

Tenascin and Fibronectin Expression in Human Mesothelial Cells and Pleural Mesothelioma Cell-Line Cells

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Fibronectin (Fn) and tenascin (Tn) are two major extracellular matrix (ECM) glycoproteins that may have important roles both in fibrotic lung diseases and in lung tumors. The significance of Fn and Tn in human pleural mesothelial cells and pleural diseases is unclear. Transformed human pleural mesothelial cells (Met5A), primary cultures of mesothelial cells, and cultured mesothelioma cell lines were investigated for Fn and Tn immunoreactivity. Mesothelial cells were exposed for 48 to 96 h to transforming growth factor- β (TGF- β), tumor necrosis factor- α (TNF- α), amosite asbestos fibers, or oxidants (H_2O_2 and menadione, a compound that auto-oxidizes to produce superoxide). Immunofluorescence and Western blotting with monoclonal anti-Fn and anti-Tn antibodies, and Northern blotting with a complementary DNA (cDNA) probe for Tn showed that mesothelial cells are capable of producing Fn and Tn. The mRNA level and immunoreactivity of Tn was enhanced by TGF- β and TNF- α , whereas Fn was intensified only by TGF- β . A wide range of amosite, H_2O_2 , or menadione concentrations had no clear effect on Fn or Tn reactivity. Fn and Tn were present at low or undetectable concentrations in five of six mesothelioma cell lines, whereas the organization of Fn immunoreactivity in these cell lines was variable. Furthermore, results obtained with the tumor tissue of these same mesothelioma patients suggested that Fn and Tn expressions do not necessarily parallel either each other or results obtained with the cultured cells. **Kinnula, V. L., A. Linnala, E. Viitala, K. Linnainmaa, and I. Virtanen. 1998. Tenascin and fibronectin expression in human mesothelial cells and pleural mesothelioma cell-line cells. *Am. J. Respir. Cell Mol. Biol.* 19:445–452.**

The extracellular matrix (ECM) glycoproteins fibronectin (Fn) and tenascin (Tn) are important molecules in cell adhesion and tissue repair (1–5). The role of Fn in cells and tissues has been relatively well elucidated (6–11), whereas the functions of Tn are less defined. Usually, the Tn level in healthy adult tissues is low or undetectable (12). Tn is upregulated during lung morphogenesis (13, 14), at the sites of active growth of bronchial tubules, and in cultured lung cells by inflammatory cytokines such as tumor necrosis factor- α (TNF- α) (15) and transforming growth factor- β (TGF- β) (16). Tn has significant antiadhesive properties, and it is known to inhibit cell adhesion to fibronectin (5). Tn has been detected in carcinomas, mainly in the

stromal tissue of the tumor (12, 17–21), and it may be related to the invasion capability of the tumor (22).

Highly invasive local growth and resistance to all forms of conventional cancer treatments are typical characteristics of mesothelioma (23). Recent treatment strategies have focused on immunotherapy and cytokine therapy, despite poor understanding of their role in this disease (24, 25). Mesothelioma is strongly associated with many cytokines and growth factors, the most important of them being TGF- β and platelet-derived growth factor (PDGF) (23–25), both of which may induce the synthesis of various adhesive and antiadhesive molecules and contribute to the invasiveness of this tumor. No systematic comparative studies are available on the expression and regulation of Tn and Fn in human mesothelial cells or in human mesothelioma.

We investigated the regulation of Tn and Fn in human mesothelial cells by exposing these cells to TGF- β , TNF- α , oxidants, and asbestos fibers, all of which have been suggested to play roles in the pathogenesis of mesothelioma. The transformed mesothelial cells (Met5A) that were used in the study are simian virus 40-immortalized, nonmalignant diploid human cells with typical mesothelial morphology (26). Additional experiments were conducted on human pleural mesothelial cells in primary culture. Tn and

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Abbreviations: extracellular matrix, ECM; fibronectin, Fn; glyceraldehyde-3-phosphate dehydrogenase, GAPDH; sodium dodecyl sulfate–polyacrylamide gel electrophoresis, SDS–PAGE; standard saline citrate, SSC; transforming growth factor- β , TGF- β ; tenascin, Tn; tumor necrosis factor- α , TNF- α .

Fn immunoreactivities were investigated in human mesothelioma cell-line cells established from the tumors of mesothelioma patients.

Materials and Methods

Cells and Tissues

Cells of an immortalized human mesothelial cell line (Met-5A) (26) were provided by Dr. Curtis Harris (Laboratory of Human Carcinogenesis, National Cancer Institute, Bethesda, MD). The cells were cultured on uncoated plastic dishes, using RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 0.03% L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin at 37°C in a 5% CO₂ atmosphere. Confluent cultures were subcultured with trypsin-ethylenediaminetetraacetic acid (EDTA) (0.05%). Primary cultured mesothelial cells (passages 1 to 4) established from nonmalignant pleural cells were cultured as described earlier, using the same RPMI 1640 medium supplemented with 15% FCS. Cultured tumor cell lines were originally established from the tumor tissue of mesothelioma patients (27). The cytogenetic and histologic characterization of the cell lines (M9K, M10K, M14K, M28K, M33K, M38K) has been described previously, and a summary is shown in Table 1. The mesothelioma cells were cultured with the culture medium and under the conditions described previously (27).

Exposures

Cells were grown until they were subconfluent, and the medium was then changed to one that contained human recombinant TGF-β1 (1 ng/ml), TNF-α (10 ng/ml), H₂O₂ (10 to 50 µM), menadione (0.1 to 5 µM), amosite asbestos fibers (1 to 10 µg/cm²), or a combination of TGF-β and various concentrations of the oxidants or fibers. The concentrations of the oxidants and fibers were selected on the basis of preliminary experiments and previously published studies of the cytotoxicity of these fibers and oxidants in Met5A mesothelial cells (28–30). Exposure times varied from 24 h to 96 h. Two different oxidants were chosen: H₂O₂ is transported through the plasma membrane and consumed by intracellular antioxidant pathways, whereas menadione is a quinone that generates superoxide intracellularly by autooxidation in the so called redox-cycling reaction.

Immunohistochemistry

Cultured mesothelial cells and mesothelioma cells were grown on small coverslips. The plates were fixed in metha-

nol at –20°C for 10 min, and were incubated for 30 min with undiluted monoclonal antibodies (mAbs) for Tn (100EB2) or Fn (52DH1). The 100EB2 antibody reacts with the two major Tn isoforms and has been characterized earlier (20); mAb 52DH1 recognizes extradomain A (EDA) of Fn (31). After washing, the cells were incubated for 30 min with fluorescein isothiocyanate (FITC)-coupled sheep anti-mouse immunoglobulin (1:300; Jackson Immunoresearch, West Chester, PA). Tumor biopsy specimens were examined with the alkaline phosphatase-antialkaline phosphatase (APAAP) technique as described (32). Shortly thereafter, frozen tumor samples were cut at 6-µm thickness and the resulting sections were fixed in acetone pre-cooled to –20°C. In the APAAP technique, incubations with the specific antibodies were preceded by treatment with nonimmune rabbit serum for 15 min. Sections were incubated with primary antibody as described previously, and then incubated with rabbit antimouse antibody (Dako, Glostrup, Denmark) for 30 min and with the APAAP complex (Dako) for another 30 min. Immunoreactivity was visualized by exposure to a substrate solution containing naphthol AS-BI phosphate and new fuchsin (both from Sigma Chemical Co., St. Louis, MO), reaction with NaNO₂ (E. Merck, Darmstadt, Germany), and dilution in Tris-buffered saline, pH 8.7. The specimens were examined with a Leitz Aristoplan microscope equipped with appropriate filters. The relative immunoreactivity assessed independently by two investigators was expressed as weak (+), moderate (++), or strong (+++).

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Western Blotting

To obtain whole-cell extracts, confluent cells were scraped into electrophoresis sample buffer. ECM constituents were isolated as described (15). The samples were boiled for 5 min and electrophoresed under reducing conditions, using 6.5% slab gels according to Laemmli (33). The amount of cell protein loaded for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and calculated from co-cultured plates was 400 µg for Tn and 100 µg for Fn. The polypeptides were transferred onto nitrocellulose sheets according to Towbin and colleagues (34). After the transfer, Tn was detected with mAb 100EB2 and EDA-Fn with mAb 52DH1. For immunostaining, peroxidase-conjugated rabbit antimouse antibody (1:200) (Dako) was used, and the polypeptides were detected by reaction with 3,3'-diaminobenzidine tetrahydrochloride (Sigma). For molecular weight

TABLE 1
Patient histories and the origins of the mesothelioma cell lines

Patient/Cell Line	Origin of Sample	Histologic Subtype/Clinical Stage	Growth Pattern <i>In Vitro</i>	Fiber Content in Lung/g Dry Weight	Therapy Before Sample Chemotherapy/Radiotherapy
1/M9K	Metastasis	Mixed/I	Mixed	3.1 × 10 ⁶	+ / –
2/M10K	Metastasis	Mixed/I	Mixed	13.0 × 10 ⁶	+ / +
3/M14K	Tumor	Epithelial/IIB	Epithelial	26.0 × 10 ⁶	– / –
4/M28K	Tumor	Epithelial/IIA	No data	150 × 10 ⁶	– / –
5/M33K	Tumor	Mixed/IV	Epithelial	4.0 × 10 ⁶	– / –
6/M38K	Tumor	Mixed	Epithelial	6.2 × 10 ⁶	– / –

standards, a high-molecular-weight kit from Sigma was used. Proteins were analyzed according to the micromethod of Bio-Rad (Hercules, CA).

Northern Blotting

Total RNA was isolated according to Chomczynski and Sacchi (35). For the analysis, polyadenosine (poly[A])-rich RNA was obtained from total RNA by using Dynabeads Oligo(dT)25 beads (Dyna, Oslo, Norway). The mRNA was denatured in the presence of 1.8 M formaldehyde, and was electrophoresed on 1.0% agarose (Agarose NA; Pharmacia, Uppsala, Sweden) in the presence of 2.1 M formaldehyde with a 1×3 -(*N*-morpholino) propanesulfonic acid (MOPS) running buffer at 40 V for 3 h. After electrophoresis, the gel was washed twice with $20 \times$ standard saline citrate (SSC) for 15 min, and the messenger RNA (mRNA) was transferred to a Hybond Nylon membrane (Amersham, Buckinghamshire, UK) by capillary blotting. The membrane was washed briefly with $6 \times$ SSC, air-dried for 30 min, and baked at 80°C for 2 h. Prehybridization and hybridization were conducted as described earlier. A complementary DNA (cDNA) probe covering a 2,124-bp sequence of Tn (nucleotides 793 to 2,916) was provided by professor L. Zardi (Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy). The detection of the hybridized probe was conducted as described (16). The mRNA expression of Tn was compared with the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression by scanning densitometry, using the 300A Computing Densitometer and Image Quant Software v3.0 Fast Scan (Molecular Dynamics, Sunnyvale, CA).

Results

Mesothelial Cells

Met5A cells were exposed to 1 to $10 \mu\text{g}/\text{cm}^2$ amosite, 10 to $50 \mu\text{M}$ H_2O_2 or to 0.1 to $5 \mu\text{M}$ menadione. Preliminary experiments conducted in duplicate indicated that $1 \mu\text{g}/\text{cm}^2$

amosite, $10 \mu\text{M}$ H_2O_2 , and $0.1 \mu\text{M}$ menadione did not have any effects on the number of viable cells when cells were trypsinized and counted after the 48-h exposure. Amosite concentrations of 4 and $10 \mu\text{g}/\text{cm}^2$ caused 18% and 75% decreases, respectively, in the cell number of viable cells after 48 h. Corresponding decreases after exposure to $25 \mu\text{M}$ and $50 \mu\text{M}$ H_2O_2 were 20% and 64%, and after exposure to $1 \mu\text{M}$ and $5 \mu\text{M}$ menadione were 18% and 45%, respectively.

Immunofluorescence microscopy indicated that Met5A cells had no significant matrix by comparison with primary cultured mesothelial cells (not shown). Correspondingly, Tn immunoreactivity of Met5A cells appeared in the whole-cell extracts but not in the ECM preparations or in immunoprecipitates from spent cultures. Immunoblotting of the whole-cell extracts showed Tn in control Met5A cells only when at least $400 \mu\text{g}$ protein/lane was loaded; both Tn (280 kD and 190 kD) isoforms could be detected. After a 48-h exposure, the Tn level was increased by TGF- β and marginally by TNF- α (Figure 1A) but not by the lowest oxidant concentrations (Figure 1B). Additional experiments conducted with the other concentrations of menadione ($1 \mu\text{M}$ and $5 \mu\text{M}$) and H_2O_2 ($25 \mu\text{M}$ and $50 \mu\text{M}$), and with the three concentrations of the fibers, showed no induction of Tn in any case. Exposure of the cells to the combination of TGF- β ($1 \text{ ng}/\text{ml}$) and the three concentra-

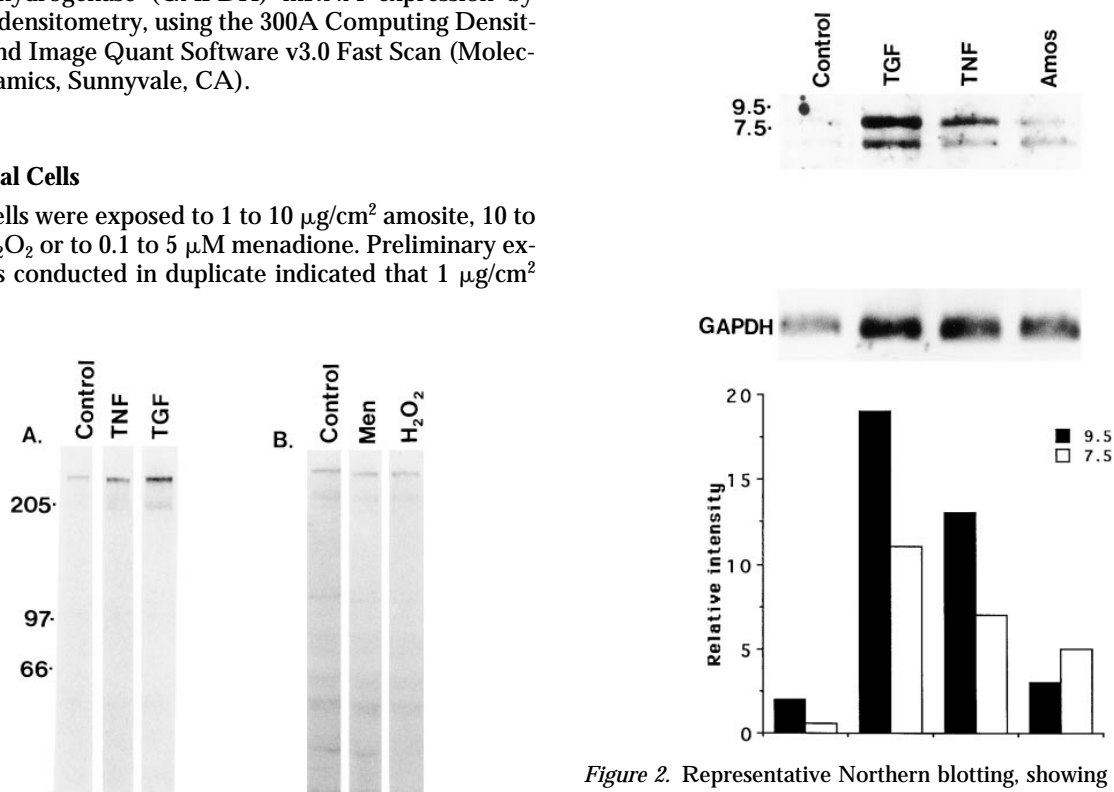


Figure 1. Immunoblotting of SDS-PAGE-separated polypeptides of whole mesothelial Met5A-cell extracts ($400 \mu\text{g}$ protein/lane) for Tn after 48-h exposures. Tn was intensified by TNF- α ($10 \text{ ng}/\text{ml}$) and TGF- β ($1 \text{ ng}/\text{ml}$) (A), but not by menadione (Men) ($0.1 \mu\text{M}$) or H_2O_2 ($10 \mu\text{M}$) (B). The molecular weights of the standard proteins (in kD) are indicated on the left.

Figure 2. Representative Northern blotting, showing Tn mRNA in control, TGF- β ($1 \text{ ng}/\text{ml}$)-, TNF- α ($10 \text{ ng}/\text{ml}$)-, and amosite ($1 \mu\text{g}/\text{cm}^2$)-exposed Met5A cells after 48 h. The results show enhanced signal for both transcripts in TGF- β - and TNF- α -exposed cells, and a very marginal response in amosite-exposed cells. The loading was standardized relative to a GAPDH cDNA probe. Numbers on the left indicate the size of the standards in kilobases.

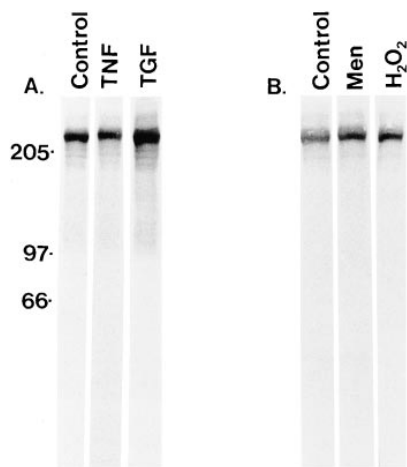


Figure 3. Fn immunoblotting of SDS-PAGE-separated polypeptides of whole mesothelial Met5A-cell extracts (100 μ g protein/lane). Control cells and cells exposed to TNF- α (10 ng/ml) or TGF- β (1 ng/ml) for 48 h revealed one Fn band of M_r 240 kD (A). Fn was intensified by TGF- β but not by TNF- α . Neither H₂O₂ (10 μ M) nor menadione (0.1 μ M) had any effect under these experimental conditions (B).

tions of the oxidants or fibers showed no difference in Tn immunoreactivity as compared with the effect of TGF- β alone (data not shown). Northern blotting revealed that both Tn transcripts (6 kb and 8 kb) were upregulated by TGF- β and TNF- α , and very marginally by amosite fibers (Figure 2).

Fn immunoreactive polypeptides were found in the whole-cell extracts and in the precipitates from the culture medium. The characteristic 240-kD band of Fn was intensified by TGF- β but not by TNF- α (Figure 3A) or by the

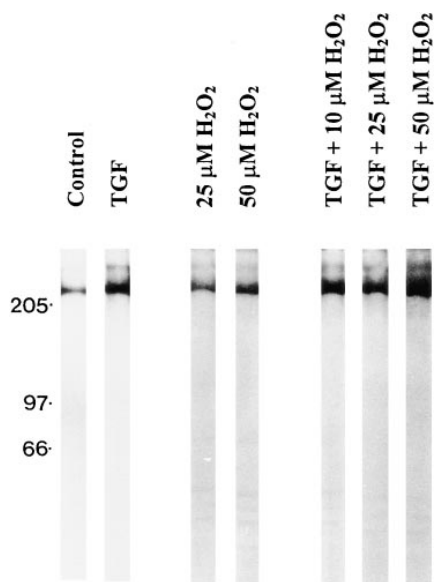


Figure 4. Fn immunoblotting of SDS-PAGE-separated polypeptides of whole mesothelial Met5A-cell extracts exposed to H₂O₂ for 48 h. Fn was intensified by TGF- β , and marginally by 25 μ M and 50 μ M H₂O₂.

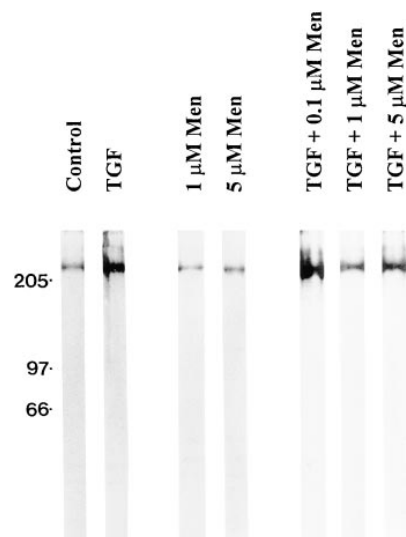


Figure 5. Fn immunoblotting of SDS-PAGE-separated polypeptides of Met5A-cell extracts. Cells were exposed to TGF- β , menadione, or a combination of TGF- β and menadione for 48 h. Menadione had no effect on Fn immunoreactivity.

nontoxic oxidant concentrations (Figure 3B). Higher concentrations of H₂O₂ (Figure 4) but not of menadione (Figure 5) caused slight increases in Fn immunoreactivity.

Asbestos fibers (4 μ g/cm² and 10 μ g/cm², 48 h) caused slight decreases in Fn immunoreactivity, possibly because of the toxicity of the fibers. Similarly, Fn levels were decreased in the cells exposed to the combination of TGF- β and the fibers (10 μ g/cm²), as compared with exposure to TGF- β alone (Figure 6). Given that Fn has been shown to be induced by asbestos fibers (4 μ g/cm²) in rat pleural mesothelial cells during 72- to 96-h exposure (36, 37), addi-

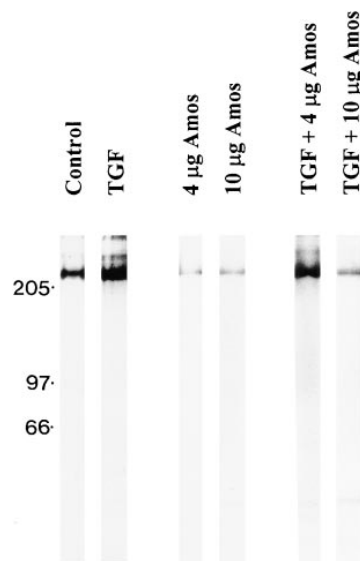


Figure 6. Fn immunoblotting of SDS-PAGE-separated polypeptides of Met5A-cell extracts. Cells were exposed to TGF- β , asbestos fibers, or a combination of TGF- β and the fibers for 48 h. Asbestos fibers had no effect on Fn immunoreactivity.

tional experiments were also conducted, in which Met5A cells were exposed to amosite asbestos fibers ($4 \mu\text{g}/\text{cm}^2$) for 96 h. However, in accord with the findings in our previous study (30), this dose and duration of exposure caused a 50% loss in cell viability. Furthermore, this exposure decreased Fn immunoreactivity when standardized against the cell protein (not shown). Because the $4 \mu\text{g}/\text{cm}^2$ dose of asbestos was toxic to these cells, further experiments were conducted in which the cells were exposed for 72 h to a nontoxic ($1 \mu\text{g}/\text{cm}^2$) dose of the fibers. No change in Fn or Tn reactivity was observed (Figure 7).

Because Met5A cells are transformed cells, and do not present detectable ECM, experiments were also conducted on primary-cultured human pleural mesothelial cells. These results showed positive Tn immunoreactivity in the ECM and also in the culture medium precipitates. The results with the matrix preparations of primary-cultured cells confirmed the induction of Tn with TGF- β but not with the nontoxic concentrations of asbestos fibers or H_2O_2 (Figure 8A). Experiments with the primary-cultured cells also confirmed the induction of Fn by TGF- β (Figure 8B).

Mesothelioma Cell Lines

Western blotting experiments showed that Fn was prominently expressed in M33K mesothelioma cells but not in the other mesothelioma cell lines examined in the study (M9K, M14K, M38K) (Figure 9). Tn could be detected only in M9K mesothelioma cells (Figure 9). In addition to the cell lines shown in Figure 9, two further cell lines (M10K, M28K) had weak Fn expression and were negative for Tn (not shown).

Immunofluorescence microscopy indicated that all mesothelioma cell lines were positive for Fn, but the pattern of Fn immunofluorescence varied, with M33K cells presenting an intense extracellular Fn network, whereas Fn was intracellularly localized in M9K and M38K cells (Figure 10). Tn immunoreactivity was undetectable in M33K cells and very low in M9K cells and M38K cells.

Because the cell culture itself may have effects on protein expression, and ECM proteins may be excreted not only by tumor cells but also by adjacent nonmalignant cells, immunostaining was also done, using tissues from tu-

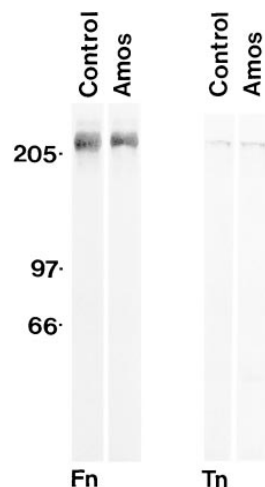


Figure 7. Fn and Tn immunoblotting of SDS-PAGE-separated polypeptides of whole-cell extracts of control and amosite ($1 \mu\text{g}/\text{cm}^2$)-exposed Met5A cells after 72 h exposure. Asbestos fibers had no effect on Fn or Tn immunoreactivity.

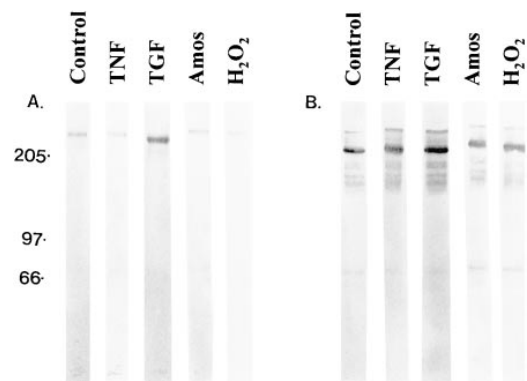


Figure 8. Tn (A) and Fn (B) immunoblotting of SDS-PAGE-separated polypeptides of primary-cultured mesothelial-cell-matrix preparations. Exposures to TNF- α , TGF- β , amosite, and H_2O_2 are the same as shown in Figures 1 and 3. Tn and Fn were intensified by TGF- β .

mor material obtained at autopsy of these same patients. No constant pattern could be observed: Tn and Fn immunofluorescence were highly variable. This variation was observed especially with Tn; Fn reactivity was moderate or abundant in five of six cases (Table 2). Tn and Fn immunoreactivity varied in different areas of the same biopsy, and both intracellular and stromal Tn and Fn immunoreactivity could be detected.

Discussion

This study showed that human pleural mesothelial cells are capable of producing low levels of Fn and Tn. Both glycoproteins were induced by TGF- β , and Tn was also marginally induced by TNF- α and Fn by H_2O_2 . Immunofluorescence microscopy of the mesothelioma cell-line cells used in the study showed low Fn and Tn immunoreactivities in most cases. In agreement with the findings in previous studies (18, 38), these results suggest that the low im-

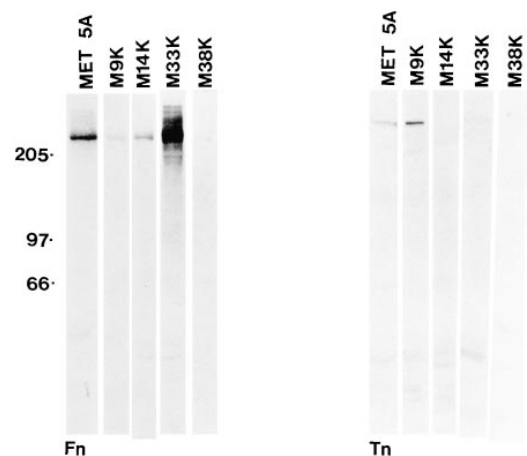


Figure 9. Fn and Tn immunoblotting of SDS-PAGE-separated polypeptides of whole mesothelial cell (Met5A) and mesothelioma cell (M9K, M14K, M33K, M38K) extracts. Fn immunoreactivity was low or undetectable, with the exception of M33K cells. Tn was low or undetectable in all cell lines.

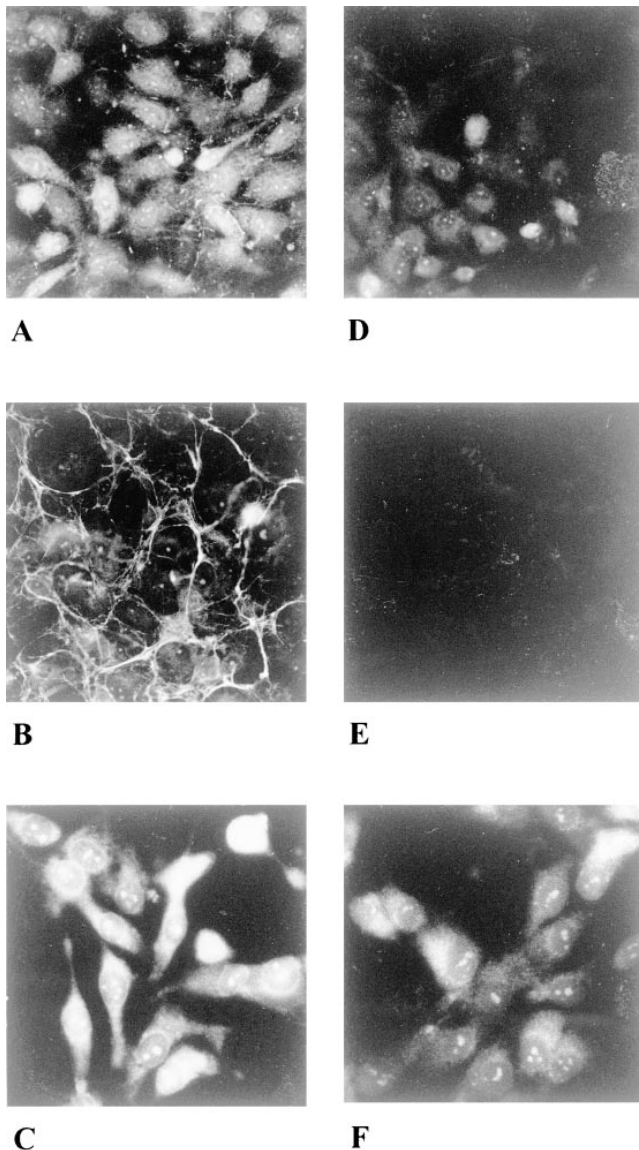


Figure 10. Photomicrographs demonstrating Fn (A, B, C) and Tn (D, E, F) immunofluorescence in M9K cells (A, D), M33K cells (B, E), and M38K cells (C, F). Enhanced extracellular fibrillar (M33K cells) or intracytoplasmic immunoreactivity was observed for Fn. Faint cytoplasmic immunostaining was seen for Tn in M9K and M38K cells, whereas M33K cells were negative for Tn.

munoreactivity of these glycoproteins in cultured cells might not reflect the situation *in vivo*.

Tn appears to be upregulated in fibrosing alveolitis (39, 40), and it may be involved in the pathogenesis of fibrotic pleural diseases as well. We have recently detected Tn in cultured human bronchial epithelial cells (15, 16), and other researchers have suggested that Tn is produced by alveolar epithelial cells (39). In the present study TGF- β and TNF- α increased Tn immunoreactivity in Met5A mesothelial cells. Northern blotting showed that both 8.0-kb and 6.0-kb transcripts of Tn were increased, indicating transcriptional regulatory control or prolongation of the half-life of the mRNA for Tn. The observed regulation of

TABLE 2
Relative abundance of tenascin and fibronectin immunofluorescence in corresponding autopsy material of the six mesothelioma patients

Corresponding Mesothelioma Cell Lines	Tn Expression	Fn Expression
M9K	+	++
M10K	+	+
M14K	+++	+++
M28K	++	++
M33K	+	+++
M38K	++	+++

Definition of abbreviations: Fn = fibronectin; Tn = tenascin.
+ = weak, ++ = moderate, +++ = abundant immunoreactivity.

Tn is in agreement with the findings in our earlier studies with bronchial epithelial cells (15, 16), and with the finding of enhanced Tn expression in chick embryo fibroblasts in response to TGF- β (41). Tn expression appears to be tightly regulated in a cell-type-specific manner (42), and this is the first study to show that TGF- β is a potent inducer of Tn in human pleural mesothelial cells.

Although the regulation of Fn in various cells and tissues is elucidated relatively well, and rat pleural mesothelial cells have been shown to produce Fn (36, 37, 43, 44), no systematic studies are available on the regulation of Fn in human mesothelial cells. Our results with human mesothelial cells contradict earlier findings, because Kuwara and coworkers (36, 37) found that crocidolite and chrysotile asbestos fibers (4 $\mu\text{g}/\text{cm}^2$) induced prominent Fn release into the conditioned media of rat mesothelial cells during 72 to 96 h exposure. In our study, neither 1 μg (nontoxic) nor 4 μg (toxic) doses of amosite caused any increase in Fn or Tn immunoreactivity in Met5A mesothelial cells. The discrepancy between these results may be related to different species, the immortalized cell line used in our study, a different fiber type, or fiber toxicity. Our results do not exclude Fn or Tn induction by chronic asbestos exposure, but they suggest that growth factors and inflammatory cytokines are more important than oxidants or fibers in causing Fn and Tn induction in human mesothelial cells.

Tn expression has not been investigated previously in human mesothelioma. TGF- β , which is a potent inducer of Tn *in vitro*, is one potential cytokine involved in the pathogenesis of mesothelioma. Furthermore, TGF- β contributes not only to the pathogenesis but possibly also to the aggressive growth of this tumor (25, 45–49). The TGF- β level in healthy lungs and airways is low, whereas this cytokine is strongly expressed in pulmonary fibrosis, and present at high levels in malignancies, one of the latter being mesothelioma (47–49). It has also been suggested that Tn accumulation in malignant neoplasms can be caused by growth factors, such as TGF- β , and may be associated with the invasiveness of the tumor (22). We found that Tn was negative in most mesothelioma cell lines but was induced by TGF- β both in transformed Met5A mesothelial cells

and in primary-cultured human mesothelial cells. Given that cultured tumor cells may secrete much lower amounts of Tn than the tumor cells *in vivo*, and that Tn may be produced by nonmalignant cells in the close vicinity of mesothelium, we also assessed Tn expression in tumor tissues obtained at autopsy from the same patients from whom the cultured mesothelioma cells had come. The data showed a high variation of Tn in these tumor tissues, but also in different areas of one tumor-tissue specimen. These results also suggested that downregulation of Tn may not be a universal response to cell culture, because Tn was detected in one mesothelial cell line (M9K) by Western-blot analysis, whereas Tn immunostaining of the corresponding tissue was very weak. It has to be emphasized, however, that autopsy material does not represent ideal material with which to investigate the expression of these proteins in malignant tumor.

The significance of Fn and its immunohistochemical pattern in human mesothelioma cells is unknown. Fn expression and organization can be changed during neoplastic conversion to mesothelioma in rat lungs (44, 50). In our study, M33K mesothelioma cells showed intense and M9K mesothelioma cells showed low Fn reactivity. Also, the organization of Fn immunoreactivity in M33K cells differed from that in the other cell lines, because these cells contained high levels of Fn in their ECM, whereas in M9K mesothelioma cells Fn was intracellularly localized. The significance of this finding remains unclear. Three histologic subtypes of mesothelioma can be identified: epithelioid, sarcomatoid, and mixed type. Both M33K and M9K mesothelioma cells represented the mixed histologic subtype. Recently, Ferriola and colleagues (44) have shown that the organization of Fn fibrils can be heterogenous in rat pleural mesothelioma cells, and it has been suggested that ECM components such as Fn may contribute to the invasive behavior of mesothelioma (44, 50).

In summary, human pleural mesothelial cells produce Fn and Tn, the production of which can be modulated *in vitro* by TGF- β . The expression of both of these glycoproteins in cultured mesothelial and mesothelioma cells was low in most cases, but did not necessarily parallel either each other or their expression in autopsy material. More studies are needed to investigate further the role of these glycoproteins in human mesothelium and mesothelioma *in vivo*.

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