control for the presence of the exon-6-derived sequence (Matoskova et al., 1989). Amplification of this cDNA with these primers resulted in a DNA fragment of 250 bp, as shown in Figs 1 – 3. Non-reverse-transcribed RNA

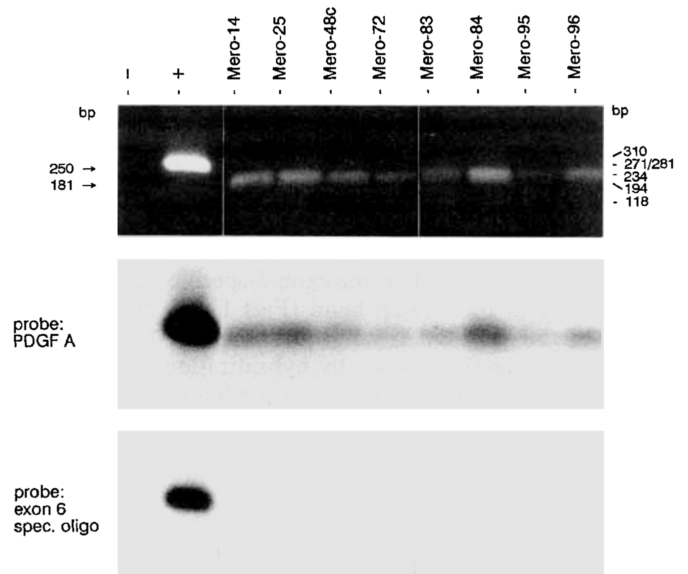

Fig. 2. Reverse-transcription PCR analysis with RNA from malignant mesothelioma cell lines Mero-14, Mero-25, Mero-48c, Mero-72, Mero-83, Mero-84, Mero-95 and Mero-96 on an ethidium-bromide-stained agarose gel. cDNA from glioma cell line U-343 was used as a positive control (+), and RNA from normal mesothelial cell line NM-2 as a negative control (−) for the PCR reaction. After blotting of the gel, the filter was hybridized to a PDGF A-chain probe and to an exon-6-specific oligomer (spec. oligo), as described in the legend to Fig. 1A.

of cell line NM-2 was used as a negative control for the PCR reaction. In the normal, as well as in the malignant, mesothelial cell lines studied, PCR-mediated amplification resulted in a predominant band of 181 bp on ethidium-bromide-stained agarose gels (Figs 1 – 3). Only in some cases was a faint band of 250 bp detected (data not shown). Band size was confirmed by electrophoresis on a 10% polyacrylamide

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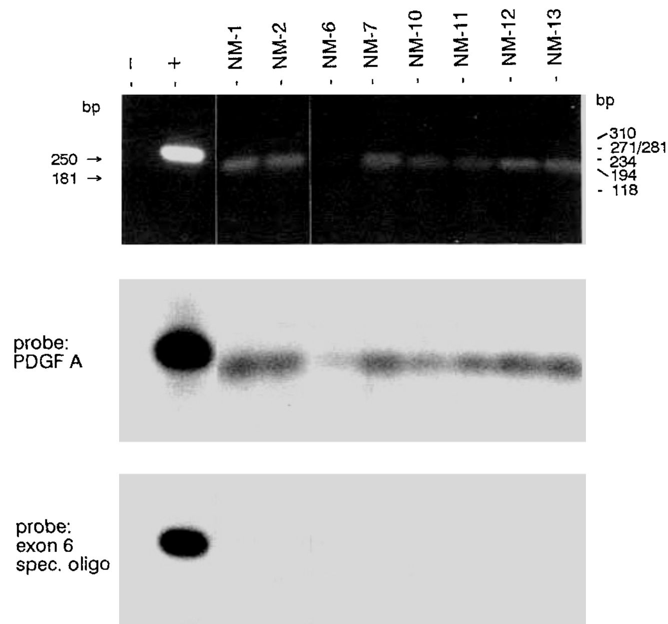

Fig. 3. Reverse-transcription PCR analysis with RNA from normal mesothelial cell lines NM-1, NM-2, NM-6, NM-7, NM-10, NM-11, NM-12 and NM-13 on an ethidium-bromide-stained agarose gel. cDNA from glioma cell line U-343 was used as a positive control (+) and RNA from normal mesothelial cell line NM-2 as a negative control (−) for the PCR reaction. After blotting of the gel, the filter was hybridized to a PDGF A-chain probe and to an exon-6-specific oligomer (spec. oligo), as described in the legend to Fig. 1.

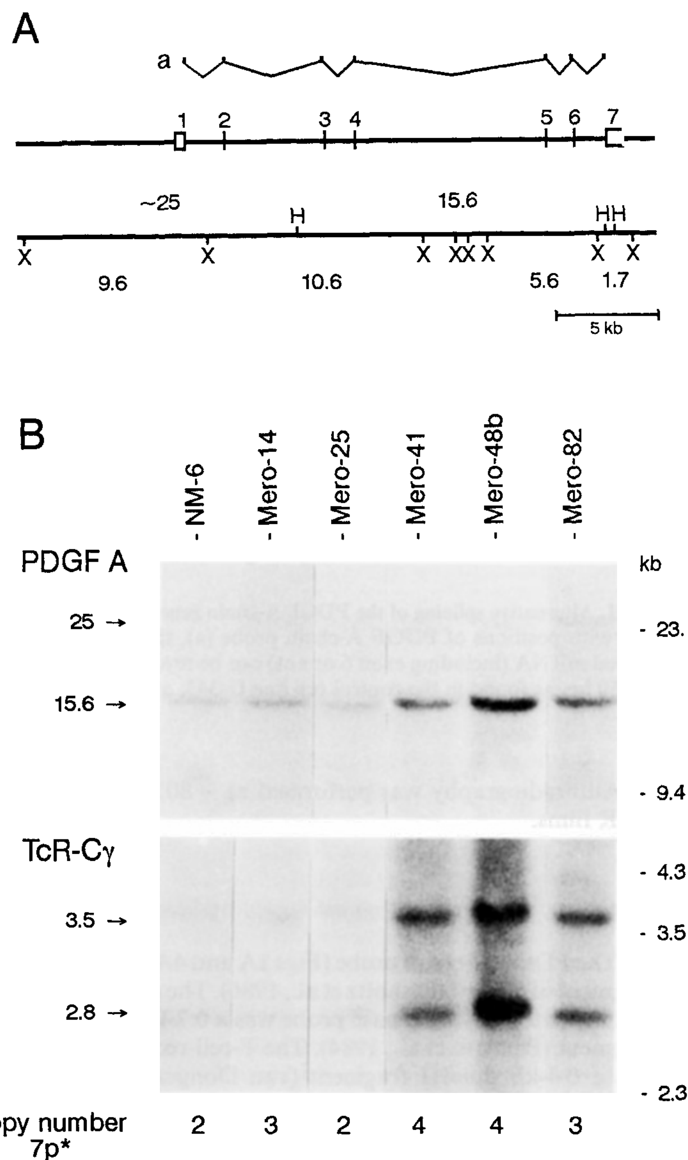

Fig. 4. Southern-blot analysis of the PDGF A-chain gene in normal and malignant mesothelial cell lines. (A) Part of the PDGF A-chain locus with PDGF A-chain cDNA probe (a), restriction sites for the enzymes *Xba*I (X) and *Hin*dIII (H) and exons numbered 1−7. Adapted from Bonthron et al. (1988). (B) Southern-blot analysis with DNA from normal mesothelial cell line NM-6 and malignant mesothelioma cell lines Mero-14, Mero-25, Mero-41, Mero-48b and Mero-82. DNA was hybridized to PDGF A-chain and T-cell-receptor (TcR) Cγ probes. *, number of normal and rearranged copies of chromosome 7p.

gel (data not shown). The nature of the bands on agarose gels was studied by hybridization to an exon-6-specific oligonucleotide primer and to the PDGF A-chain cDNA probe. As expected, the PDGF A-chain probe hybridized to the 181-bp and the 250-bp fragments, and not to the marker DNA (Figs 1−3). However, the exon-6-specific oligomer hybridized only to the 250-bp band (Figs 1−3), showing the absence of the exon-6-derived sequence in the 181-bp fragment. It was therefore shown by hybridization that normal and malignant mesothelial cell lines predominantly contain the 181-bp fragment variant of the PDGF A-chain. On longer exposure, however, in some cell lines faint bands of 250 bp were also seen. A summary of these data is given in Fig. 1B.

Malignant mesothelioma cell lines do not show structural rearrangements or amplification of the PDGF A-chain gene

As a possible reason for elevated PDGF A-chain mRNA expression in malignant mesothelioma cell lines, structural rearrangement or amplification of the PDGF A-chain gene were considered. *Xba*I/*Hin*dIII-digested DNA from the normal mesothelial cell line NM-6 and from the malignant mesothelioma cell lines Mero-14, Mero-25, Mero-41, Mero-48b and Mero-82 was used for Southern-blot analysis with a PDGF A-chain cDNA probe (Fig. 4A). *Xba*I/*Hin*dIII-digested DNA of these cell lines did not reveal structural rearrangements in the PDGF A-chain gene. In normal as well as malignant cells, a band of about 15.6 kb was detectable after *Hin*dIII digestion (Fig. 4B). On longer exposure, a second band of about 25 kb, probably corresponding to a fragment upstream of the 15.6-kb fragment, was detected in the investigated cell lines (Fig. 4B). The bands seen on Southern blots of *Xba*I-digested (data not shown) and *Hin*dIII-digested (Fig. 4B) DNA correspond to DNA fragments in the PDGF A-chain locus, which are indicated in Fig. 4A.

The same filters were subsequently hybridized with a T-cell-receptor Cγ probe, since this locus, like the PDGF A-chain, has been mapped to chromosome 7p. This resulted in bands of 2.8 bp and 3.5 kb in all cell lines investigated after *Hin*dIII digestion (Fig. 4B). The intensities of the bands correlated with those after PDGF A-chain hybridization, which suggests that there is no amplification of the PDGF A-chain gene in malignant mesothelioma cell lines. Correlation of the intensities of the bands from the various cell lines with the copy number of chromosome 7p (Fig. 4B) showed that variation in

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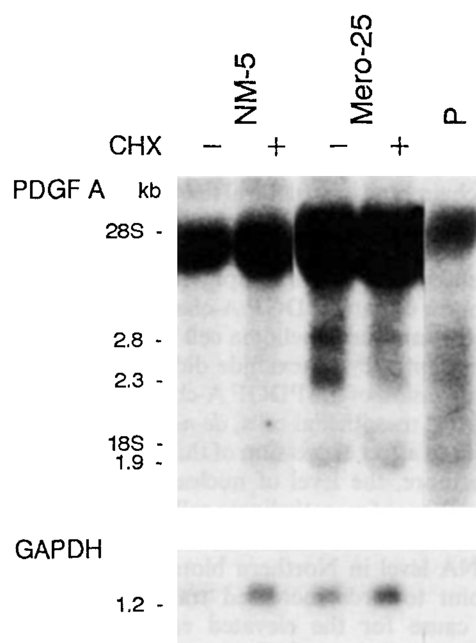

Fig. 5. Northern-blot analysis with 25 μg total RNA from placenta (P), normal mesothelial cell line NM-5 and malignant mesothelioma cell line Mero-25, cultured in the presence (+) or absence (−) of 10 µg/ml cycloheximide (CHX). RNA was hybridized to PDGF A-chain and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) probes.

copy number is the major cause of the difference in band intensity seen in the various lanes.

Increased mRNA stability does not seem to be the cause of elevated PDGF A-chain levels in malignant mesothelioma cell lines

Elevated expression of mRNA on Northern blots can, in general, be accounted for by increased transcription or by increased stability of the transcript in the cytoplasm. To discern between these possibilities, the human malignant mesothelioma cell lines Mero-25, Mero-48c and Mero-82 and the normal mesothelial cell lines NM-5, NM-7 and NM-9 were cultured in the absence or presence of a protein synthesis inhibitor, cycloheximide, for 2 h. RNA isolated from these cell lines was analyzed for the expression of PDGF A-chain mRNA. Placental RNA was used as a positive control. Addition of cycloheximide did not affect levels of PDGF A-chain transcripts (2.8, 2.3 and 1.9 kb) significantly in the three normal or in the three malignant mesothelial cell lines investigated. This is shown for the normal mesothelial cell line NM-5 and the malignant mesothelioma cell line Mero-25 (Fig. 5). Approximately equal amounts of RNA were loaded in each lane, which was demonstrated by rehybridization of the filter with glyceraldehyde-3-phosphate dehydrogenase (Fig. 5).

Nuclear RNA expression levels of the PDGF A-chain were studied in the malignant mesothelioma cell lines Mero-25 and Mero-82 and in the normal mesothelial cell lines NM-5 and NM-7. By nuclear run-off analysis, nuclear PDGF A-chain RNA levels were found to be increased in the two malignant mesothelioma cell lines, compared to the two normal mesothelial cell lines. In Fig. 6, this is demonstrated for NM-5 and Mero-25. This increase was comparable to the earlier observed increase on Northern blots between the two cell types

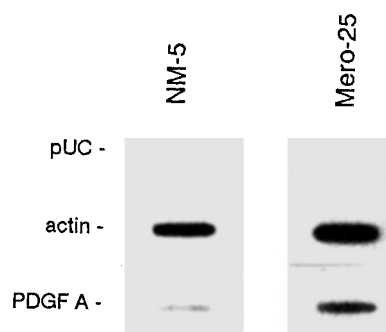

Fig. 6. Nuclear run-off analysis with ^{32}P-labeled nuclear RNA from normal mesothelial cell line NM-5 and malignant mesothelioma cell line Mero-25, on nitrocellulose blots containing the plasmids pUC, pUC plus actin and pUC plus PDGF A chain at the indicated positions.

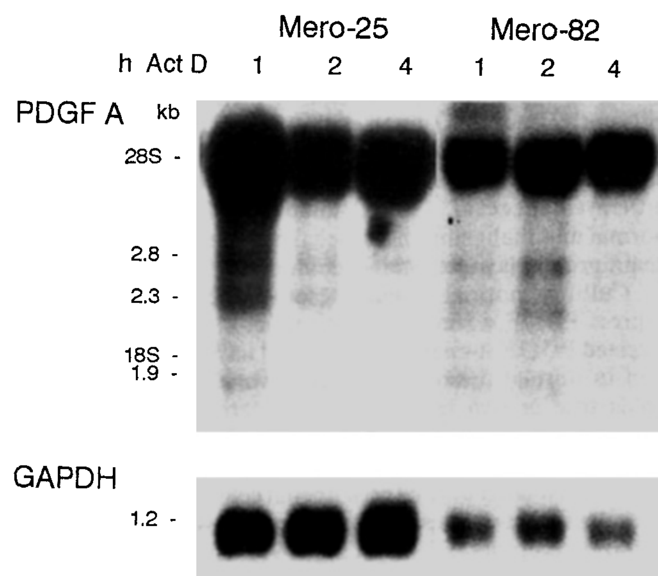

Fig. 7. Northern-blot analysis with 25 μg total RNA from malignant mesothelioma cell lines Mero-25 and Mero-82, cultured in the presence of 5 μg/ml actinomycin D (Act D) for the indicated time. RNA was hybridized to PDGF A-chain and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) probes.

(data not shown). Concomitant analysis of the constitutively transcribed actin gene revealed that the same amounts of nuclear RNA were analyzed in the various cells. Non-specific hybridization to the pUC vector was not observed (Fig. 6). Thus, the difference in PDGF A-chain mRNA expression between normal and malignant mesothelial cell lines, as detected by Northern-blot analysis, was also reflected in the amount of nuclear transcripts.

Northern-blot analysis of RNA from the malignant mesothelioma cell lines Mero-25 and Meso-82, cultured for various times with the transcription inhibitor actinomycin D, revealed a sudden decrease in PDGF A-chain mRNA expression, after 2–4 h treatment with actinomycin D, which was not accompanied by a similar decrease in the glyceraldehyde-3-phosphate-dehydrogenase mRNA expression (Fig. 7). The level of expression after 1 h treatment with actinomycin D was comparable to the level found in untreated cells of Mero-25 and Mero-82 (data not shown). These results suggest a half-life of less than 4 h for the PDGF A-chain mRNA in these cell lines. Unfortunately, we did not succeed in isolating

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enough RNA from actinomycin-D-treated normal mesothelial cells to study the half-life of the A-chain transcript in normal cells.

DISCUSSION

Alternatively spliced PDGF A-chain transcripts, which differ in the presence or absence of exon-6-derived sequences, have been described. In some publications, transcripts including this sequence were thought to be tumour specific (Collins et al., 1987; Rorsman et al., 1988). The results in this study suggest that in both normal and malignant mesothelial cell lines, PDGF A-chain mRNA without exon 6 was the predominant transcript. In some cell lines, only tiny amounts of the alternative mRNA, which includes the exon-6-derived sequence, were observed. Thus, the results obtained in mesothelial cells are consistent with earlier described data by Matoskova et al. (1989), who suggested that usage of exon-6 was not tumour specific. Of the two proteins formed, the PDGF A-chain protein encoded by the smaller transcript is efficiently secreted (Östman et al., 1991). The protein encoded by the larger transcript, however, is retained in the cell due to a cell-retention signal in the exon-6-derived domain of this protein (Maher et al., 1989; Östman et al., 1991). Thus, in both normal and malignant mesothelial cells, secretable PDGF A-chain protein is most often encountered.

Cultured normal human mesothelial cells predominantly express PDGF α receptors (Versnel et al., 1991). Therefore, secreted PDGF A-chain protein could have an autocrine function in normal mesothelial cells *in vitro*. Whether this also holds true *in vivo* remains to be determined, since PDGF receptors are easily induced in culture (Terracio et al., 1988), and no data are currently available about PDGF α receptors in freshly isolated normal mesothelial cells. Human malignant mesothelioma cell lines, however, were shown to produce only PDGF β receptors (Versnel et al., 1991). The secretable PDGF A-chain protein, produced by malignant mesothelioma cell lines, might therefore act as a paracrine growth factor. Possible target cells for this paracrine growth activity could be mesothelial cells, fibroblasts or smooth muscle cells.

PDGF A-chain mRNA levels were earlier shown to be elevated in human malignant mesothelioma cell lines, compared to cultured normal mesothelial cells (Gerwin et al., 1987; Versnel et al., 1988). In this paper, the possible reason for this elevation was studied. The culture conditions for normal and malignant mesothelial cell lines are almost identical, except for the addition of epidermal growth factor and hydrocortisone to the culture medium of normal mesothelial cells. Recently, PDGF A-chain mRNA and protein expression were reported to be decreased in hepatoma cells cultured in the presence of the glucocorticoid dexamethasone (Haraguchi et al., 1991). Malignant mesothelioma cell lines cultured in the presence of epidermal growth factor and hydrocortisone, alone or in combination, however, showed a similar level of PDGF A-chain mRNA to that found in the absence of these agents (data not shown). Moreover, the absence of epidermal growth factor or hydrocortisone in the culture medium of normal mesothelial cells did not result in an increase in PDGF A-chain mRNA to the level seen in malignant cells (data not shown).

Alterations at the genomic level were considered a possible reason for elevated expression of the PDGF A-chain gene. However, by cytogenetic analysis, the copy number of chromosome 7 was previously shown to be variable between the ten different human malignant mesothelioma cell lines, but not to be related to the PDGF A-chain mRNA level. Moreover, no consistent specific marker of chromosome 7, which might be involved in PDGF A-chain expression, could be found in these cell lines (Versnel et al., 1988). In this study, Southern-blot analysis did not reveal amplification of the PDGF A-chain gene or structural rearrangements in the investigated region.

Based on these observations, increased transcription and increased stability of the transcript were studied as the possible mechanism for elevated PDGF A-chain mRNA expression in human malignant mesothelioma cell lines. Since the protein-synthesis inhibitor cycloheximide did not significantly influence the expression of the PDGF A-chain transcript in normal and malignant mesothelial cells, *de-novo*-synthesized proteins do not seem to affect expression of this gene in these cell types.

Furthermore, the level of nuclear PDGF A-chain transcripts in malignant mesothelioma cells compared with normal mesothelial cells was elevated to the same extent as the steady-state mRNA level in Northern blots. Taken together, these results point towards increased transcription as the most probable cause for the elevated expression in malignant mesothelioma cell lines. Differences in mRNA stability are probably less important in this respect.

Many different regulators of PDGF A-chain mRNA expression have been found in various cell types *in vitro*. PDGF A-chain expression in microvascular endothelial cells was shown to be increased by transforming growth factor β and phorbol ester (Starksen et al., 1987), and by acidic fibroblast growth factor, interleukin-1, interleukin-6, tumour necrosis factor α and phorbol ester in human endothelial cells (Gay and Winkles, 1990). In smooth muscle cells, PDGF A-chain mRNA was positively modulated by acidic fibroblast growth factor, tumour necrosis factor α, transforming growth factor β, phorbol ester and serum (Winkles and Gay, 1991), and in fibroblasts by PDGF and interleukin-1 (Paulsson et al., 1987; Raines et al., 1989). Some of these regulators are thought to act by transcriptionally activating the PDGF A-chain gene. One or more might also be involved in modulation of PDGF A-chain gene expression in malignant mesothelioma cell lines. However, this remains to be demonstrated in further experiments.

A half-life of less than 4 h was observed for PDGF A-chain mRNA in human malignant mesothelioma cell lines. This is similar to the previously described half-life of about 4 h in rat aortic smooth muscle cells (Majesky et al., 1988) and differs slightly from the half-life of 2.4 h in human umbilical-vein endothelial cells (Gay and Winkles, 1991).

In conclusion, in this paper, we demonstrate that both normal and malignant human mesothelial cells predominantly produce PDGF A-chain transcripts without exon-6-derived sequences. This means that normal and malignant mesothelial cells probably secrete PDGF A-chain proteins, due to the absence of the sequence encoding a cell-retention signal in the transcript. We suggest that the PDGF A-chain protein, produced and secreted by malignant mesothelioma cell lines, probably has a paracrine function. Furthermore, we also demonstrate that the elevated PDGF A-chain mRNA expression in human malignant mesothelioma cell lines is presumably caused by an increased transcription of this gene. The causes of this increased transcription rate will be further investigated in the future by localisation of regulatory regions in the PDGF A-chain gene and characterisation of the possible factor(s) acting on them.

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