Tumor/Stromal Caveolin-1 Expression Patterns in Pleural Mesothelioma Define a Subgroup of the Epithelial Histotype With Poorer Prognosis

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ABSTRACT

Objectives: Malignant pleural mesothelioma (MPM) is a highly aggressive disease for which new prognostic biomarkers need to be identified. Caveolin-1 (CAV1), the most important member of caveolae, has been described as deregulated in MPM at the genomic level, but detailed histologic information on its distribution and prognostic role is still lacking.

Methods: A series of 131 MPMs (91 epithelial, 17 biphasic, and 23 sarcomatous histotype) were investigated for CAV1 expression with immunohistochemistry and correlated with clinical-pathologic variables and outcome.

Results: CAV1 was detected in neoplastic cells of 70 (77%) of 91 epithelial, 17 (100%) of 17 biphasic, and 23 (100%) of 23 sarcomatous MPMs. Furthermore, in the epithelial group, CAV1 expression in spindle-shaped stromal cells was detected in 61 (67%) of 91 cases.

Conclusions: The presence of stromal CAV1 expression was associated with a worse patient outcome. In MPM, CAV1 is differentially expressed according to low- to high-grade histotypes. Furthermore, in the MPM epithelial group, additional stromal CAV1 expression is associated with a worse prognosis.

Malignant pleural mesothelioma (MPM) is a highly aggressive and fatal disease that is still considered a rare tumor, but its worldwide incidence is expected to increase over the next decades.1 Its prognosis is still extremely poor, with a median survival ranging between 11 and 13 months. The number of long-term survivors, however, is increasing,2 probably because of the improved multimodal therapeutic approaches in recent years.3

MPM is a heterogeneous disease, with three histologic types that yield different outcomes, with the epithelial type being associated with a better survival than the biphasic and sarcomatous types.1 Additional adverse prognostic factors include male sex and older age, but new prognostic and predictive biomarkers need to be identified. Emerging data on both protein and gene expression levels in specific molecular alterations in MPM tissues and cell lines should lead investigators to the identification of new biomarkers.4,5 In a recent meta-analysis of published gene expression profiling microarray studies comparing MPM with normal mesothelial cells, 78 of 387 altered genes reached a highly significant threshold level and, among others, Caveolin-1 gene was described as specifically deregulated in MPM.6

Caveolin-1 (CAV1) is the most important member of a family of membrane proteins that are the coating proteins of caveolae,7 small invaginations in the plasma membrane, which play an important role in the endosomal degradation of several molecules and receptors as well as in intracellular signaling transduction.8,9 In human diseases, CAV1 deletion may participate in the pathogenesis of lung fibrotic disorders, such as systemic sclerosis and idiopathic pulmonary fibrosis.10 In neoplastic disorders, CAV1 was recently reported as a marker of extracellular matrix (ECM) remodeling11 and
epithelial-to-mesenchymal transition (EMT) mechanisms. So far, only one study was previously performed to test CAV1 protein expression in MPM. It has been proposed that CAV1 be used as a biomarker to differentiate mesothelioma from lung adenocarcinoma, but its tissue distribution and prognostic role in MPM have never been addressed.

In this study, we aimed to investigate the expression of CAV1 in a large series of MPM, with special reference to its distribution among different MPM histotypes and its potential role as a novel prognostic biomarker. We were able to show that a significantly different distribution of CAV1 is detectable among different histologic MPM types, not only in neoplastic cells, but also in cells of the tumor-associated stroma. Furthermore, CAV1 expression in the stroma (but not in neoplastic cells) was significantly associated with a worse prognosis in an epithelial MPM subgroup.

**Materials and Methods**

**Patient Series**

From a database of 630 histologically proven MPMs diagnosed at San Luigi Hospital, Orbassano, Turin, Italy, between 1989 and 2010, a total of 131 cases with recorded clinical, therapeutic, and outcomes data and with available archival tissue material were selected. Histopathologic diagnoses were reviewed and histotype subclassification reassessed in all cases by two of us (L.R. and M.C.C.). Epithelial type MPM cases were morphologically reclassified according to newly reported guidelines and grading score count as recently reported.

For clinicopathologic statistical correlation, patients were grouped as follows: group A, a consecutive series (called test series) of 63 MPM cases (54 epithelial and 9 biphasic) diagnosed between 2004 and 2010 including patients who underwent pemetrexed-based chemotherapy at the Division of Thoracic Oncology of San Luigi Hospital, as previously reported; group B, an independent series (called validation series) of 45 MPMs (including 37 epithelial and 8 biphasic types) treated with different chemotherapy schemes, selected from a larger previously reported series; and group C, a series of 23 sarcomatous MPMs with no treatment information, selected as an additional control group. Furthermore, a series of 40 consecutive biopsy samples of pleuritis were selected as a control group.

All histologic material was deidentified and cases were anonymized by a pathology staff member not involved in the study. Clinical data were compared and analyzed using coded data. The study was approved by the institutional review board of San Luigi Hospital.

**Cell Lines**

MPP89, REN, and MSTO cell lines were supplied by the American Type Culture Collection (ATCC, Rockville, MD). All cells were cultured in RPMI (Sigma Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (Biowest, Nuaillé, France) and 1% l-glutamine (Sigma Aldrich). Cell lines were cultured in triplicate into six-well plates for 72 hours and used for RNA extraction.

**Immunohistochemistry**

Five-µm-thick paraffin sections of each case were collected onto charged slides, deparaffinized, and rehydrated in water. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 15 minutes. Slides were washed and incubated with a blocking reagent (1% bovine serum albumin in tris-buffered saline) for 10 minutes at room temperature to block nonspecific antibody reactions and then reincubated for 40 minutes at room temperature with the following antihuman primary antibodies: CAV1 (1:800; rabbit polyclonal, Santa Cruz Biotechnology, Santa Cruz, CA), calretinin (1:400; rabbit polyclonal, Thermo Scientific, Waltham, MA), Ki67 (1:1500; mouse clone MIB-1 Dako, Glostrup, Denmark), CD31 (1:30; mouse clone JC/70A, Dako), smooth muscle actin (SMA, 1:4500, mouse clone 1A4, NeoMarkers, Fremont, CA), pancytokeratin (PanCK, 1:150; mouse clone AE1/AE3, Dako), podoplanin (1:150; mouse clone D2-40, Dako), WT1 (1:10; mouse clone 6F1H2, NeoMarkers), and vimentin (1:1,000; mouse clone V9, Dako). After a prolonged wash, the slides were incubated with biotin-free polymer preconjugated with goat antirabbit (or antimouse) and peroxidase (En Vision Plus antirabbit or mouse-HRP System from Dako) at room temperature for 30 minutes. The reaction product was developed with 3-3′-diaminobenzidine tetrahydrochloride (Dako) as chromogen. The specificity of the reaction was validated in serial negative control sections by omitting the primary antibody for each immunohistochemical run. The internal reference control was represented by endothelial cells.

Two observers who were blinded to the clinical data scored all the cases, and a consensus was reached in cases with significant discrepancy between the individual scores. CAV1 expression in neoplastic cells (N-CAV1) was quantified using the histo-score (HS) method, as previously reported. Both the extent of expression (in percentage) and the staining intensity were taken into account, and for each tumor a semiquantitative value ranging from 0 to 300 was obtained. Subsequently the median HS value was considered to classify samples as having “high” or “low” N-CAV1 expression. CAV1 expression in stromal cells (S-CAV1) was recorded as absence (score 0) or presence, with weak (score 1), moderate (score 2), or strong (score 3) staining intensity and no difference in expression. The Ki67 labeling...
index was expressed as the percentage of positive nuclei among 1,000 cells. Samples were categorized as having a high vs low proliferation index based on the median nuclear count of the group distribution.

Double Immunofluorescence Staining Reactions

In selected cases, double immunofluorescence staining reactions were performed to investigate the nature of S-CAV1–expressing cells, by testing the coexpression of CAV1 and mesothelial, epithelial, and endothelial markers including podoplanin, WT1, PanCK, and CD31, respectively, and further assessing the proliferative potential with the Ki67 index. Briefly, after the appropriate antigen retrievals (see above), sections were incubated with each primary antibody and the immune reaction was detected using a green Alexa Fluor 488-anti-rabbit (1:200, Invitrogen, Carlsbad, CA) or red Alexa Fluor 568-anti-mouse (1:200, Invitrogen) secondary antibody, counterstained with DAPI (Abbott Laboratories, North Chicago, IL) and mounted. CAV1 staining had a fine dot-like green diffuse staining, whereas PanCK, podoplanin, WT1, Ki67, and CD31 staining had a red mesh-like appearance.

mRNA Gene Expression Analysis of MPM Cell Lines

Total RNA was extracted using Qiazol reagent (Qiagen, Germantown, MD) according to the manufacturer’s protocol. Relative cDNA quantification of markers was done using a fluorescence-based real-time detection method with measurements done in triplicate and the comparative cycle threshold (Ct) method used, as previously described.17 Quantitative real-time polymerase chain reaction (qPCR) was performed with an ABI Prism 7900HT sequence detection system (Life Technologies, Applied Biosystems, Carlsbad, CA) on 384-well plates. All qPCR mixtures contained 1.2 μL of cDNA template (approximately 20 ng of retrotranscribed total RNA) diluted in distilled sterile water, 0.6 μL of a ×20 Caveolin-1 TaqMan Gene expression assay (code Hs00971716_m1), and TaqMan Gene Expression Master Mix (Life Technologies), to a final volume of 11 μL.

Cycling conditions were 50°C for 2 minutes, 95°C for 10 minutes, followed by 46 cycles at 95°C for 15 seconds and 60°C for 1 minute. Baseline and threshold for Ct calculation were set manually with ABI Prism SDS 2.1 Software (Life Technologies). The cell line with lowest levels of CAV1 mRNA (MPP89) was used as calibrator. β-actin was used as the internal reference gene. The fold change in gene expression levels, expressed in unitless values, was evaluated using the 2^−ΔΔCt method.19

Statistical Analyses

To test the significant association between HS values (continuous variable) and clinical and pathologic features (dichotomous variables), Kruskal-Wallis, Mann-Whitney U, or one-way analysis of variance tests were used. Correlation among continuous variables was estimated using the Spearman test. Correlation among categorical variables was analyzed with the Pearson χ² or Fisher exact tests. Survival curves were estimated by means of the Kaplan-Meier method and verified using the log-rank test in univariate analysis. Statistical analyses were performed using GraphPad 4.0 software (GraphPad, La Jolla, CA) with a level of significance of P < .05.

Results

Case Series

Patient characteristics are summarized in Table 1. A total of 131 patients with MPM, 93 men and 38 women, with a mean age of 65 years were included in the study. Based...
on histologic findings, 91 tumors were epithelial, 17 were biphasic, and 23 were sarcomatous type. The 91 epithelial MPM were morphologically classified as follows: 32 (35%) solid, 25 (28%) acinar, 12 (13%) trabecular, 10 (11%) tubulopapillary, 5 (5%) adenomatoid, 3 (3%) adenoid cystic, and 1 each (1%) lymphohistiocytoid, deciduoid, micropapillary, and pleomorphic. According to the nuclear grade score, they were distributed as follows: score 2, 8 cases (9%); score 3, 43 cases (47%); score 4, 26 cases (29%); score 5, 14 cases (15%). Ki67 mean value of this subtype was 25% (range, 5%-80%). The 17 MPMs of the biphasic type contained variable percentages of epithelial and sarcomatous components merging into one another. The epithelial component was composed of polygonal cells having abundant eosinophilic cytoplasm, high nuclear grade, and prominent nucleoli; the Ki67 mean value of this component was 43% (range, 20%-70%). The sarcomatous component was made of moderately atypical polygonal

or spindle cells invading the surrounding fat, with moderate inflammatory infiltrate, hyaline stroma, and Ki67 mean value of 38% (range, 20%-70%). Finally, the 23 sarcomatous MPMs included 4 (17%) desmoplastic and 19 (83%) classic spindle cell variants and were associated with high proliferative Ki67 index (mean, 55%; range, 30%-85%).

The control series of pleuritis were distributed as follows: 8 chronic sclerosing pleuritis; 10 pneumothorax-associated pleuritis with mesothelial proliferation and fibrosis; 6 exudative pleuritis associated with organizing fibrotic tissue; and 16 unhyalinized chronic pleuritis with mesothelial hyperplastic proliferation.

CAV1 Expression in Mesothelioma Cells

In tissue samples, CAV1 immunoreactivity was detected in neoplastic cells (N-CAV1) of all three MPM histotypes with different HS values: in epithelial
To confirm our observation, we measured mRNA levels in three mesothelioma cell lines and found that the biphasic-derived MPM cell line (MSTO) expressed significantly higher levels of CAV1 mRNA than the two epithelial-derived MPM cell lines (REN and MPP89) (Kruskal-Wallis, $P = .02$) Figure 2I. To further support these findings, CAV1 immunostaining was performed in cell blocks, and MSTO cells had a higher CAV1 protein expression than MPP89 and REN cell lines Image 2I.

**CAV1 Expression in Stromal Cells of the Epithelial MPM Histotype**

In the epithelial MPM group, a finely granular dot-like cytoplasmic staining was documented in some stromal cells associated with the neoplastic epithelial cell nests Image 3I, with weak to moderate high staining intensity. This S-CAV1 expression was detected in 61 (67%) of 91 epithelial MPM cases, which made it possible to distinguish a novel epithelial MPM subgroup, defined by the presence of S-CAV1–positive component (Epi-S), from the total group of epithelial MPM cases. Furthermore, separating the two components of the biphasic MPM group and considering N-CAV1 levels in the whole spectrum of MPM subgroups thus defined, a significantly different distribution was found ($P < .0001$) among different groups, with N-CAV1 expression increasing from the “pure” epithelial subgroup (Epi) to the sarcomatous histotype Image 3I. Moreover, correlating N-CAV1 HS levels with the different S-CAV1 score intensities, the trend showed increasing N-CAV1 with increasing S-CAV1 expression levels, but the values did not reach statistical significance ($P = .2$, data not shown). No correlations were found between S-CAV1 and other clinicopathologic variables, including nuclear grading score and the Ki67 proliferative index, which had no significant difference between the S-CAV1–positive and –negative groups.

CAV1-positive stromal cells associated with the epithelial MPM proliferation had a peculiar spindle-shaped appearance Image 4AI, bunching in small foci within the ECM associated with mesothelial tumor cells. These cells were mildly atypical with scant and clear cytoplasm, pale roundish nuclei, finely dispersed chromatin, and few small nucleoli. The ECM in which they were embedded was loose, edematous, sometimes myxoid, and constituted by soft collagen matrix, with no dense fibrosis or hyalinization. These cells were present in all the epithelial MPM morphologic variants, with a higher prevalence in the solid and acinar types (data not shown). They occurred in the tumor stroma, but not in the infiltrated fat tissue. The percentage of these stromal cells was variable (5%-35%) among different cases and within stromal areas of the same case. These peculiar stromal cells were not traced in epithelial MPM cases lacking stromal CAV1 expression, which had a rather...
sclerohyaline, paucicellular connective tissue. According to the immunoprofile, these stromal CAV1-positive cells showed diffuse and intense vimentin (data not shown), WT1, SMA, reactivity, but only focal and weak calretinin, cytokeratin, podoplanin, and Ki67 expression; double immunofluorescence staining revealed that scattered CAV1-positive stromal cells were coexpressing cytokeratin, podoplanin, and WT1, and. Very few CAV1-positive cells were found to be proliferating, whereas CD31 was coexpressed solely in endothelial cells along vascular channels (data not shown).

All 8 chronic sclerosing pleuritis cases and 10 pneumothorax-associated pleuritis cases with mesothelial proliferation and fibrosis were negative for CAV1 expression. In addition, two of six exudative pleuritis cases associated with organizing fibrotic tissue were negative for proliferative fibroblastic tissue, and the other four had a weak focal CAV1 expression in some spindle cells. Finally, of
16 nonhyalinized chronic pleuritis cases with mesothelial hyperplasia, four were negative, six were only weakly and focally positive, and six were positive for stromal CAV1 expression (data not shown).

CAV1 Prognostic Role

The prognostic role of CAV1 expression was assessed by separating the series of 63 MPM cases treated with pemetrexed-based therapy from the control group of 45 cases with no uniform treatment modalities to avoid treatment bias. The survival of sarcomatous MPM cases was used as the reference. In the group of 63 patients with MPM, N-CAV1 expression had no significant prognostic role (Figure 4A). In contrast, a detailed subanalysis of 54 epithelial MPMs treated with pemetrexed showed that S-CAV1 was associated with a worse prognosis (irrespective of the staining intensity scores) (mean survival time of 17 months vs 37.8 months; hazard ratio, 0.27; confidence interval 0.14-0.5; \(P < .0001\)) (Figure 4B). Interestingly, comparing the survival rates of epithelial MPMs having S-CAV1 expression (Epi-S) and those of the other MPM histotypes, the outcome of the former group was comparable to that of biphasic MPM (rather than to epithelial MPM without S-CAV1) (Figure 4C). A similar survival pattern was observed in the control series. In fact, N-CAV1 HS values had no significant prognostic role either in survival curves (Figure 5A) (Kaplan Meier \(P\) value not significant) or when cases were divided by long- and short-term survivors (based on an arbitrary cut-off of 24 months’ survival, which may reasonably identify long-term survivors (Figure 5B), Mann-Whitney \(P\) value not significant). In contrast, a higher number of S-CAV1-positive cases was observed in the group of short-term survivors compared with long-term survivors (\(\chi^2\) test, \(P = .0002\)) (Figure 5C). Moreover, segregating S-CAV1-expressing cases in the epithelial MPM cases, the survival of S-CAV1 cases (Epi-S) overlapped that of biphasic MPM, rather than that of S-CAV1-negative epithelial MPM (Epi) (Figure 5D).

Discussion

With the aim of defining the role of CAV1 expression in MPM, we report herein the differences in immunohistochemical reactivity in the different MPM histotypes. We also describe specific expression patterns in neoplastic vs stromal MPM cells that have an influence on patient outcomes. CAV1 was previously described in MPM as an immunohistochemical marker for differentiating MPM from lung adenocarcinoma. However, to the best of our knowledge, this is the first time that the intratumoral CAV1 expression was assessed and its potential prognostic role investigated.

In our experience, CAV1 had variable immunohistochemical expression with a finely granular pattern, as described in epithelial tumor cells of various organs. When the neoplastic cells were considered, CAV1 expression in epithelial MPM had the lowest expression levels, with a mean HS value of 70, thus indicating that not all cases expressed the protein at detectable levels; in most of these cases, a focal distribution was detected. Interestingly, in biphasic MPM, an intermediate CAV1 expression level was detected, comparable in both components to those of the corresponding pure histotypes. It is well known that histotype is one of the most important prognostic factors in MPM. The increased CAV1 expression, according to low-grade epithelial to high-grade sarcomatous histotypes, is in line with the known correlation of increased CAV1 expression with a more aggressive behavior in various epithelial and nonepithelial malignancies, including non–small cell lung cancer, brain tumors, renal cell carcinoma, and, recently, gastric cancer. Furthermore, expression of CAV1 was described in several soft tissue and bone sarcomas as a marker associated with the degree of differentiation. In line with this observation, our series of sarcomatous MPMs were all positive (100%) with the highest N-CAV1 levels (mean, 215). We thus confirmed that this neoplasia exhibits a CAV1 gain along with dedifferentiation when the spectrum of...
Image 4: Immunoprofile of caveolin-1 (CAV1)-positive stromal cells. Stromal cells (A) show cytoplasmic granular CAV1 expression (B), focal calretinin (C), and high smooth muscle actin (D). Furthermore, stromal cells expressed cytokeratin (E), podoplanin (F), and WT1 (G), and had a low proliferative index with Ki67 (H). Double immunofluorescence staining demonstrated coexpression (I, CAV1 green/keratin red; J, CAV1 green/podoplanin red; K, CAV1 green/WT1 red; L, CAV1 green/Ki67 red. (A, H&E, x20; B-H, immunoperoxidase, x20; I-L, fluorochrome staining, x100.)
mesothelial histotype, from the low-grade differentiated epithelial to the high-grade sarcomatous histotype, is considered a Figure 6. As a further confirmation, we tested three MPM cell lines, two (MPP89 and REN) derived from epithelial MPM and one (MSTO) from biphasic MPM pleural effusion (as stated by ATCC). The different CAV1 distribution according to the histotype observed in patient tissue samples was confirmed at both protein and mRNA levels in cell line models, thus also proving the reliability of the CAV1 antibody used.

A unique finding of our investigation was the detection of a peculiar CAV1 expression in stromal cells of a fraction of epithelial MPMs (67% of cases). S-CAV1 expression allowed the identification of a particular type of spindle-shaped stromal cells characterized by bland atypical morphology and embedded in a soft stroma; this S-CAV1 expression was absent in cases having a poorly cellular sclerohyaline stroma. Immunostaining revealed that S-CAV1–positive cells expressed diffuse SMA and vimentin, and coexpressed scattered mesothelial markers calretinin, cytokeratin, podoplanin, and WT1 in the same cells. This suggests that such cells could originate from mesothelial cells able to transdifferentiate into stromal activated cells with low proliferative potential. Double immunofluorescence staining seemed to confirm this hypothesis. It is not clear whether these cells represent early transforming neoplastic cells into a biphasic/sarcomatous phenotype, thus confirming the mesothelioma histotypes as a continuous transformation of the mesothelial cell. Although the low proliferative index may not support this notion, the clinical behavior of epithelial MPM having stromal CAV1 expression is more aggressive, overlapping that of biphasic MPM type. These findings lead to a question on the real nature of biphasic MPM compared with epithelial MPM with areas of “reactive” stroma. In particular, could MPM having a stroma enriched for CAV1-expressing cells represent a real, more aggressive variant, irrespective of these cases being classified as epithelial or biphasic histotypes?

An additional aim of our study was to investigate the potential prognostic role of CAV1 expression in MPM. Because prognosis in MPM is greatly affected by MPM histotype and type of treatment administered, we specifically grouped the cases to overcome biases related to these two factors. The sarcomatous MPM group (group C) was in fact used as control only, and specific subanalyses were conducted separately in the series of patients with MPM who were treated with the same chemotherapy regimen represented by platinum-pemetrexed (group A) and control cases with unknown treatment regimens (group B). No association with survival was detected for N-CAV1 expression in pemetrexed (PEM)-treated patients with malignant pleural mesothelioma (MPM). In 54 patients with epithelial (Epi) MPM who received PEM-based treatment, neoplastic CAV1 (N-CAV1) expression did not play a prognostic role (A), whereas stromal CAV1 (S-CAV1) positivity allowed the recognition of a subgroup with a worse outcome (B, continuous line; $P < .0001$); this group had an outcome comparable with that of patients with biphasic (Biph) and sarcomatous (Sarc) MPM (C; $P < .0001$).
of the epithelial MPM type in both groups. On the contrary, the detection of the S-CAV1 positivity identified a subgroup of epithelial MPMs characterized by a worse outcome compared with those devoid of stromal positivity, and with a survival outcome comparable with that of biphasic and sarcomatous histotypes. Together these findings led to the identification of a specific population of stromal cells in epithelial MPM subtype with peculiar morphologic and phenotypic characteristics and linked to prognostic information.

The exact mechanism of CAV1 action in tumor cells is incompletely understood and even more obscure is its role in stromal cells. Physiologically, CAV1 acts as a multifunctional scaffolding protein with multiple binding partners and is associated with cell surface caveolae in the regulation of lipid raft domains. But it also seems to be involved in cancer growth and progression, modulating tissue responses through architectural regulation of the microenvironment. Our findings are in line with prior evidence by Goetz and coworkers, showing an involvement of CAV1 in biochemical remodeling of the microenvironment that favors tumor invasion and metastases. These authors showed that stromal tissue associated with human carcinomas and melanoma metastases is enriched in CAV1-expressing carcinoma-associated fibroblasts (CAFs) that favor biochemical remodeling of the microenvironment that favors tumor invasion and metastases.

Figure 5: Prognostic role of caveolin-1 (CAV1) in validation series. In 37 patients with epithelial malignant pleural mesothelioma (MPM), neoplastic CAV1 (N-CAV1) expression did not play a prognostic role (A) (Kaplan-Meier). If cases were divided by long-term (LS; >24 mo) and short-term (SS; <24 mo) survival groups, distribution levels of stromal CAV1 (S-CAV1) (C) were found to be significantly different ($\chi^2$ test, $P = .0002$), but not those of N-CAV1 levels (B) (Mann-Whitney, $P$ value not significant). Also in this series, the group of epithelial (Epi) MPM cells with S-CAV1 positivity (Epi-S) had an outcome comparable with that of patients with biphasic (Biph) MPM (D) (Kaplan-Meier test, $P < .0001$).
directional migration and invasiveness of carcinoma cells in vitro.27 In ovarian serous carcinomas, which share morphology, biological, and histogenetic similarities with MPM, the presence of CAFs has been associated with a poor prognosis.28 However, the opposite association has been found in other tumor models such as breast cancer and melanoma.29,30 Furthermore, CAV1 was also recently described to promote progression of gastric cancer by upregulating EMT by cross-talk of signaling mechanisms.13 More specifically in MPM, SMA-positive tumor-associated fibroblast was previously described to promote mesothelioma cell progression in vitro and in vivo through cell recruitment and activation by the malignant cytokine network31; however, the exact nature of these cells still remains undefined. Normal mesothelium is of a complex cell type, sharing epithelial and mesenchymal properties that are maintained in neoplastic conditions, and having potential EMT features. Based on this particular nature of the mesothelial cells, we think that they could acquire a (myo)fibroblast-like phenotype through an EMT-like process or, more appropriately, a mesothelial-to-mesenchymal transition process, as described in a recent study in peritoneal mesotheliun.m.32 Yanez-Mo et al33 reported that mesothelial cells in the course of peritoneal dialysis could acquire a mesenchymal phenotype through a mesothelial-to-mesenchymal transition. Sandoval et al32 later showed that cancer-associated fibroblasts could originate from mesothelial cells, allowing carcinoma cells to invade the peritoneal compact zone. Furthermore, mesothelial cells with a mesenchymal phenotype acquire the capacity to synthesize inflammatory and angiogenic factors as well as ECM components. Thus these mesothelial cells contribute to the deterioration of the peritoneum during peritoneal injury. CAV1 expression was also detected in mesothelial cells reactive to Freund’s adjuvant therapy injected into the peritoneal cavity of rats.34 This experimental model was used as an in vivo system to investigate the steps of the EMT mechanism: the possibility that mesothelial cells are not entirely differentiated and might undergo EMT was suggested, also based on a documented change of the distribution of caveolae during inflammation. These data support the idea that CAV1/caveolae might be involved in the regulation of signal transduction, leading to transdifferentiation of mesothelial cells and an increase of cytoplasmic caveolae internalization.34 Finally, upregulation of EMT-related transcription factors in MPM was recently described35 as playing a significant role in morphologic tumor features.36

In our opinion, the presence of stromal CAV1-positive cells also observed in some pleuritis cases is not in contrast with our model; these findings expand the concept that mesothelial cells might transdifferentiate into a (myo)fibroblastic or mesenchymal phenotype (including CAV1 expression), but do not modify our data and interpretation of the peculiar function of these particular cells in the neoplastic setting.

In summary, CAV1 may represent a marker of differentiation and transition from epithelial to sarcomatous MPM, and an early marker of aggressive behavior in epithelial MPM, independent of the treatment, which needs further validation.

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