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Podoplanin Expression in Cancerous Stroma Induces Lymphangiogenesis and Predicts Lymphatic Spread and Patient Survival

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Abstract

Context.——Podoplanin is a mucin-type glycoprotein and a lymphatic endothelial marker. Immunohistochemical staining for podoplanin is currently used as a routine pathologic diagnosis

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tool in Japan to identify lymphatic invasion of cancer cells. Recent reports suggest that podoplanin and other proangiogenic molecules are expressed in stromal fibroblasts and myofibroblasts.

Objective.——To analyze the distribution of podoplanin expression in tumor stroma and its clinical and biologic significance.

Design.——We performed immunohistochemistry for podoplanin on tissue microarrays from 1350 cases of 14 common cancer types.

Results.——Two hundred eighty-seven of 662 cases (43%) showed podoplanin expression in the stromal cells within cancer nests. Stromal podoplanin expression in 14 common cancer types was significantly associated with tumor stage ($P < .001$), lymph node metastases ($P < .001$), lymphatic invasion ($P = .02$), and venous invasion ($P < .001$). The stromal cells positive for podoplanin were also positive for a-smooth muscle actin but negative for desmin, confirming a myofibroblasts phenotype. In contrast, myofibroblasts in inflammatory fibrotic lung diseases were podoplanin negative. Lymphatic vessel density was greater in the stromas with podoplanin expression than in the stroma lacking podoplanin-expressing stromal cells ($P = .01$). Survival data were available for non-small cell lung cancer. Stromal podoplanin expression was associated with poorer prognosis in adenocarcinoma ($P < .001$) and remains statistically significant after adjustment for sex, age, and stage ($P = .01$).

Conclusion.——Our data indicate that podoplanin expression in stromal myofibroblasts may function as a proangiogenic biomarker and may serve as a predictive marker of lymphatic/vascular spread of cancer cells and a prognostic marker of patient survival.

The recent identification of lymphatic endothelial markers has enabled the specific identification of lymphatic vessels.[1-3] Among them, the mucin-type transmembrane glycoprotein podoplanin (also known as T1, gp38, D2-40, or Aggrus) is a well-established marker specific for lymphatic-endothelial cells and is not expressed in blood vessel endothelial cells.[3]

Identification of lymphatic, as well as vascular, invasion by cancer cells is often a negative prognostic factor[4,5] and is used in routine diagnostic pathology in Japan. Podoplanin is often used to detect lymphatic involvement by cancer cells in various cancer types. For colon cancer, use of podoplanin to identify lymphatic involvement is recommended by some Japanese cancer treatment guidelines.[6] With increasing routine use of immunohistochemistry for podoplanin, we have observed frequent podoplanin expression in cancerous stromal cells, most commonly in association with colon cancer. Apart from lymphatic endothelial cells, expression of podoplanin in tumor cells has been reported in some cancer types including squamous cell carcinoma in the lung,[7-10] malignant mesothelioma,[11,12] Kaposi sarcoma, angiosarcoma,[13] hemangioblastoma,[14] dysgerminoma,[7] and brain tumors.[15-17] Recent reports suggest that podoplanin expression in cancer cells may be associated with tumor invasion, metastases, or poor prognosis, although the detailed mechanisms remain obscure.[3,10,17]

Previous publications have reported that podoplanin expression may contribute to lymphatic metastasis in intrahepatic cholangiocarcinoma and have called attention to their presence in cancer-associated fibroblasts of lung adenocarcinoma.[18,19] In our study, we focused on

podoplanin expression in stromal cells and investigated its clinicopathologic characteristics among common cancer types and its association with prognosis in lung cancer.

MATERIALS AND METHODS

Clinical Samples

A total of 1350 cancer cases (400 lung cancer cases; 100 cases each of breast, kidney, biliary tract, thyroid, liver, colon, and stomach cancer; and 50 cases each of prostate, pancreas, bladder, ovary, and uterine body cancer) were selected from the pathology case archive of Toyama University Hospital based on the diagnosis and the quality of the available tissue on the paraffin block. This study was approved by the ethics committee at Toyama University. A total of 1152 patients had adequate clinical and pathologic information. These patients did not receive neoadjuvant treatment. Survival time data and outcome were limited to 211 of 400 lung cancers. The tumors were pathologically staged according to the International Union Against Cancer's TNM classification and histologically divided and graded according to the 2004 World Health Organization guidelines.[20]

Composition of Tissue Microarrays

Two high-density tissue microarrays (TMAs) were designed. The first TMA has 1150 cores from 14 common cancer types (multiple cancer TMA). The second contains 1200 cores representing 400 lung cancer cases in duplicate along with nonneoplastic lung tissue cores from the same patients (lung cancer TMA). For each case, the area with the most representative histology was selected from review of hematoxylin-eosin-stained slides. The cylindrical tissue samples (0.6 mm) were cored from the previously described areas in the donor blocks and extruded into the recipient array blocks using a manual tissue microarrayer (Beecher Instruments, Silver Spring, Maryland) as previously described.[20,21] Multiple 4-μm sections were cut with a microtome using an adhesive-coated tape (Instrumedics, St Louis, Missouri) and stored until use as previously described.[20] Hematoxylin-eosin staining of TMA slides was examined every 50th section to confirm the presence of tumor cells.

Immunohistochemical Staining for TMAs

For all antibodies, the same protocol was used. After sections were deparaffinized and hydrated, antigen retrieval was performed using a pressure chamber (Pascal, DAKO, Kyoto, Japan) in which tissues were heated to 125°C, kept for 1 minute, and cooled to 90°C. After rinsing, slides were placed in an Autostainer (DAKO) and an Envision+ detection system was applied as suggested by the manufacturer's protocol (DAKO). The anti-human podoplanin monoclonal antibody was generated in Kyoto University.[22] The specificity and sensitivity of the antibody, clone 7B10, was examined and reported using enzyme-linked immunosorbent assay, Western blot analyses, and immunohistochemistry.[22] Anti-cytokeratin (AE1/AE3, DAKO) and anti-vimentin (Vim3B4, DAKO) antibodies were used to exclude cores with questionable antigenicity from further analysis, and α-smooth muscle actin (SMA) (1A4, DAKO) and desmin (D33, DAKO) were used to identify cell types in the cancerous stroma. Dilutions for each antibody were 1:200 for cytokeratin, 1:200 for vimentin, 1:100 for SMA, 1:200 for desmin, and 1:10 000 for podoplanin.

Specificity of the immunohistochemical staining for podoplanin was confirmed by staining an in vitro grown cell line that was formalin fixed and paraffin embedded.[23] The cell line NIH3T3 was cultured and transfected with podoplanin gene using FuGENE6 (Roche, Tokyo, Japan) as previously described.[22] After the transfection, the cells were scraped, and the pellet was fixed in formalin and embedded in a paraffin block as an ordinary clinical tissue sample.[20] As a negative control, wild-type NIH3T3 cell line was identically processed. Four-micron thick sliced specimens from both the transfected cell line block and the wild-type cell line block were stained with podoplanin as described previously.

Analysis Using Conventional Pathology Sections

Consideration of tissue heterogeneity must be taken into account when analyzing TMAs. With markers that demonstrate remarkable tissue heterogeneity, staining with 2 to 3 different cores is frequently required.[24] To validate stromal podoplanin staining results seen in TMAs, we compared the immunohistochemical staining results between selected cores and the corresponding original whole tissue sections. Three each of positive and negative cases with podoplanin staining in the TMA were selected at random and compared with whole sections of the same specimens. The staining pattern was concordant between the TMA and the whole sections analyzed. To confirm if stromal podoplanin staining is unique to malignant processes, we also stained specimens from 6 inflammatory lung diseases including usual interstitial pneumonia, atelectasis, and organizing pneumonia, which have a combination of inflammatory cells, myofibroblastic proliferation, and collagen deposition.

Evaluation of Lymphatic Vessel Density in Relation to Podoplanin Expression

In a manner similar to the reported method to evaluate microvessel density,[25] 20 fields were selected in the areas with high number of lymphatic vessels, and lumens of lymphatic vessels were counted in the ×20 objective field (1.3 mm_2). Mean numbers of microlymphatic vessels were calculated and compared using *t* tests.

Scoring of Immunostaining Results in TMA

We scored immunohistochemical staining as previously described.[23] Specifically, the distribution score, which reflects the distribution of the positive signal among stromal cells, was scored as 0 (0%), 1 (1%–50%), or 2 (51%–100%) to reflect the percentage of positive staining among stromal cells seen in the same tissue core. The intensity score, intensity of the signal, was scored as 0 (no signal), 1 (weak), 2 (moderate), or 3 (marked). The sum of distribution and intensity scores (distribution score + intensity score; range, 0–5) was converted into total score (TS): TS = 0 (sum, 0), TS = 1 (sum, 2), TS = 2 (sum, 3), and TS = 3 (sum, 4 or 5). TS 0 and TS 1 were considered negative, whereas TS 2 and TS 3 were considered positive.[20,23] In case of discrepancy in scores between duplicated cores from the same patient, the higher score was assigned as the final score.

Statistical Analysis

Using the χ^2 test, we compared the positive and negative groups of podoplanin staining of multiple cancer TMA and lung cancer TMA on the basis of clinicopathologic factors that included age at diagnosis, sex, histologic type, pathologic stage, and TNM. When one arm

of expected numbers was less than 5, Fisher exact test was applied. Survival analysis of lung cancer patients was performed using the log-rank test for comparing positive and negative groups of staining. Kaplan-Meier curves for the 2 groups were plotted based on overall survival. We accounted for clinical factors by fitting Cox proportional hazards models. The *P* value for the survival analysis corresponds to the log-rank test. *P* values were considered significant when they were less than .05.

RESULTS

The staining of podoplanin in transfected NIH3T3 showed specific membranous signal in approximately 10% to 20% of the cells (Figure 1, A), whereas wild-type NIH3T3 cells were negative (data not shown). Examining the staining in the reactive stroma in response to cancer, 43% of cores in multiple cancer TMA (Figure 1, B) and 33% of cores in lung cancer TMA were positive for podoplanin (Figure 1, C). Stromal cells (Figure 1, D) coexpressed podoplanin (Figure 1, E) and SMA (Figure 1, F) but were negative for desmin, confirming them as myofibroblasts. Inflammatory cells, mainly lymphocytes and macrophages, present in the same stroma were negative for podoplanin. Lymphatic vessels stained more intensely with podoplanin than did podoplanin-positive stroma (Figure 1, E and G). The mean numbers of lymphatic vessels were significantly higher in the stroma with podoplanin-positive expression (5.2 ± 1.23) than in stroma with podoplanin-negative expression (1.9 ± 1.12) ($P = .01$) (Figure 2).

A total of 1152 patients had clinical and pathologic information. Of 1152 cases, 126 cases were excluded as inadequate for scoring. We applied a criteria that tissue cores that were immunohistochemically negative for both cytokeratin and vimentin would be excluded from analysis based on the assumption that most carcinomas should demonstrate pancytokeratin staining, and most interstitial cells should be reactive for vimentin. By this method, 3 cores were excluded from the experiment. An additional 145 cases were also excluded due to absence of cancerous stromal tissue in the core by hematoxylin-eosin light microscopic examination. Most renal cell carcinomas (87 of 94 cases) lacked cancerous stromal cells, which is not an unanticipated result considering the histologic features of renal cell carcinoma. Therefore, all cases of renal cell carcinoma were excluded from further analysis. Ultimately, 784 cases were analyzed. The number of cases eventually extracted for final analysis is listed in Tables 1 and 2 along with their clinical data.

Upon examination of multiple cancer TMA, the distribution of immunohistochemical status in each cancer type was determined (Table 3). The most frequent expression was found in colon cancer (90%), followed by stomach cancer (82%), cancer of the biliary tract (73%), and pancreatic cancer (73%). Associations between podoplanin expression and T status ($P < .001$), lymph node metastases ($P < .001$), and stage ($P < .001$) were found. Podoplanin expression was observed in 49% of T2 to T4 cancers compared with 26% of T1, 52% of cancers with lymph node metastases compared with 39% of cancers without metastasis, and 50% stage II to IV cancers compared with 28% of cancers in stage I. Associations between podoplanin expression and lymphatic invasion ($P = .02$) and venous invasion ($P < .001$) were also observed (Table 4).

Upon examination of lung cancers in lung cancer TMA including all histologic types, there were no significant associations between podoplanin expression and T status ($P = .06$), lymph node metastases, or stage. However, when the analysis was limited to pulmonary adenocarcinomas (n = 157), podoplanin expression was associated with lymph node metastases ($P = .05$), lymphatic invasion ($P = .006$), venous invasion ($P = .008$), and trended with stage ($P = .06$) (Table 5). No association was observed in squamous cell carcinoma (n = 88). Case numbers in other histologic types were insufficient for statistical analysis.

Follow-up data were only available for non-small cell lung cancer. Survival analysis demonstrated podoplanin expression in stroma correlated with increased risk of death for non-small cell lung cancer ($P = .02$) (Figure 3, A). After adjustment for sex, age, and stage by the Cox proportional hazards regression model, the association with survival was not significant. In patients with adenocarcinoma, expression was associated with increased risk of death ($P < .001$) (Figure 3, B), and Cox proportional hazards modeling showed persistent significance in lung adenocarcinoma ($P = .01$) (Table 6). In squamous cell carcinoma, however, the expression was not associated with prognosis (Figure 3, C).

COMMENT

In this study we found that podoplanin was expressed in myofibroblasts of desmoplastic stroma in a great variety of cancer cell types. Expression of podoplanin within desmoplastic stroma correlated with T and lymph node status. In examining lung cancer specifically, we demonstrated that podoplanin expression is not statistically correlated with stage and other factors when all histologies are considered. Subset analysis, by histology, demonstrated podoplanin expression in the stroma associated with adenocarcinoma is correlated with T status, lymph node status, stage, and patient survival and remained statistically significant with multivariate analysis. We examined podoplanin expression in tumor cells, but the associations between podoplanin expressions in cancer cells and clinicopathologic factors or patients' survival in lung cancer were not found.

In our cohort of non-small cell lung cancer, we only observed the survival significance of podoplanin expression in pulmonary adenocarcinoma but not in pulmonary squamous cell carcinoma. Biologically there appears to be a difference in the stromal cells between adenocarcinoma and squamous cell carcinoma. As for utility, podoplanin expression in adenocarcinomas may have some value in guiding therapy as well as in being a predictive marker of survival. For example, detection of stromal podoplanin expression in a preoperative small biopsy might function to guide the extent of lymph node dissection at the time of definitive resection.

Although expression of different molecules has been widely explored in tumor cells, expression within the stroma has received little attention. [7,26,27] Our results support a unique model in which podoplanin expression in the stromal myofibroblasts is associated with lymph node metastases and lymphatic invasion. They indicate that podoplanin expression in myofibroblasts may be associated with lymphangiogenesis and lymphatic spread of cancer cells. Tumor lymphangiogenesis has been reported as a prognostic indicator and a predictor of lymph node metastases in non-small cell lung cancer,[28] bladder cancer,[29] colorectal

cancer, and head and neck cancer.[25,30] Aishima et al[18] reported the antibody D2-40, against human podoplanin, stained myofibroblasts and was associated with lymphatic metastasis in intrahepatic cholangiocarcinoma.

Kawase et al[19] also reported the prognostic significance of stromal podoplanin expression in lung adenocarcinoma, consistent with our findings. However, they presented no data in squamous cell carcinoma or other common cancers. They additionally observed that expression of podoplanin in carcinoma-associated fibroblasts was not found in noninvasive adenocarcinoma.[19] We similarly observed that in 9 cases with predominant bronchioloalveolar features, all demonstrated podoplanin-negative stromal cells.

We showed that the mean numbers of lymphatic vessels in the cancerous stroma with positive podoplanin staining was higher. The results suggest the hypothesis that podoplanin expression in myofibroblasts predicts prognosis due to lymphangiogenesis associated with stromal podoplanin expression. The hypothesis is partially supported by the data in the study by Kawase et al[19] in which cases with high-grade podoplanin expression in myofibroblasts was associated with lymphatic invasion. In contrast, Aishima et al[ls] reported similar data examining expression of vascular endothelial factor C in tumor cells and D2-40/podoplanin in myofibroblasts for intrahepatic cholangiocarcinoma. They showed that lymphatic vascular density is not associated with patients' survival or vascular endothelial factor C expression in intrahepatic cholangiocarcinoma; however, they did not examine stromal podoplanin expression in reference to lymphatic vascular density. They concluded that lymphangiogenesis does not play a direct role in lymph node metastasis. The clinical significance of lymphangiogenesis in relation to lymph node metastasis is controversial and may differ by cancer type.[31] According to the report by Renyi-Vamos et al[28] published recently, lymphangiogenesis in non-small cell lung cancer significantly correlated with lymph node metastasis, which supports our findings.

There are few reports describing molecular expression in stromal cells in relationship to patients' prognosis[19,30,32-14]; however, most of those reports use the term *activated fibrosis*[45] and do not define the phenotype of the stromal cells. Using SMA and desmin staining in conventional whole specimen analysis, we confirmed that most of the podoplanin-positive stromal cells are myofibroblasts. In contrast, myofibroblasts and/or fibroblasts in inflammatory lung diseases were negative for podoplanin expression (data not shown). Myofibroblasts within wound sites have been demonstrated to express podoplanin, similar to carcinoma-associated fibroblasts.[45] Orimo et al[45] described that carcinoma-associated fibroblasts enhanced tumor angiogenesis and exhibited increase of SMA expression as well as contractility, which indicates that the fibroblasts they described were myofibroblasts. Proliferation of myofibroblasts in peritumoral areas appears with invasion by the adenocarcinoma tumor cells and may play an important role in lymphangiogenesis.[19,46]

The origin of myofibroblasts in cancerous stroma remains unclear. One theory is that they are derived from bone marrow. Alternative models suggest that they may be tumor cells with epithelial mesenchymal transition or endothelial cells with endothelial mesenchymal

transition.[47-49] Additional analysis to unveil the pathogenesis of myofibroblasts in cancerous stroma is needed.

The importance of tumor stroma to tumor behavior is well demonstrated. Tumor stroma consists of a complex admixture of cells and extracellular matrix. Approaches where tumor stroma is evaluated as a homogenous unit are inadequate. The different cell types that compose the tumor stroma have biologic significance and require interrogation at the cellular level and are not amenable to evaluation at the homogenous tissue level. Evidence of this is the contribution of bone marrow–derived stem cells contributing to tumor cells and tumor stroma, with cells that have phenotype characteristics of vascular endothelium, fibroblasts, and myofibroblasts.[48,49] We anticipate that cytomorphologic analysis is necessary to identify the exact cell types contributing to the tumor stroma biology. Biologic events in stroma are potential molecular targets for therapy. Modulation of stromal cells derived from the normal host has advantages over targeting multimutated genetically unstable tumor cells. Tumor cells routinely acquire multiple mutations. A tumor may contain different clonal populations with different phenotypes, different gene expression patterns, and differences in response to the environment.[50] Currently, most anticancer drugs target tumor cells. One exception is bevacizumab (Avastin), a drug that targets stromal vessels within cancerous stromal tissues. Additional studies on the role of podoplanin in lymphangiogensis may help the development of new drugs targeting stromal tumoral vessels.

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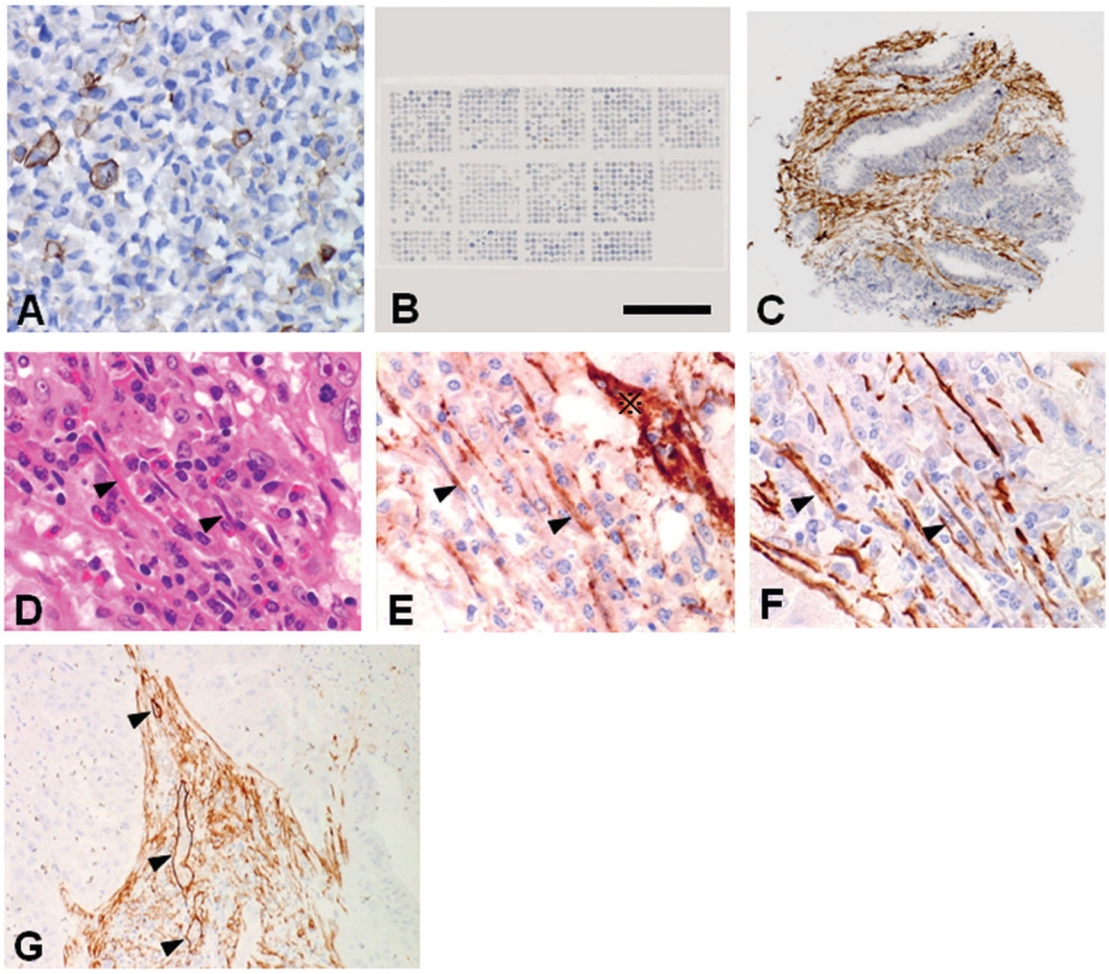

Figure 1.

Immunohistochemical detection of podoplanin in cancerous stroma. A, Validation of podoplanin immunohistochemical staining using podoplanin transfected NIH3T3 cells. Paraffin-embedded cells demonstrate membranous signals in 10% to 20% of cells (original magnification ×400). B and C, Images of a tissue microarray. B, Overview of multiple cancer tissue microarray with podoplanin staining. Each group has either 100 or 50 cores from 1 cancer type. A total of 1150 cases from 14 different cancer types are included (scale bar = 1 cm). C, Representative core positive for anti-podoplanin staining in cancerous stromal cells (diameter of the core is 0.6 mm). D through F, High-power view of cancerous stroma stained with hematoxylin-eosin (D), anti-podoplanin (E), and anti-α-smooth muscle actin (F) using consecutive sections (original magnifications ×400 [D through F]). Arrowheads indicate nonneoplastic spindle cells seen in the cancerous stroma. Identical cells are positive for podoplanin and α-smooth muscle actin. Spindle cells positive for podoplanin were considered as myofibroblasts based on the staining patterns and morphology (asterisk in E, lymphatic vessel stained with podoplanin). G, Cancerous stromal cells and lymphatic vessels (arrowheads) are positive for podoplanin (original magnification ×100). Lymphatic vessels stained more intensely with podoplanin than did stromal spindle cells.

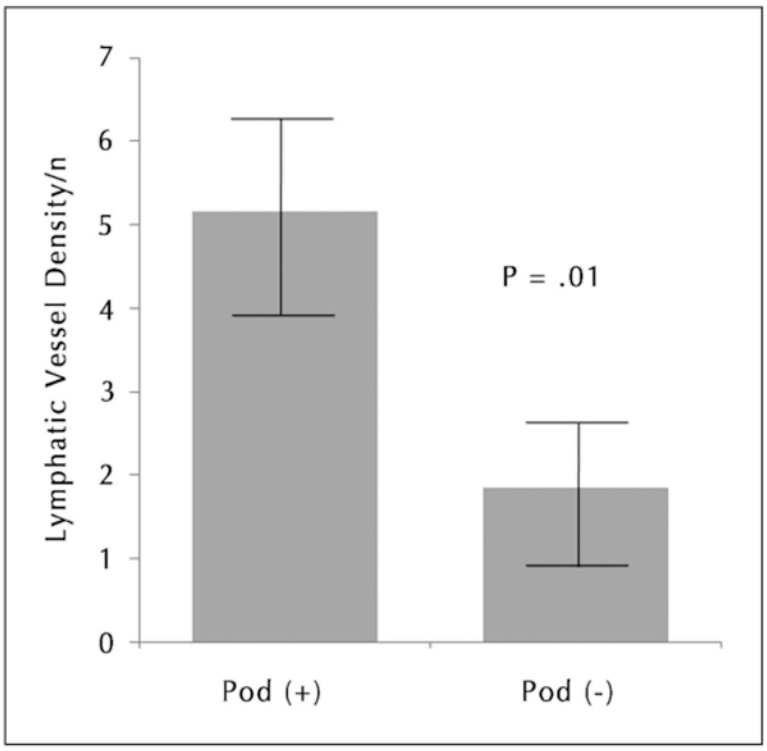

Figure 2.

*The mean numbers of lymphatic vessels in the stroma with podoplanin (Pod) positive (+) and negative (−) expression. The numbers were counted in the area of a ×20 (1.3 mm^2) objective. Lymphatic vessel densities in the podoplanin-positive stroma were higher than those in podoplanin-negative stroma (*P = *.01,* t *tests).*

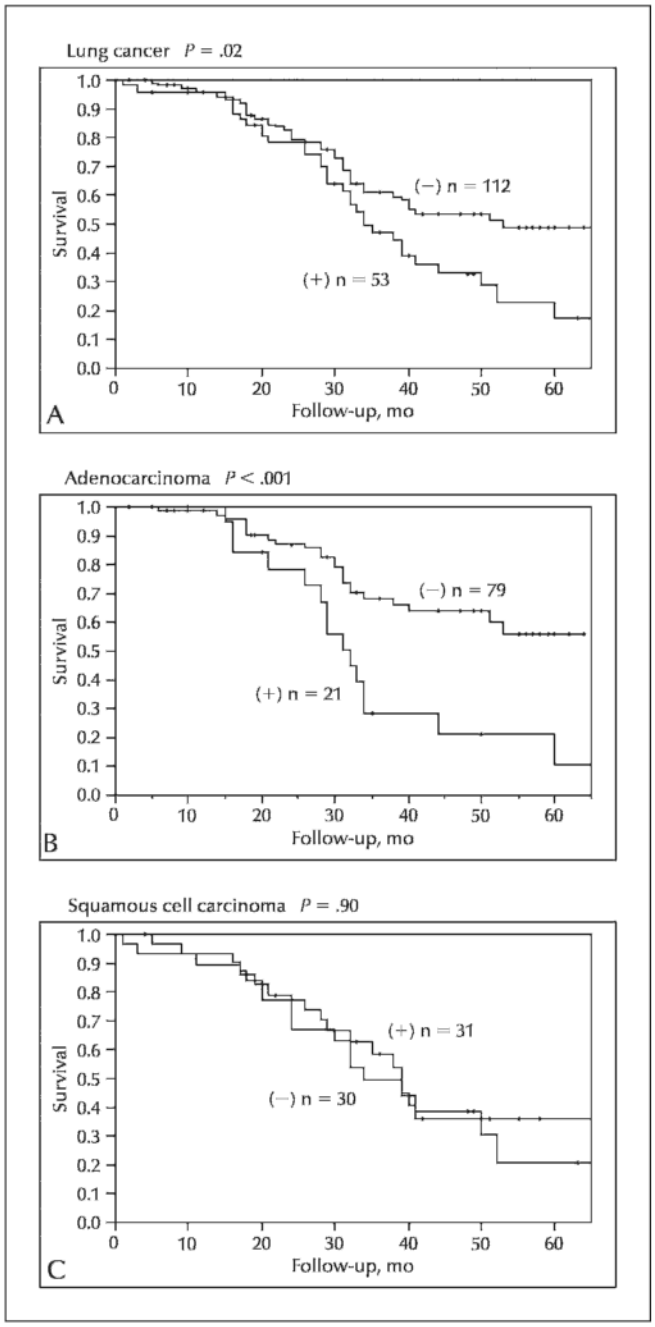

Figure 3.

Survival analysis of podoplanin expression in cancerous stromal cells in lung cancer patients. Kaplan-Meier (KM) curves comparing survival between cases with podoplanin negative (−) in cancerous stroma and those with podoplanin positive (+). A, KM curve in non-small cell lung cancer cases. B, KM curve in adenocarcinoma cases. C, KM curve in squamous cell carcinoma cases.

Table 1.

Clinicopathologic Characteristics of the Patients in Multiple Cancer Tissue Microarray[a]

	Sex		Age	Tumor Stage, No.			
	Male, No.	Female, No.	(mean ± SD), y	I	II	III	IV
Prostate	31	…	68 ± 5.8	0	12	15	2
Thyroid	11	57	55 ± 16.3	15	11	39	0
Bladder	22	7	67 ± 9.3	11	4	9	4
Breast	1	75	57 ± 12.3	23	39	6	0
Lung (AD)	45	37	65 ± 9.7	49	16	14	3
Uterine body	…	29	59 ± 12.2	15	6	6	2
Stomach	42	13	67 ± 10.7	7	13	27	6
Ovary	…	37	54 ± 13.2	20	1	15	1
Liver	16	6	64 ± 10.0	5	6	2	5
Colon	46	32	69 ± 11.3	7	27	40	4
Lung (SCC)	60	2	66 ± 9.3	34	22	6	0
Biliary tract	38	34	68 ± 11.5	7	11	8	46
Pancreas	14	7	68 ± 10.1	0	0	14	5

Abbreviations: AD, adenocarcinoma;SCC, squamous cell carcinoma.

[a]Numbers of cases listed are after exclusion due to tissue loss, lack of cancerous stroma, or negative result of validation for antigen retardation.

Table 2.

Clinicopathologic Characteristics of the Lung Cancer Patients

	No. of Cases	No. Cases With Follow-up
Sex		
Male	182	122
Female	84	59
Age, y		
Mean ± SD	65.6 ± 9.3	64.9 ± 9.2
Stage		
I	146	97
II	54	44
III	62	37
IV	4	3
Tumor type		
Adenocarcinoma	157	107
Squamous cell carcinoma	88	70
Large cell carcinoma	12	0
Adenosquamous carcinoma	8	4
Small cell carcinoma	1	0

Table 3.

Podoplanin Expression in Stromal Spindle Cells Using Multiple Cancer Tissue Microarray

		Total Score, No.				
	Total, No.	0	1	2	3	Positive Rate, %
Colon	77	1	7	14	55	90
Stomach	55	1	9	19	26	82
Biliary tract	66	1	17	16	32	73
Pancreas	22	1	5	11	5	73
Bladder	29	7	7	2	13	52
Lung (SCC)	68	13	29	15	11	38
Liver	22	5	9	6	2	36
Breast	76	18	36	18	4	29
Uterine body	29	1	20	7	1	28
Prostate	31	7	16	8	0	26
Ovary	37	13	16	5	3	22
Lung (AD)	82	36	32	9	5	17
Thyroid	68	54	14	0	0	0
Total	662	158	217	130	157	43

Abbreviations: AD, adenocarcinoma; SCC, squamous cell carcinoma.

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Table 4.

Association Between Podoplanin Expression and Clinicopathologic Factors in Multiple Cancer Tissue Microarray

	No.	Positive, No. (%)	Negative, No. (%)	*P* Value
T1	150	39 (26)	111 (74)	<.001
T2–T4	486	239 (49)	247 (51)	
NO	382	149 (39)	233 (61)	<.001
N1–N3	240	124 (52)	116 (48)	
Stage I	193	54 (28)	139 (72)	<.001
Stage II–IV	447	225 (50)	222 (50)	
Ly (−)	128	39 (30)	89 (70)	.02
Ly (+)	164	72 (44)	92 (56)	
v (−)	166	44 (27)	122 (73)	<.001
v (+)	121	67 (55)	54 (45)	

Abbreviations: Ly, invasion to the lymphatic vessel; v, invasion to the blood vessel.

Table 5.

Association Between Podoplanin Expression and Clinicopathologic Factors in Lung Cancer Tissue Microarray

	All Cases				Adenocarcinoma			
	No.	**Positive, No. (%)**	**Negative, No. (%)**	***P* Value**	**No.**	**Positive, No. (%)**	**Negative, No. (%)**	***P* Value**
T1	113	32 (28)	81 (72)	.06	80	14 (18)	66 (83)	.09
T2–T4	153	60 (39)	93 (61)		77	22 (29)	55 (71)	
NO	161	51 (32)	110 (68)	.22	96	17 (18)	79 (82)	.05
N1–N3	105	41 (39)	64 (61)		61	19 (31)	42 (61)	
Stage I	146	44 (30)	102 (70)	.13	93	16 (17)	77 (83)	.06
Stage II–IV	120	47 (39)	73 (61)		64	20 (31)	44 (69)	
Ly (−)	111	36 (32)	75 (68)	.96	60	6 (10)	54 (90)	.006
Ly (+)	116	38 (33)	78 (67)		77	22 (29)	55 (71)	
v (−)	140	36 (26)	104 (74)	.008	91	12 (13)	79 (87)	.008
v (+)	82	36 (44)	46 (56)		42	14 (33)	28 (67)	

Abbreviations: Ly, invasion to the lymphatic vessel; v, invasion to the blood vessel.

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Table 6.

Multivariate Analysis with Cox Proportional Hazards Model for Prediction in Lung Cancer Patient With Adenocarcinoma

	Hazard Ratio (95% Confidence Interval)	*P* Value
Podoplanin		
Negative	1	.01
Positive	1.72 (1.13–2.60)	
Age, y		
<60	1	.42
≥60	0.86 (0.60–1.69)	
Sex		
Female	1	.57
Male	1.12 (0.75–1.69)	
Stage		
I	1	<.001
II	1.46 (0.89–2.33)	
III	2.78 (1.80–4.35)	
IV	2.20 (0.86–4.31)	