Published in final edited form as:
Am J Surg Pathol. 2009 November ; 33(11): 1673–1682. doi:10.1097/PAS.0b013e3181b6bde3.

Tenascin-X is a novel diagnostic marker of malignant mesothelioma

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Abstract

TNXB was previously identified as a gene that is more highly expressed in malignant mesothelioma compared to ovarian/peritoneal serous carcinoma based on gene expression array analysis. The objective of the present study was to validate this finding at the mRNA and protein level. Effusions (n=91; 71 ovarian carcinomas, 10 breast carcinomas, 10 malignant mesotheliomas) were assayed for *TNXB* mRNA expression using quantitative PCR. Tenascin-X protein expression was studied in 183 effusions (137 carcinomas of different origin, 37 mesotheliomas, 9 reactive effusions) and 178 solid lesions (122 ovarian/ peritoneal carcinomas, 56 mesotheliomas) using immunohistochemistry. Quantitative PCR analysis showed significantly higher *TNXB* mRNA level in mesotheliomas compared to ovarian and breast carcinomas ($p<0.001$). By immunohistochemistry, tenascin-X protein expression was significantly higher in malignant mesothelioma compared to metastatic carcinoma in effusions (34/37 vs. 31/137 positive cases; sensitivity = 92%, specificity = 77%; $p<0.001$). Reactive mesothelial cells had focal or no tenascin-X expression. Tenascin-X protein was detected 41/56 mesothelioma biopsy specimens, and was uniformly absent from all 122 ovarian carcinomas (sensitivity = 73%, specificity = 100%; $p<0.001$). Our data suggest that tenascin-X may be a new diagnostic marker of malignant mesothelioma in the differential diagnosis of cancers involving the serosal cavities, particularly in the differential diagnosis between this tumor and ovarian/peritoneal serous carcinoma.

Keywords

TNXB; serous effusions; adenocarcinoma; malignant mesothelioma

Introduction

The serosal cavities are frequently affected by cancer, often concomitantly in the form of solid lesions and malignant effusion. Involvement of the peritoneal, pleural and pericardial spaces by metastatic cancer is most frequently encountered in ovarian carcinoma (OC), breast carcinoma or lung carcinoma [2]. The serosal cavities are additionally the site of origin of

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malignant mesothelioma (MM) and primary peritoneal carcinoma (PPC). The presence of cancer cells at this anatomic site is associated with poor prognosis [8].

Recent improvements in the immunohistochemistry panels used in effusion cytology and surgical pathology currently allow for correct diagnosis of the majority of tumors [8,11,14–16,18]. However, difficulties may be still encountered in determining the site of origin for metastatic adenocarcinoma, in differentiating reactive mesothelial cells (RMC) from MM, and in the distinction between serous OC/PPC and MM [8,14,15,18]. Identifying molecular characteristics that are unique for one cancer type may aid in optimizing the diagnosis of serosal tumors, as well as in designing molecular therapies for specific malignancies.

We recently reported on the differential gene expression of 189 genes in OC/PPC compared to diffuse peritoneal MM (DMPM) using cDNA microarray technology [7]. Among the differentially expressed genes, *TNXB*, coding for the tenascin-X protein, was identified as a potential marker of DMPM.

The tenascins are a family of four glycoproteins located in the extracellular matrix, consisting of tenascin-C, -X, -R and -W, that are considered to be unique for vertebrates. Tenascins are large molecules of >300 kDa, although smaller splice variants have been observed in animals. Tenascin family members share considerable homology in structure, containing amino-terminal heptad repeats, epidermal growth factor-like repeats, fibronectin type III domain repeats, and a carboxy-terminal fibrinogen-like globular domain. The main role of tenascins is in modulating cell-matrix interactions, mediating an anti-adhesive phenotype promoting motility [12,19]. Tenascin-C, the most studied family member, is involved in connective tissue morphogenesis in the embryo, but has been additionally shown to be expressed in the tumor stroma in several cancers, where it is postulated to promote tumor progression and metastasis [17]. Tenascin-X is mainly expressed in loose connective tissue, including the dermis, epimysium and blood vessels, in both the embryo and the adult [19]. Inactivating mutations in the *TNX* gene are the genetic cause of some cases of the Ehlers-Danlos syndrome [4]. The expression and role of tenascin-X in cancer are largely unknown to date.

The aim of the present study was to validate the gene expression array data for *TNXB*, obtained in analysis of 15 effusions, studying larger material using quantitative PCR (qPCR). In addition to DMPM and OC/PPC effusions, the qPCR material included pleural MM and breast carcinoma effusions. Findings were further validated on protein level using Western blotting and immunohistochemistry (IHC).

Material and methods

Effusions

Specimens were submitted for routine diagnostic purposes to the Division of Pathology at the Norwegian Radium Hospital during the period 1998–2006. Effusions were centrifuged immediately after tapping and cell pellets were fresh-frozen in RPMI 1640 medium with 50% fetal calf serum and 20% dimethylsulfoxide. Cell blocks were prepared using the thrombin clot method. Diagnoses were established based on smear and cell block morphology and immunohistochemistry [5,6].

The Regional Committee for Medical Research Ethics in Norway approved the study.

qPCR

A total of 91 effusions (71 OC/PPC, 10 breast carcinomas, 10 MM) were analyzed for *TNXB* mRNA expression. OC/PPC effusions (n=71; 54 peritoneal, 17 pleural) were obtained from 68 patients (3 patients with 2 effusions each) diagnosed with advanced-stage (FIGO III-IV)

OC (n=58), PPC (n=7) or the closely-related serous carcinoma of the fallopian tube (n=3). The majority of OC/PPC specimens (61/71; 86%) were of the serous type.

Ten pleural effusions from patients diagnosed with histologically verified infiltrating duct carcinoma of the breast were analyzed. The 10 MM effusions consisted of 7 pleural and 3 peritoneal specimens. All were from patients diagnosed with MM of the epithelioid or biphasic type in biopsy specimens.

Total RNA was extracted using the Trizol reagent (Invitrogen, Carlsbad, CA). mRNA isolation was performed using the Dynabeads Oligo (dT)25 kit (Dynal, Oslo, Norway). mRNA was reverse-transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA).

Primers for *TNXB* (NM_019105) were located at exon 4–5. Primer specificity was validated by running in parallel genomic DNA and cDNA as template, and viewing the results by gel electrophoresis. The assays were controlled for primer dimers using the NetPrimer software by PREMIER Biosoft, as well as for single nucleotide polymorphisms through the NCBI database. Primer efficiency was tested using Power SYBR® Green (Applied Biosystems) and a dilution series of synthetic oligonucleotides as template and subsequently as standard curve. The qPCR reaction was run using the Platinum® qPCR SuperMix-UDG with ROX solution (Invitrogen) and quantified on the Applied Biosystems 7900HT Sequence Detection System. *TNXB* primer and probe sequences were as follows:

Sense: 5'-CCAAGACCATCACCACCATGA -3'

Antisense: 5'-GTTGTCGGTGTCACAGCCA-3'

Probe: Fam 5'- ATGGGCCCCAGGACCTCCGAGT -3' Non fluorescent Quencher An array of 12 reference genes (TaqMan low density array human endogenous control panel; Applied Biosystems) was tested in order to identify the most uniformly expressed transcript in the effusion specimens. Based on this assay, *beta-glucuronidase* (*GUS*) was used as housekeeping gene in these assays. The *GUS* primer and probe sequences have been published elsewhere [3]. Standard curves for the *GUS* assay were from Ipsogen (Marseille, France).

IHC

A total of 183 effusions were analyzed. Diagnoses and effusion site are detailed in Table 1. As in the qPCR material, the majority of OC/PPC specimens were of the serous type. Breast carcinomas were predominantly of the infiltrating duct type (n=45), the remaining specimens being lobular (n=2) or mixed (=2) carcinomas. The primary tumor was not available for type determination in 3 cases. The 37 MM effusions were from patients diagnosed with MM of the epithelioid or biphasic type in biopsy specimens.

A series of 178 solid lesions, consisting of 122 OC/PPC and 56 MM, was additionally immunostained. The former were in a tissue microarray (TMA) containing 280 2mm cores from 42 primary carcinomas and 80 solid metastases from patients operated at the Norwegian Radium Hospital. Metastases were to the omentum (n=46), peritoneum (n=17), intestines (n=12) or various other sites (n=5). MM consisted of 8 peritoneal and 48 pleural specimens. Forty-one tumors were of the epithelioid type, 3 were sarcomatous and 12 were biphasic. Following microwave pretreatment in Tris-EDTA buffer (pH=10), sections were incubated for 30min with a mouse polyclonal anti-tenascin-X antibody (Abcam, Cambridge, UK), diluted 1:100. Visualization was achieved using the EnVision ™+ peroxidase system (Dako, Glostrup, Denmark). Negative controls consisted of sections that underwent similar staining procedures with a non-relevant antibody of the corresponding isotype (mouse IgG). Positive control

consisted of a high-grade fibrosarcoma and a malignant fibrous histiocytoma that demonstrated focal immunoreactivity for the studied antigen in a pilot study.

All specimens containing <100 tumor cells were excluded. Staining was considered positive when localized to the membrane or cytoplasm. Staining extent was scored on a scale of 0–4, as follows: 0=no staining, 1=1–5%, 2=6–25%, 3=26–75%, 4=76–100% of tumor cells. Slides were scored by a surgical pathologist with diagnostic experience in cytopathology (BD).

Western blotting

The specificity of the anti-tenascin-X antibody used in IHC was studied using Western blotting of the MSTO-211H MM cell line (purchased from ATCC, East Greenwich, RI) and 13 effusions (4 MM, 5 OC/PPC, 4 breast carcinomas). Cells were lysed in ice-cold NP-40 lysis buffer (1% NP-40; 10% glycerol; 20mM Tris-HCl, pH 7.5; 137mM sodium chloride, 100mM sodium fluoride; 1mM sodium vanadate, 1mM phenylmethylsulfonyl fluoride (PMSF); 0.02mg/ml each of aprotinin, leupeptin and pepstatin; and 10μl/ml each of phosphatase inhibitor cocktail I and II (Sigma-Aldrich, St. Louis, MO). Lysates were sonicated and clarified by centrifugation. Protein quantification was done by Bradford analysis and 25μg protein/lane was resolved by SDS polyacrylamide gel electhrophoresis and blotted onto Immobilon-P membranes (Millipore, Bedford, MA). To visualize even loading, membranes were stained with naphtol-blue black (Sigma-Aldrich). Membranes were blocked overnight with 5% nonfat dry milk in Tris-buffered saline-Tween (TBST). Tenascin XB (TNXB) protein expression was studied using a mouse polyclonal anti-TNXB antibody (Abcam, Cambridge, England) diluted 1:1000 in 5% milk-TBST. Rabbit polyclonal anti-β-actin (Cell Signaling Technology, Danvers, MA) served as loading control. HRP-conjugated anti-mouse and HRP-conjugated anti-rabbit IgG secondary antibodies were from Promega (Madison, WI). The ECL plus Western blotting detection system (GE Healthcare, Buckinghamshire, England) was used for visualization.

Statistical analysis

Statistical analysis was performed applying the SPSS-PC package (Version 16.0, Chicago, IL). Probability of <0.05 was considered significant. The association between tumor type (OC/PPC, breast carcinoma or MM) and *TNXB* mRNA levels by qPCR was studied using the Kruskal-Wallis H test. The same test was used for analysis of the relationship between tenascin-X protein expression and tumor type using IHC. The relationship between tenascin-X protein expression by IHC and specimen type (effusion vs. biopsy) and anatomic site (pleura vs. peritoneum) in MM was studied using the Mann-Whitney U Test.

Results

TNXB mRNA expression is higher in MM compared to OC/PPC and breast carcinoma

TNXB mRNA was expressed in all 91 effusions, irrespective of tumor type, using qPCR. However, considerable differences were observed with respect to copy number (Figure 1). *TNXB* copy number values were as follows: OC/PPC: range = 0.18–148.36, median = 5.43; breast carcinoma: range = 0.71–97.71, median = 4.37; MM: range = 14.58–551.98, median = 78.52. Values of the reference gene *GUS* showed little variation among samples.

Statistical analysis showed significantly higher expression of both genes in MM compared to the 2 other cancers ($p<0.001$). Values of <10 copies were found exclusively in carcinoma effusions (64.8% of OC/PPC and 80% of breast carcinomas).

Tenascin-X protein expression is higher in MM compared to carcinomas involving the serosal cavities

IHC analysis of the 183 effusions showed frequent tenascin-X expression in MM, with focal (score=1) or no staining in the majority of carcinomas and in RMC (Figure 2, Table 2). With the exception of one breast carcinoma, staining in >25% of tumor cells was not observed in any of the carcinoma effusions, as opposed to 65% of MM effusions. Statistical analysis showed significant difference in tenascin-X expression ($p<0.001$) between MM and carcinomas using different groupings of the latter (all MM vs. all carcinomas grouped together, MM vs. OC/PPC, and MM vs. OC/PPC, breast carcinomas, lung carcinomas, GI-tract carcinomas and non-ovarian gynecological carcinomas). Positive and negative controls gave satisfactory results in all experiments. Western blotting confirmed the expression of tenascin-X in MM effusions and its general absence in OC/PPC specimens, with some expression in breast carcinoma effusions. The size of the larger fragment (>200kDa) detected is in agreement with data regarding splice variants in animals [12], while the smaller one (80kDa) is at the size specified by the manufacturer. The MSTO-211H MM cell line was negative (Figure 3).

Interpreting any staining extent as positive, the sensitivity of tenascin-X in differentiating MM from metastatic carcinoma was 92%, with a specificity of 77%. Using a cut-off of 5% for classifying specimen as positive resulted in sensitivity of 76% and specificity of 97% for this marker.

In order to further evaluate the role of tenascin-X in differentiating OC/PPC from MM, a series of 178 solid lesions was immunstained. Tenascin-X stained 41/56 MM biopsies and was uniformly absent from all 122 ovarian carcinomas (sensitivity = 73%, specificity = 100%; $p<0.001$).

Comparative analysis of MM specimens with respect to specimen type and site showed significantly higher tenascin-X expression in effusions compared to solid specimens ($p<0.001$). Expression in DMPM and pleural MM was comparable ($p=0.6$)

Tenascin-X performs well compared to established markers in the differential diagnosis between MM and adenocarcinoma in effusions

Based on our experience, only few markers available to date are helpful in the differential diagnosis between OC/PPC and MM in effusion cytology. Our panel in this setting includes Ber-EP4 (mouse monoclonal, Dako), B72.3 (mouse monoclonal, BioGenex, San Ramon CA), EMA (mouse monoclonal, clone E29, Dako), and calretinin (mouse monoclonal, clone DAK Calret 1, Dako). In order to further assess the value of tenascin-X in the differential diagnosis of malignant effusions, we compared its performance with that of these four markers in the differentiation between MM and OC/PPC, as well as in differentiating MM from breast carcinoma.

Data for 130 effusions from cases in which previous immunohistochemistry was performed on the same specimen analyzed in the present study (6 cases were diagnosed in different specimens) are shown in Table 3 and illustrated in Figure 4. As may be appreciated from this table, tenascin-X had comparable performance to that of calretinin, currently the best positive MM marker in the market in our opinion, when any degree of staining was considered positive. Notably, tenascin-X performed better than calretinin in terms of specificity (89% vs. 81%) when the differential diagnosis was only between OC/PPC and MM. Tenascin-X had comparable sensitivity to that of thick membrane EMA expression, with lower specificity. However, some OC/PPC specimens had only focal cytoplasmic EMA expression, making the detection of staining pattern more difficult than determining staining *per se*.

Data regarding the epithelial markers were in agreement with our previous observations [5, 6]. B72.3 staining was highly specific for carcinoma in effusions, with a sensitivity that was much higher for OC/PPC than for breast carcinoma. Ber-EP4 was highly sensitive for detection of both tumors, but stained a considerable number of MM.

Discussion

Despite recent improvements in the immunohistochemistry panels used for the differentiation between MM and metastatic carcinoma in surgical pathology and effusion cytology, few markers are entirely specific for either one of these entities. One major factor in the sub-optimal performance of many antibodies in the differential diagnosis of serosal cancers is the variety of carcinomas involving this anatomic site. Among these, OC/PPC is unequivocally the most difficult to distinguish from MM, due to overlapping clinical presentation (especially within the peritoneal cavity), morphological resemblance, and co-expression or lack of expression of multiple markers. Markers that are co-expressed by both tumors in surgical specimens include podoplanin, calretinin, CK5/6, WT1, and mesothelin, while both tumors are CEA-negative [reviewed in 15,18]. We have previously shown that these similarities apply to effusion cytology as well [1,6]. However, based on our gene expression array data [7], we have been able to identify several new potential markers that are significantly more highly expressed in OC/PPC effusions compared to MM and/or RMC, including the gap junction protein claudin-3 [13], MUC-4 [9], cyclin E [10] and folate receptors [20]. In the present study, we investigated the diagnostic role of the potential MM marker tenascin-X.

Significantly higher levels of *TNXB* mRNA were found in MM effusions compared to OC/PPC, in agreement with our previous observation using Affymetrix arrays [7], with comparable differences between MM and breast carcinoma effusions. While no high cut-off levels that were diagnostic for MM were found, low cut-off expression levels below which the diagnosis of MM was unlikely were observed for *TNXB*. These data suggest that this assay may have a role in selected difficult cases in effusion cytology.

The diagnostic role of tenascin-X was subsequently studied at the protein level. IHC analysis of tenascin-X protein expression in effusions showed very good performance of this marker in differentiating MM from metastatic carcinoma, as reflected in sensitivity and specificity of 76% and 97% using a staining cut-off at 5%. Staining of >25% of cells essentially ruled out the diagnosis of metastatic carcinoma in our series, irrespective of the organ of origin, and was not observed in RMC either.

The diagnostic value of tenascin-X as a positive marker of MM was compared with the two best markers available to date in our opinion, calretinin and EMA. Calretinin expression is most frequently diffuse in MM and focal, when present, in serous OC/PPC, making it a useful differentiator between these two cancers. However, negative vs. positive staining is easier to appreciate, and using this scoring cut-off, Tenascin-X staining was more specific than that for calretinin in our material. As discussed above, the detection of EMA expression in a thick membrane pattern supports the diagnosis of MM, a finding that was as sensitive as, but more specific than tenascin-X staining in our series. However, the distinction between pure membrane localization and combined cytoplasmic and membrane staining with accentuation at the membrane may be difficult in some cases, making tenascin-X a useful adjunct to the diagnostic panel.

In view of the observed differences in effusions, we studied a large series of solid MM and OC/PPC for tenascin-X expression. Tenascin-X performance in this material was still better than in effusions, as reflected in sensitivity and specificity of 73% and 100%, respectively.

Notably, the universal absence of tenascin-X from OC/PPC cells was true for both primary and metastatic tumors.

Finally, potential differences in tenascin-X expression between DMPM and pleural MM were assessed. We found comparable expression of this protein in peritoneal and pleural tumors. Interestingly, expression in effusions was higher compared to solid lesions, suggesting a role in the inhibition of adhesion and thereby formation of metastasis.

In conclusion, quantitative PCR analysis confirmed the higher levels of *TNXB* mRNA in MM compared to OC/PPC and breast carcinoma effusions. Using immunohistochemistry, pronounced differences in tenascin-X expression were found between MM and metastatic carcinomas of various origin in effusions, as well as between solid MM and OC/PPC, suggesting that this protein should be considered as a novel marker in the diagnostic panel of serosal tumors.

Acknowledgments

Financial acknowledgement: This work was supported by grants from the Norwegian Cancer Society and the Health Region of South-Eastern Norway; C. Wu is supported by NIH grant GM65188

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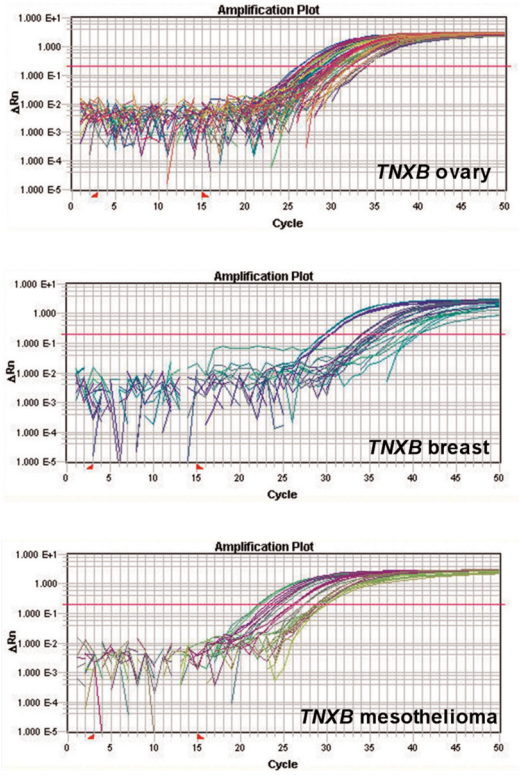

Figure 1. *TNXB* mRNA expression in serous effusions

Quantitative PCR amplification plots for *TNXB* mRNA in 47 effusions (29 ovarian carcinomas, 9 malignant mesotheliomas, 9 breast carcinomas). Cases were run in triplicate. The cycle threshold value is clearly lower for MM compared to carcinoma effusions, denoting higher mRNA expression in the former tumor. Final values were established as ratio with level of the reference gene *GUS* (see text).

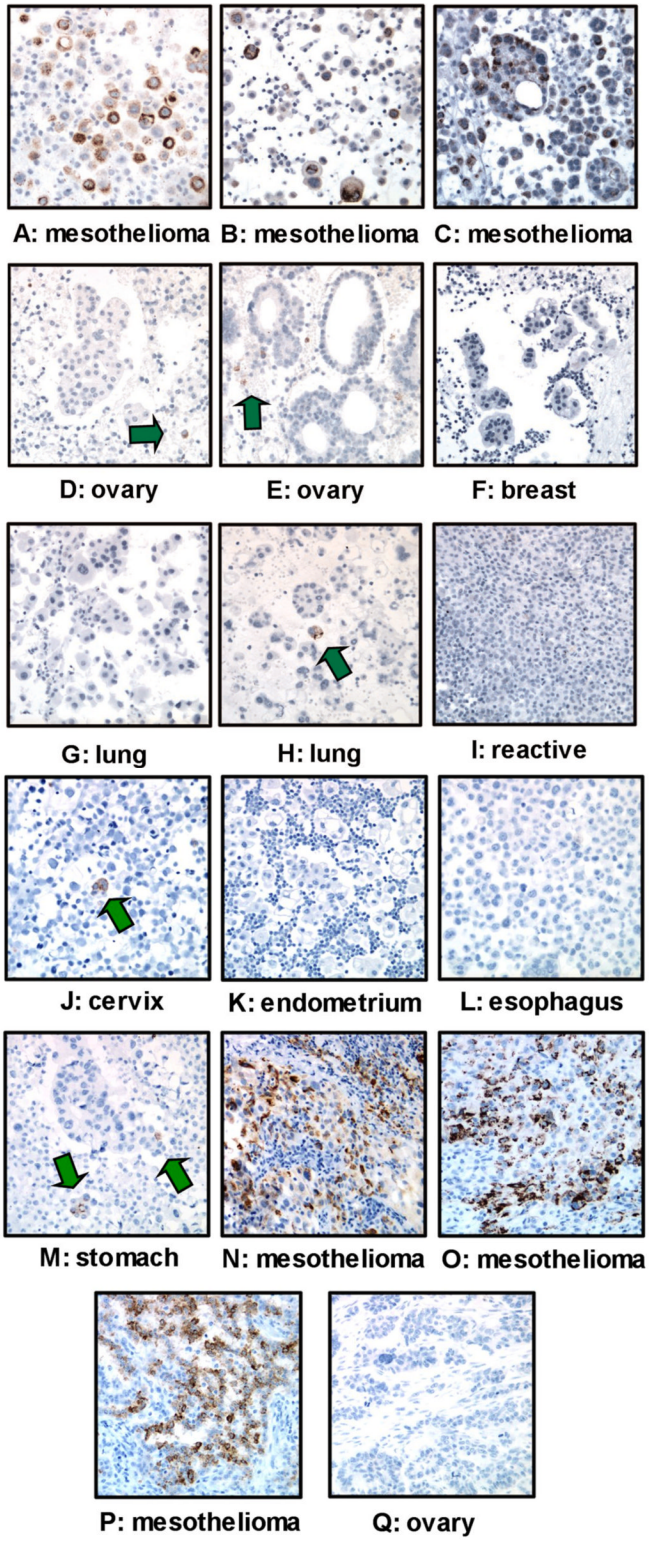

Figure 2. Tenascin-X protein expression in serous effusions and solid tumors

Representative examples of tenascin-X expression by immunohistochemistry: (A–C), malignant mesothelioma: staining in three pleural effusions, all showing tenascin-X expression

in >25 of tumor cells; (D–E), ovarian carcinoma: two peritoneal effusions showing no expression of tenascin-X in tumor cells. Few reactive mesothelial cells are stained (arrows); (F), breast carcinoma: Tenascin-X-negative pleural effusion; (G–H), lung carcinoma: A tenascin-X-negative tumor is shown in G, focally positive (<5%, arrow) specimen in H; (I), Reactive: Pleural effusion with a large number of reactive mesothelial cells showing lack of tenascin-X expression; (J–M), carcinomas of other origin: Tenascin-X-negative carcinomas of the endometrium (K) and esophagus (L), focally positive (<5%, arrows) cervical (J) and gastric (M) carcinoma; (N–P), malignant mesothelioma: staining in three solid pleural lesions, all showing tenascin-X expression in >25 of tumor cells; (Q), primary ovarian carcinoma: Tenascin-X-negative serous carcinoma.

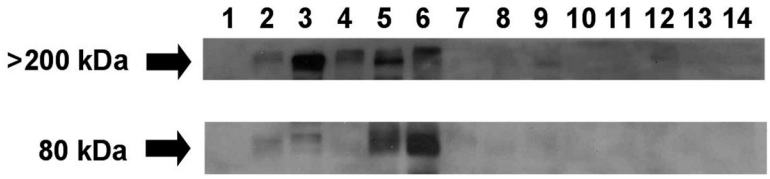

Figure 3. Tenascin-X protein expression by Western blotting

Validation of the tenascin-X antibody used in the immunohistochemical staining in the MSTO-211H mesothelioma cell line (lane 1) and 13 effusion specimens, obtained from patients with malignant mesothelioma (lanes 2–5), breast carcinoma (lanes 6–9) and ovarian carcinoma (lanes 10–14). Three mesothelioma effusions (lanes 2, 3 and 5) and one breast carcinoma effusion (lane 6) express the 80 kDa form of tenascin-X, whereas all four mesotheliomas (lanes 2–5) and two breast carcinoma effusions (lanes 6 and 9) express a larger (>200kDa) form of the protein. Ovarian carcinomas and the MSTO-211H cell line are negative.

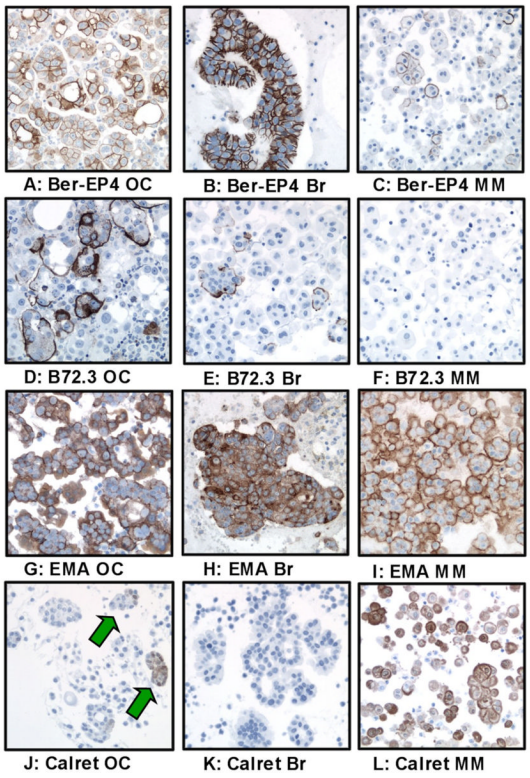

Figure 4. Expression of established diagnostic markers in cancer cells in effusions

Representative examples of immunohistochemical staining using the Ber-EP4, B72.3, EMA and calretinin antibodies: (A–C), Ber-EP4: Ovarian (A) and breast (B) carcinoma cells are immunostained, but staining is also present in some of the mesothelioma cells in figure (C); (D–F), B72.3: Ovarian (D) and breast (E) carcinoma cells are immunstained, whereas mesothelioma cells are negative (F); (G–I), EMA: All three tumor types express EMA. However, staining is localized to both cytoplasm and membrane in carcinomas (G–H), as compared to strong membrane accentuation in mesothelioma (I); (J–L), calretinin: Focally-positive (arrows) ovarian carcinoma specimen (J), negative breast carcinoma effusion (K), and diffusely positive malignant mesothelioma (L) are shown.

Table 1

Case distribution based on diagnosis and anatomic site

Clinical diagnosis	No. of cases	Site		
		Peritoneum	Pleura	Pericardium
Breast carcinoma	52	3	48	1
ovarian carcinoma [a]	47	43	4	0
Malignant mesothelioma	37	13	24	0
Other gynecological carcinomas [b]	15	13	2	0
Lung carcinoma	12	0	12	0
Gastrointestinal carcinomas [c]	10	3	7	0
Reactive	9	3	6	0
Prostate carcinoma	1	0	1	0
Total	**183**	**78**	**104**	**1**

[a] Including 42 ovarian carcinomas and 5 primary peritoneal carcinomas

[b] 5 uterine cervical and 10 endometrial carcinomas.

[c] 5 esophageal, 1 colon, 3 gastric and 1 pancreatic carcinoma.

Table 2

Tenascin-X immunostaining results

Diagnosis	Tenascin-X staining extent				
	0%	1–5%	6–25%	26–75%	76–100%
		Effusions (174)			
Breast carcinoma (52)	36 (9%)	13 (25%)	2 (4%)	1 (2%)	0 (0%)
Ovarian carcinoma [a] (47)	42 (89%)	4 (9%)	1 (2%)	0 (0%)	0 (0%)
Malignant mesothelioma (37)	3 (8%)	6 (16%)	4 (11%	15 (41%)	9 (24%)
Other gynecological carcinomas [b] (15)	11(73%)	4 (27%)	0 (0%)	0 (0%)	0 (0%)
Lung carcinoma (12)	10 (83%)	2 (17%)	0 (0%)	0 (0%)	0 (0%)
Gastrointestinal carcinomas [c] (10)	6 (60%)	4 (40%)	0 (0%)	0 (0%)	0 (0%)
Reactive (9)	6 (67%)	2 (22%)	1 (11%)	0 (0%)	0 (0%)
Prostate carcinoma (1)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
		Solid tumors (178)			
Ovarian carcinoma [d] (122)	122 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Malignant mesothelioma (56)	15 (27%)	22 (39%)	12 (21%)	7 (13%)	0 (0%)

[a] Including 42 ovarian carcinomas and 5 primary peritoneal carcinomas

[b] 5 uterine cervical and 10 endometrial carcinomas.

[c] 5 esophageal, 1 colon, 3 gastric and 1 pancreatic carcinoma.

[d] 42 primary carcinomas, 80 metastases.

Table 3

Comparison between tenascin-X and established diagnostic markers in differentiating malignant mesothelioma (MM) from ovarian/peritoneal carcinoma (OC/PPC) and breast carcinoma in effusions (n=130) [a]

Antibody (target tumor)	Number of staining cases (%) [b]			Sensitivity [c]	Specificity [c]
	OC/PPC	Breast	Mesothelioma		
Ber-EP4 (carcinoma)	47/47 (100%)	45/47 (96%)	13/36 (36%)	98%	64%
B72.3 (carcinoma)	42/47 (89%)	23/47 (49%)	0/36 (0%)	69%	100%
EMA - membrane (MM)	0/47 (0%)	0/47 (0%)	33/36 (92%)	92%	100%
EMA - cytoplasm + membrane (carcinoma)	47/47 (100%)	47/47 (100%)	2/36 (6%)	100%	94%
Calretinin (MM)	9/47 (19%)	11/47 (23%)	35/36 (97%)	97%	79%
Tenascin-X (MM)	5/47 (11%)	15/47 (32%)	33/36 (92%)	92%	79%

[a] Immunohistochemistry performed on a different specimen in 6 of 136 cases, which are therefore not shown in this table.

[b] Any number of cells

[c] For the differentiation between carcinoma and mesothelioma