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Independent value of fascin immunoreactivity for predicting lymph node metastases in typical and atypical pulmonary carcinoids

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Summary Immunoreactivity for fascin, an actin-bundling protein related to cell motility, has been reported in breast, ovary, pancreas, skin, and non-small cell carcinomas, and associated with more advanced disease stage and poorer prognosis. Data on pulmonary neuroendocrine (NE) tumors, however, are lacking. We evaluated the expression of fascin by immunohistochemistry—using two different monoclonal antibodies—in surgical specimens of pulmonary NE tumors of all the diverse histological types from 128 consecutive patients recruited between 1987 and 2001, and investigated its relationship with the presence of lymph node metastases. Overall, fascin immunoreactivity was detected in 5% of 38 typical carcinoids (TC), 35% of 23 atypical carcinoids (AC), 83% of 40 large-cell neuroendocrine carcinomas (LCNEC), and 100% of 27 small-cell lung carcinomas (SCLC) ($P < 0.001$). Normal NE cells or hyperplastic NE tumorlets were consistently unreactive. No statistically significant differences in fascin immunoreactivity were found between the two antibodies. In TC and AC but not high-grade NE tumors, fascin immunoreactivity closely correlated with the occurrence of lymph node metastases, the pN class and the number of involved lymph nodes ($P < 0.001$). It was also significantly associated with an increased proliferative activity (Ki-67 labeling index $> 5\%$) ($P = 0.020$), and

Abbreviations: AC, atypical carcinoids; ACTH, adrenocorticotrophic hormone; alpha-HCG, alpha-subunit of human chorionic gonadotropin; GRP, gastrin-related peptide; IGF-I, insulin growth factor-I; LCNEC, large-cell neuroendocrine carcinoma; NE, neuroendocrine; SCLC, small-cell lung carcinoma; TC, typical carcinoids.

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with either down-regulation or altered subcellular compartmentalization of E-cadherin ($P < 0.001$) and CD99 ($P = 0.030$), two cell adhesion complexes in pulmonary NE tumors. At multivariate analysis, only fascin emerged as an independent predictor of lymph node metastases in this tumor group (HR 30.28; 95% confidence intervals: 1.59–574.49; $P = 0.023$). This study indicates that fascin immunoreactivity may identify subsets of pulmonary carcinoid patients with different metastatic potential to regional lymph nodes. Targeting the fascin pathway could be a novel therapeutic strategy of pulmonary carcinoids.

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1. Introduction

Approximately one fourth to one third of lung tumors are neuroendocrine (NE), and are classified according to a three-tier clinico-pathological spectrum, ranging from relatively indolent neoplasms with long life expectation to very aggressive tumors with dismal prognosis [1,2]. According to recently refined criteria, high-grade NE tumors include small-cell lung carcinoma (SCLC) and LCNEC, whereas low- to intermediate-grade NE tumors typical carcinoids (TC) and atypical carcinoids (AC), respectively [3]. The prevalence of regional lymph node metastases at presentation is 5–15% in TC, up to 40–48% in AC and 50–80% in poorly differentiated NE tumors [1,3–6]. Several parameters, including DNA ploidy, proliferative activity, oncogene and tumor-suppressor gene alterations or chromosome deletions, have been recently suggested as prognostic factors in low to intermediately malignant NE tumors [7–15], but histological typing still remains the most powerful predictor of their clinical course [4,5,16].

Interactions among cancer cells, extracellular matrix proteins and endothelial cells play a pivotal role in tumor invasiveness and metastasis [17]. Invasive tumor cells are often characterized by changes in cell shape resulting in membrane protrusions, loss of anchorage dependency, and reduction of either cell–cell adhesion or junctional communications. Many of these changes are due to re-arrangements of the cytoskeletal actin microfilaments at the cell periphery, where several types of actin cross-linking proteins accumulate [18,19]. Among these molecules, fascin is a highly conserved 55-kDa actin-bundling protein that play an important role in the organization of several types of actin-based structures such as filopodia, spikes, lamellipodial ribs, dendrites and microvilli (reviewed by Kureishy et al., 2002) [20]. In fact fascin is normally expressed by cells characterized by different types of membrane protrusions, such as

neurons, glial cells and dendritic cells [21–23], or by actively migrating cells, such as endothelial cells and macrophages [22,24,25]. In epithelial cells, the fascin expression level is usually low, but is often up-regulated in transformed cells [26] and in several types of human neoplasms, such as breast [27–29], ovary [24], skin [30], pancreas [31,32], and non-small cell lung carcinomas [33,34].

E-cadherin is the key functional component of adherent junctions between epithelial cells [35]. Loss or down-regulation of E-cadherin in lung cancer usually correlate with poor tumor differentiation, more advanced disease stage, lymph node involvement, bone marrow micrometastases, and reduced life expectation [36–42], although this is possibly not true for NE tumors of the lung [43]. A functional relationship between E-cadherin and fascin has been documented in transformed epithelial cell lines, where cytoplasmic accumulation of fascin leads to loss of cell–cell adhesion via a selective disruption of the E-cadherin adhesion system [26].

CD99/MIC-2, a heavily *O*-glycosylated 32-kDa trans-membrane protein, [44], is another molecule likely involved in homophilic cell–cell adhesion properties of several cell systems [45–51], including pulmonary and gastroenteropancreatic NE tumors, where its down-regulation is associated with increased proliferative activity and occurrence of lymph node metastases [52].

Here we investigated for the first time fascin immunoreactivity by using two different monoclonal antibodies in a retrospective series of 128 stage I–IV pulmonary NE tumors. We document that it correlates with the occurrence of lymph node metastases, the pN class and the number of involved lymph nodes in low- to intermediate-grade NE tumors, in association with the disruption of E-cadherin- and CD99-mediated cell–cell adhesion systems.

Table 1 Antibody panel used in the current study

Antibodies	m/p	Clone	Source	Dilution	Pretreatment
Fascin (p55)	m	IM20	Novocastra Laboratories	1:400	MWO-EDTA
Fascin (p55)	m	55k-2	DAKO	1:400	MWO-EDTA
CD99	m	HO36-1.1	Novocastra Laboratories	1:500	MWO-CB
Chromogranin A	m	LK2H10	Signet Laboratories, Dedham, MA, USA	1:40	None
Alpha-HCG	p	–	Signet Laboratories	1:200	MWO-CB
Synaptophysin	m	SY 38	DAKO	1:20	MWO-CB
Gastrin releasing peptide	p	–	DAKO	1:400	None
Calcitonin	p	–	DAKO	1:20	None
Serotonin	m	5HT-H209	DAKO	1:10	None
ACTH	m	02A3	DAKO	1:100	None
E-cadherin	m	4A2 C7	Zymed Laboratories, San Francisco, CA, USA	1:400	MWO-EDTA
Ki-67 antigen	m	MIB-1	Immunotech, Marseille, France	1:400	MWO-EDTA

MWO-EDTA: microwave oven at 750 W for 12 min in EDTA buffer pH 8. MWO-CB: microwave oven at 750 W for 10 min in citrate buffer pH 6. m, monoclonal antibody; p, polyclonal antibody.

2. Patients and methods

2.1. Patients

The study population includes 128 consecutive patients (83 males and 45 females) with stage I–IV NE tumors [53] (38 TC, 23 AC, 40 LCNEC, and 27 SCLC) surgically treated at the participating Institutions, between 1987 and 2001. For each case, all paraffin blocks were retrieved and original hematoxylin and eosin-stained sections reviewed. All lymph nodes had been conventionally processed, with two sections stained with hematoxylin and eosin being examined per single paraffin block. The diagnosis of NE tumor was based on established morphological and immunohistochemical criteria (immunoreactivity for synaptophysin, chromogranin A, and a variety of respiratory tract-related hormones, including gastrin-related peptide (GRP), calcitonin, adrenocorticotrophic hormone (ACTH), serotonin and alpha-subunit of human chorionic gonadotropin (alpha-HCG)) [2,3,52,54]. In particular, AC were defined as tumors with carcinoid morphology showing 2–10 mitoses/10 HPF (each field corresponding to an area of 0.19625 mm² with a 40 × objective) [2] and/or punctate necrosis. To ensure accurate staging, all tumors were removed by radical surgery, inclusive of extensive mediastinal lymph node dissection (median value: nine excised lymph nodes per patient).

The patients' age ranged from 18 to 80 years for men (mean ± S.D.: 60.3 ± 13.4 years; median: 64 years) and from 15 to 78 years for women (mean ± S.D.: 55.1 ± 15.8 years; median: 60 years). TC and AC were more commonly (43/61) in stage I of disease, whereas most (35/67) LCNEC and SCLC were in stage II–IV ($P = 0.015$). The follow-up time

in most cases, however, was still too short (mean ± S.D.: 20.5 ± 27.2 months; median: 11 months) to perform reliable survival analysis in such types of tumors, especially in low- to intermediate-grade neoplasms.

2.2. Immunohistochemistry

Formalin-fixed and paraffin-embedded tissue samples obtained at surgery were investigated. Tumors up to 2 cm in size were entirely embedded and immunostained; at least two representative tissue blocks were investigated in larger neoplasms. Ten samples of normal pulmonary parenchyma and bronchial tree taken at different levels from patients with non-malignant lung diseases, and ten samples of pulmonary NE tumorlets and non-neoplastic peritumoral tissue from the study patients were used as control groups.

Immunohistochemical reactions were performed using the primary antibodies listed in Table 1 according to previously refined immunohistochemical methods [52,55]. In particular, fascin was immunolocalized using two monoclonal antibodies, clone IM20 (Novocastra Laboratories, Newcastle upon Tyne, UK) that recognizes the C-terminal region of the molecule, and clone 55k-2 (Dako, Glostrup, Denmark) that reacts with a 55-kDa protein in Western blots of HeLa, normal rat kidney and gerbil fibroma cell lysates [56]. The latter anti-fascin antibody was also found to immunoblot bacterially expressed *hsn* gene product and a 55-kDa protein from cell lysates of peripheral blood dendritic cells [57,58]. The specificity of the clone IM20 has been evaluated on human tonsil for Western blot analysis, with a molecular weight detected at approximately 55-kDa (Novocastra

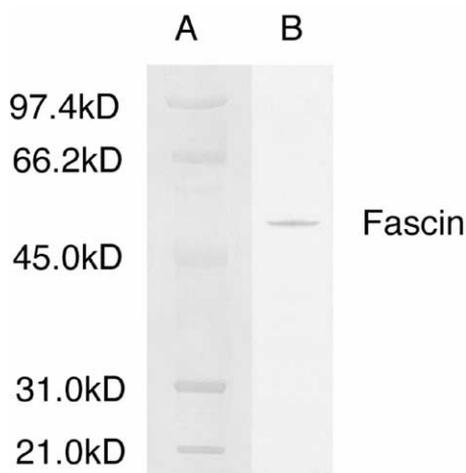


Fig. 1 Western blot analysis of human tonsil using NCL-FASCIN (clone IM20) is shown. Lane A, molecular weight markers. Lane B, tonsil immunoblotted with NCL-FASCIN antibody. (Image supplied by courtesy of Novocastra Laboratories Ltd).

Laboratories, personal communication, 2002, Fig. 1). The specificity of all immunoreactions was double-checked by substituting the primary antibody with a non-related isotypic mouse immunoglobulin at a comparable dilution, with normal serum alone [59], and using appropriate internal positive controls (dendritic cells of the bronchus-associated lymphoid tissue and endothelial cells of microvessels).

2.3. Scoring method

Two observers evaluated fascin immunoreactivity of tumor cells independently and blindly without knowledge of the patients' identity or disease stage. For each case, a numerical fascin score was recorded, based on both the percentage of immunolabeled neoplastic cells and the staining intensity, assessed by scanning at least 1000 neoplastic cells in representative fields of immunostaining. The percentage of immunoreactive cells was recorded in a 4-tier scale (0–3): tumors were considered negative if staining was either completely absent or occurring in less than 5% of the neoplastic cells; cases showing immunoreactivity in 5–30%, 31–60%, or more than 60% of the neoplastic cells were given the value of 1, 2, or 3, respectively (Table 2). Staining intensity was recorded in a 3-tier scale, as weak (1), if only a faint granular labeling was detectable throughout the cytoplasm, moderate (2), if a more distinct staining but less intense than that seen in the normal endothelial or dendritic cells was observed, or strong (3), if it was of the same or greater intensity

Table 2 Distribution of fascin immunoreactivity in 128 NE tumors of the lung

Tumor	Fascin index (%)		Fascin intensity (%)				Fascin score (%)					
	Negative		1+	2+	3+	None	Weak (1)	Moderate (2)	Strong (3)	Negative	1–4	≥ 6
TC	36 (95)		2 (5)	0	0	36 (95)	0	2 (5)	0	36 (95)	2 (5)	0
AC	15 (65)		5 (22)	3 (13)	0	15 (65)	3 (13)	5 (22)	0	15 (65)	8 (35)	0
LCNEC	6 (16)		10 (25)	9 (22)	15 (37)	6 (15)	4 (10)	18 (45)	12 (30)	6 (15)	16 (40)	18 (45)
SCLC	0		2 (7)	6 (22)	19 (71)	0	2 (7)	7 (26)	18 (67)	0	5 (19)	22 (81)
			$\chi^2 = 96.624, P < 0.001$				$\chi^2 = 98.032, P < 0.001$					

Fascin index: negative (< 5%), 1+ (5–30%); 2+ (31–60%); 3+ (61–100%). Fascin intensity: weak (1); moderate (2); strong (3) (see text for details). Fascin score: fascin index multiplied by fascin intensity.

than the latter. The fascin score was then calculated by multiplying the two values. In order to evaluate differences between the two antibodies in both normal tissue and tumor samples, the same histological fields were evaluated for fascin immunostaining on adjacent paraffin sections.

E-cadherin staining pattern was defined as linear, if a moderate to strong staining was seen along the entire cell membrane, membrane-disrupted, if only an irregular or serrated immunostaining was observed along the cell membrane, or cytoplasmic, if immunostaining was seen predominantly confined to the cytoplasm with no discernible membrane reinforcement. Likewise, CD99 labeling pattern was defined as linear, if decoration of cell membrane was seen along with variable cytoplasmic staining, or cytoplasmic, if immunostaining was seen predominantly confined to the cytoplasm with no appreciable membrane reinforcement. In a similar manner as for fascin, tumors immunostained for E-cadherin and CD99 were considered negative if less than 5% neoplastic cells were immunoreactive.

2.4. Statistical analysis

Associations of categorical variables were evaluated by the Fisher exact *t*-test or χ^2 -test. Intra-observer reproducibility was assessed by analysis of the variance, whereas inter-observer agreement was assessed calculating the slope and the intercept when regressing the readings of one observer as a function of the readings of the other one. A logistic regression analysis model served to compare explanatory variables with the occurrence of lymph node metastases. All estimates were performed using the SAS statistical software (SAS Institute, Inc., Cary, NC). All *P*-values were based on two-sided testing.

3. Results

Fascin immunoreactivity appeared as a fine, granular to diffuse cytoplasmic staining, in both normal and neoplastic cells. IM20 and 55k-2 monoclonal antibodies did not show significant differences with regards to the number of normal and neoplastic immunoreactive cells, the staining pattern and the immunostaining intensity, and a high correlation was noted between the two antibodies (Spearman's rank test: $r > 0.90$, $P < 0.0001$).

In the normal lung of both neoplastic and non-neoplastic patients, fascin immunoreactivity invariably decorated the endothelial cells of bronchial and alveolar microvessels, and the dendritic

cells of the mucosa-associated lymphoid tissue. Endothelial cells of major pulmonary vessels, and bronchial and alveolar epithelia were consistently unreactive, though the basolateral membranes of bronchial epithelial cells were occasionally decorated. The scattered bronchial mucosa-associated NE cells and the NE tumorlets did not show any fascin immunoreactivity (Fig. 2).

Overall, fascin immunoreactivity was detected in 71/128 (55%) NE tumors that included 2/38 (5%) TC, 8/23 (35%) AC, 34/40 (83%) LCNEC and 27/27 (100%) SCLC (Table 2). The percentage of immunoreactive cells, the staining intensity and the fascin score increased significantly from TC and AC to poorly differentiated NE tumors (Table 2). No preferential distribution of fascin-immunoreactive cells was seen within individual tumors in perivascular, peripheral or central areas, although it was often markedly enhanced at the tumor–host interface. Neoplastic emboli were commonly decorated by the anti-fascin antibodies, especially in AC and poorly differentiated NE tumors (Fig. 2). Evaluation of intra-observer reproducibility did not show statistically significant differences among different readings of each observer. Evaluation of inter-observer agreement showed that the slope of the regression line was 0.959 (standard error = 0.0251) and not different from 1, and that the intercept value of the regression line was 1.88 (standard error = 1.266) and not different from 0, indicating a good agreement between the two readers ($P = NS$).

In TC and AC, the fascin score correlated significantly with more advanced disease stage, membrane-disrupted or cytoplasmic E-cadherin expression, reduced CD99 labeling index and reduced linear immunostaining pattern (Fig. 3), increased proliferative activity assessed by Ki-67 labeling index, and reduced content of NE secretory granules, evaluated by cromogranin A immunostaining (Table 3). In LCNEC and SCLC, the fascin score correlated with decreased percentage of E-cadherin immunoreactive cells, membrane-disrupted or cytoplasmic E-cadherin staining patterns, increased Ki-67 labeling index, and reduced content of NE secretory granule-related hormones (calcitonin, serotonin and alpha-HCG) (Table 3). No significant associations were noted with the other clinico-pathological variables examined.

Regional lymph node metastases were present in 8/38 TC, 12/23 AC, 18/40 LCNEC and 18/27 SCLC ($P = 0.002$), without any difference in the prevalence of N1 and N2 classes. In TC and AC, the fascin score correlated significantly not only with the occurrence of lymph node metastases (Table 3), but also with the level of lymph node involvement

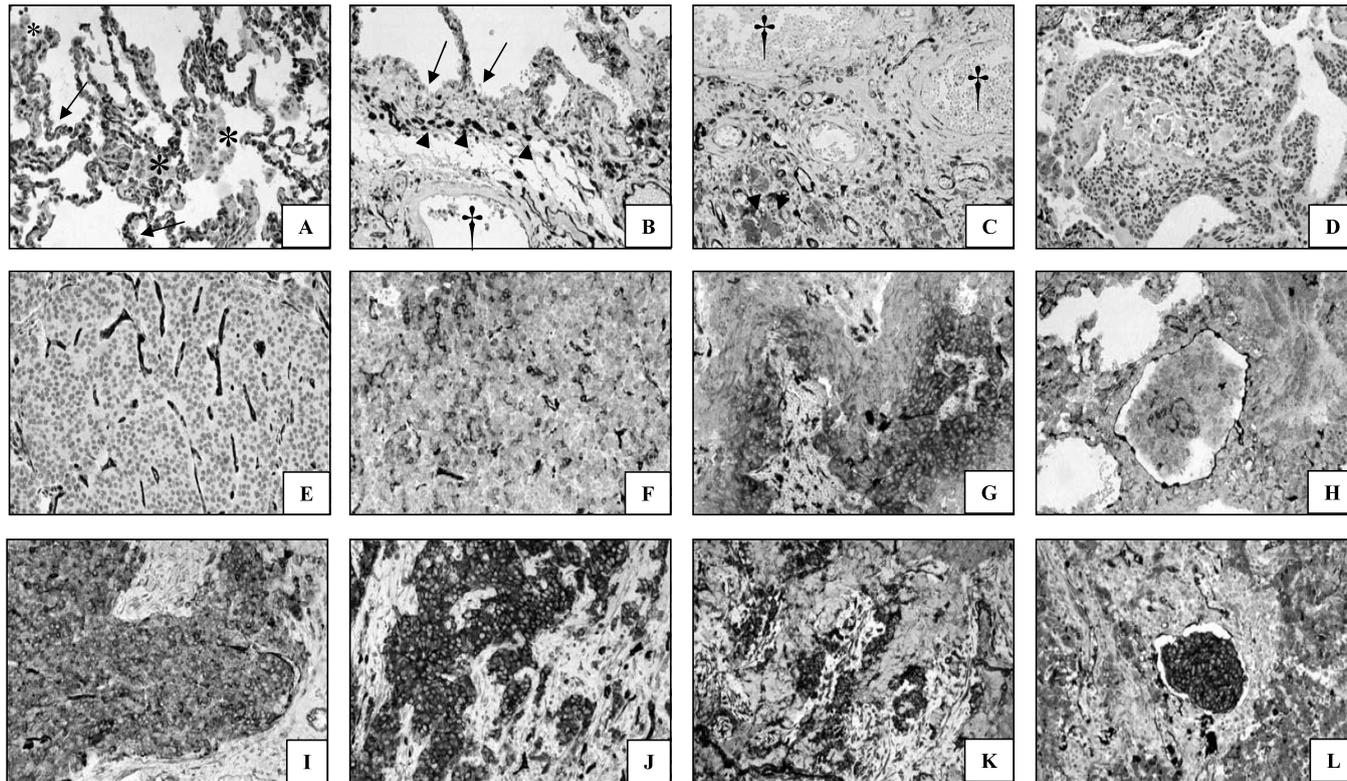


Fig. 2 Distribution of fascin immunoreactivity in non-neoplastic tissue (A–D) and NE tumors of the lung (E–L). In the normal lung, fascin immunoreactivity invariably decorated microvessel endothelial cells of the interstitium (A–C) and the bronchial wall (B), as well as several types of stromal cells (arrowheads) (B, C). Intra-alveolar macrophages (asterisks) (A), endothelial cells of major pulmonary vessels (crosses) (B, C) and broncho-alveolar epithelia (arrows) (A, B) were consistently unreactive for fascin, as well as hyperplastic cells of the NE tumorlets (D). In pulmonary NE tumors most TC exhibited negative tumor cells (microvessels were helpful internal controls) (E), but fascin could decorate tumor cells when lymph node metastases occurred (F). AC were more consistently immunoreactive for fascin than TC, and could show either staining enhancement at the tumor–host interface (G) or vascular invasion, with neoplastic emboli being immunoreactive for fascin (H). Poorly differentiated tumors, either LCNEC (I) or SCLC (J), usually showed strong and diffuse labeling for fascin and often enhancement at the periphery of invasive tumor nests (K). In these poorly differentiated tumors most neoplastic emboli appeared intensely labeled by the anti-fascin antibodies (L). (A–L, $\times 250$; all immunoperoxidase staining was performed with diaminobenzidine, and counterstained with hematoxylin).

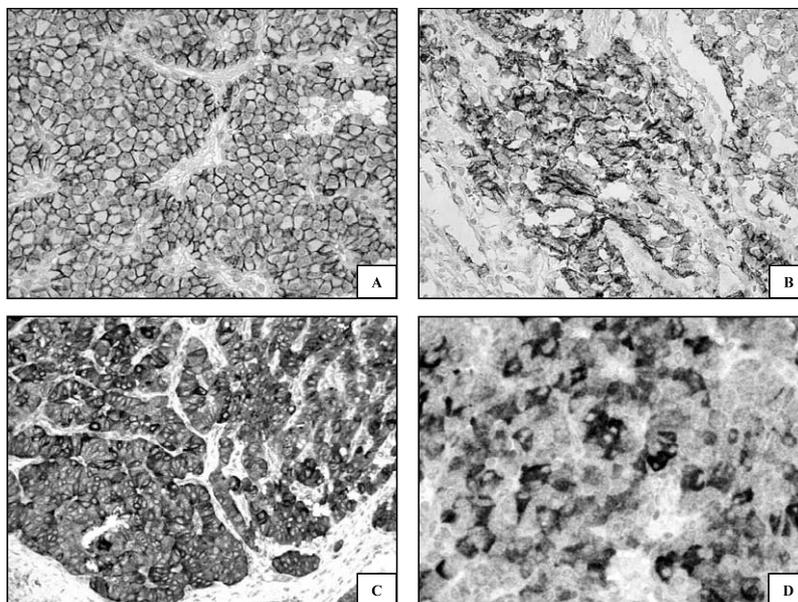


Fig. 3 E-cadherin and CD99 immunoreactivity patterns in TC and AC of the lung. For E-cadherin immunoreactivity, the linear pattern was characterized by moderate to strong labeling of the entire cell membrane (A), whereas the membrane-disrupted pattern evidenced a reduced and irregular decoration of cell membrane along with variable cytoplasmic decoration (B). For CD99 immunostaining, the linear pattern showed labeling of cell membrane along with cytoplasmic reactivity, whereas in the cytoplasmic pattern the immunostain product was predominantly confined to the cytoplasm with no appreciable membrane reinforcement (D). [All immunostains were developed with diaminobenzidine, and counterstained with hematoxylin].

(1/41 pN0 vs. 4/9 pN1 vs. 5/7 pN2) ($P < 0.001$) and the number of metastatic lymph nodes ($P < 0.001$). Other significant predictors of metastases in the same group of tumors were down-regulation of E-cadherin and CD99, membrane-disrupted or cytoplasmic E-cadherin immunostaining, and lack of staining or cytoplasmic confinement of CD99 immunostaining (Table 4). Proliferative activity predicted lymph node metastases only marginally ($P = 0.063$).

At multivariate analysis, only fascin immunoreactivity emerged as an independent predictor of lymph node metastases in CT and AC patients (hazard ratio: 30.28; 95% confidence intervals: 1.59–574.49; $P = 0.023$). No significant predictors of lymph node metastases were found in high-grade tumors.

4. Discussion

In the current investigation we first document, to our knowledge, that fascin is up-regulated in the majority (55%) of NE tumors of the lung, where correlates with increased tumor grade, more advanced disease stage, higher proliferative activity and de-regulated E-cadherin and CD99 expression. Moreover, in low- to intermediate-grade tumors fascin immunoreactivity is an independent predic-

tor of regional lymph node metastases, suggesting that it may serve as a prognostic factor for stratifying patients with different biological aggressiveness of disease. Fascin immunoreactivity, in both normal tissue and NE tumors of the lung, was substantially equivalent in terms of number of immunodetected cells and immunostaining intensity for both the two antibodies tested.

The high prevalence of fascin immunoreactivity in pulmonary NE tumors (as opposed to the lack of staining in normal and hyperplastic NE cell of the lower respiratory tract) and its significant correlations with higher tumor grade—also confirmed by the down-regulation of secretory granule-related NE differentiation markers, including chromogranin A in TC and AC and respiratory tract hormones in LCNEC and SCLC—, more advanced tumor stage and occurrence of regional lymph node metastases emphasize its possible involvement in the development and progression of these tumors, with particular reference to CT and AC. In these latter tumors, the fascin score correlated significantly not only with the occurrence of lymph node metastases, but also with the pN class and with the number of involved lymph nodes. Although one out of 41 carcinoid patients with no regional lymph node metastases showed fascin-immunoreactive primary tumor (Table 3), no systematical search for missed micrometastases was performed in this

Table 3 Immunostaining results of fascin score in 128 cases of NE tumors of the lung

Fascin score	TC/AC				LCNEC/SCLC			
	Category	Negative, n	1–4	P-value	Negative, n	1–4	≥ 6	P-value
Metastasis	Absent	40	1	< 0.001	4	11	16	NS
	Present	11	9		2	10	24	
Tumor stage	I	31	1	0.005	1	4	7	NS
	II–IV	20	9		5	17	33	
E-cadherin index ^a	≤ 90%	19	6	NS	4	6	27	0.012
	> 90%	32	4		2	15	13	
E-cadherin pattern	Absent	2	0	< 0.001	0	0	1	< 0.001
	Linear	40	0		2	1	0	
	Mem-disrup	9	9		3	18	10	
	Cytoplasmic	0	1		1	2	29	
CD99 index ^a	Negative	7	8	0.028	5	15	32	NS
	≤ 60%	20	11		1	5	7	
	> 60%	13	1		0	1	1	
CD99 pattern	Absent	7	8	0.022	5	15	32	0.042
	Cytoplasmic	11	8		1	5	7	
	Linear	23	4		0	1	1	
Ki-67 labeling index ^a	≤ 5%	28	1	0.024	5	12	14	NS
	> 5%	23	9					
	≤ 55%							
	> 55%							
Chromogranin A ^a	≤ 95%	17	7	0.040	2	5	9	NS
	> 95%	34	3					
	Absent							
	≤ 30%							
Calcitonin	Negative	31	8	NS	1	10	27	0.030
	Positive	20	2		5	11	13	
Serotonin	Negative	18	4	NS	6	17	40	0.010
	Positive	33	6		0	4	0	
Alpha-HCG	Negative	4	3	NS	1	4	26	< 0.001
	Positive	47	7		5	17	14	

Mem-disrup, membrane-disrupted immunostaining pattern.

^a Median of positive values.

case by re-cutting paraffin blocks at different levels. Whether fascin also favors distant metastases cannot be derived from the current data, for the lack of an adequate follow-up time. In stage I non-small cell lung cancer, however, we have recently found that fascin up-regulation is significantly associated with distant metastases, and is an independent predictor of both recurrent disease and shorter overall survival [34]. In the present investigation, we found that tumor emboli were often positive for fascin, independent of tumor type, suggesting a role of this molecule in the metastasis formation of pulmonary NE tumors.

To further investigate the mechanisms of invasion in NE pulmonary tumors, we investigated the relationships between fascin and two homophilic cell adhesion systems of pulmonary NE tumors, namely E-cadherin [43] and CD99 [52]. In transformed epithelial cell systems, Yamashiro et al.

have demonstrated that fascin gene transfection or protein microinjection induce the emission of microspikes on the apical surfaces and of extended lamellipodia at the basolateral surfaces, that lead to cell–cell contact disorganization and increased cell motility [26]. Some of these changes are due to down-regulation and altered cytoplasmic distribution of the E-cadherin-based cell adhesion complex, directly induced by fascin over-expression [26,60]. We found that the subcellular compartmentalization of E-cadherin changes dramatically from normal NE cells, where it is always membranous, to NE tumors, where it progressively accumulates in the cytoplasm paralleling an increased fascin expression. Although conflicting data exist regarding the prognostic implication of E-cadherin expression in NE tumors of the lung [43], we believe that the peculiar changes in the subcellular compartmentalization of E-cadherin may reflect an

Table 4 Other predictors of metastasis in 61 cases of TC and AC

Determinant	Category	Metastases		P-value
		Absent, n	Present, n	
Fascin score	Negative	40	11	< 0.001
	1–4	1	9	
E-cadherin index	Negative	0	3	0.013
	≤ 90% ^a	13	9	
	> 90% ^a	28	8	
E-cadherin pattern	Absent	0	2	0.003
	Linear	33	7	
	Linear/cytoplasmic	8	10	
	Cytoplasmic	0	1	
CD99 index	Negative	8	8	0.040
	≤ 60% ^a	20	11	
	> 60% ^a	13	1	
CD99 pattern	Absent	7	8	0.022
	Cytoplasmic	11	8	
	Linear	23	4	

^a Median of the positive values.

important role for this protein in the neoplastic transformation and tumor progression, in close association with fascin up-regulation.

Similar to E-cadherin, an abnormal immunoreactivity for CD99 was also correlated with fascin score, as well as with tumor grade and proliferative activity (data not shown), in the different types of NE tumors. Previous reports indicate that CD99 may interfere with the growth stimulatory effects of insulin growth factor-I (IGF-I) on different NE neoplasms [61,62], including gastroenteropancreatic carcinoids [63–65], neuroectodermal tumors [61], and pulmonary SCLC [66,67]. A functional relationship between fascin and CD99 has recently been found in breast cancer cell lines, where the activation of the autocrine loop of IGF-I and its cognate receptor induced fascin-rich membrane projections and promoted cell migration [68]. This might be consistent with a functional interaction between fascin, CD99 and the IGF autocrine loop in pulmonary NE tumors to regulate both their growth potential and metastatic phenotype.

Although the mechanism of this interaction between fascin and CD99 still is unknown, either protein down-regulation or abnormal subcellular localization or emission of membrane projections could hamper the homophilic interactions of CD99 molecules present on the surface of joined epithelial tumor cells. In our hand CD99 immunoreactivity was either reduced or accumulated in the cytoplasm of tumor cells in close association with fascin up-regulation. Therefore, an inverse functional relationship with opposite biological effects may

be suggested for fascin and CD99 in pulmonary NE tumors, with the former hampering and the latter promoting cell–cell adhesion and contact inhibition of tumor cell growth.

At variance with previous findings [43], either down-regulation or altered compartmentalization of E-cadherin and CD99 also significantly correlated with the occurrence of lymph node metastases in TC and AC, similarly to fascin over-expression. The latter, however, was the only independent predictor of lymph node metastases at multivariate analysis, raising the possibility that fascin may facilitate lymph node metastases in low to intermediate grade NE tumors via a mechanism which involves the disruption of CD99- and E-cadherin-mediated homophilic cell adhesion machinery.

In conclusion, the current study indicates that fascin immunoreactivity may identify subsets of pulmonary carcinoids with different metastatic potential to regional lymph nodes, and that this protein may affect cell adhesion via disruption of the E-cadherin- and CD99-mediated adhesion machinery.

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