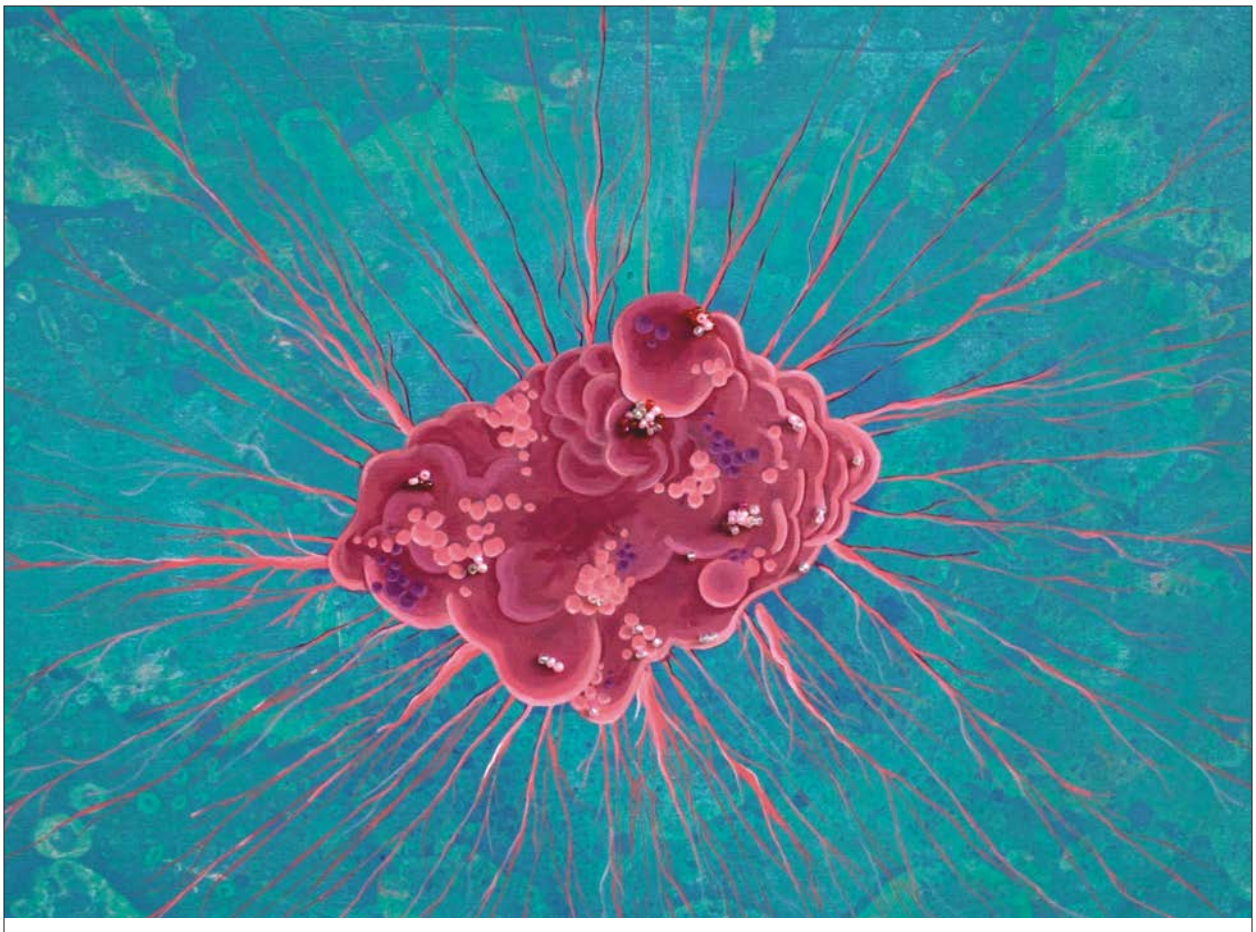

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Significance of Immunohistochemical Expression of Fascin and Caveolin-1 in Non Small Cell Lung Cancer

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ABSTRACT

Molecular mechanisms that regulate development of lung cancer are still unclear. Several risk factors may predispose to lung cancer. Fascin and caveolin-1 are important biomarkers involved in lung carcinogenesis. The aim of this study was to explore the expression pattern of Fascin and Caveolin-1 proteins as potential new diagnostic or predictive markers and its correlation with the clinical, pathological and radiological findings. Seventy eight cases of lung cancer were examined (49 males and 29 females). The patients' age ranged from 46 to 71 years. All of them were subjected to clinical examination, sputum cytology and imaging studies including plain chest×ray and multidetector chest CT scans. Biopsy was obtained from all patients via Bronchoscopy or CT guided. Histopathological examination was done to assess tumor grading. Immunohistochemical evaluation was done to evaluate expression of Fascin and caveolin-1. High expression rates of fascin and aveolin-1 in lung cancer were 67.9 and 53.8%, respectively, which were significantly lower in adenocarcinomas than in squamous cell carcinomas. Both high fascin and caveolin-1 expression was significantly correlated with tumor grade, stage and nodal metastasis. Both expression of fascin and caveolin-1 was significantly correlated ($p<0.001$). The present study suggested that fascin and caveolin-1 may play some role in the progression of non-small cell lung carcinoma.

Key words: Fascin, caveolin-1, non small cell, lung cancer, immunohistochemistry, diagnostic potential

INTRODUCTION

Lung cancer is one of the most common causes of cancer lethality worldwide. Despite recent progress, long-term survival remains poor (Fernandes *et al*., 2009). Human lung cancer is divided into two major histological types: Small Cell Lung Cancer (SCLC) and Non-small Cell Lung Cancer (NSCLC) (Minna *et al*., 2002).

The main causes of lung cancer include exposure to many carcinogens (such as those in tobacco smoke), ionizing radiation, asbestos and viral infection (Counts *et al*., 2005; Darby *et al*., 2005; Biggar *et al*., 2007). This exposure causes cumulative changes to the DNA in the bronchial epithelium leading to formation of DNA adducts, which are carcinogen metabolites bound covalently to DNA. DNA adducts result in permanent mutations with activation of oncogenes or deactivation of tumor suppressor genes. As more tissue becomes damaged, eventually a cancer

develops (Hecht, 2003). Passive smoking which is the inhalation of smoke from another's smoking causes lung cancer in nonsmokers as study of side stream smoke suggests that it is more dangerous than direct smoke inhalation (Hung *et al*., 2003).

Much attention has been directed towards the role of fascin and caveolin-1 in the progression of non small cell lung cancer. Fascin is an actin-bundling protein that induces cell membrane protrusions and increases the motility of normal and transformed epithelial cells (Choi *et al*., 2006). Fascin-1 is the most expressed form of fascin in vertebrate tissues. Very few data are available on the role of this protein in NSCLC (Pelosi *et al*., 2003). In normal esophagus, bronchus, larynx, uterine cervix and skin, fascin was mainly expressed in the basal cells or reserve cells, but variable fascin immunoreactivity was detected in lung squamous cell carcinoma, lung adenocarcinoma and large cell carcinomas (Xue *et al*., 2010). Fascin is considered as an independent prognostic predictor of unfavourable clinical course of the disease, so, targeting fascin pathway could be a novel therapeutic strategy of NSCLC (Pelosi *et al*., 2003). The evaluation of fascin immunoreactivity on the preoperative biopsy sample could be a novel therapeutic strategy for selecting the most appropriate therapy for small-size pulmonary adenocarcinomas (Choi *et al*., 2006).

In humans, caveolin-1 is a protein that is encoded by the CAV1 gene. The scaffolding protein encoded by this gene is the main component of the caveolae plasma membranes found in most cell types (Yoo *et al*., 2003). Caveolae are flask-shaped invaginations of the plasma membrane that play an important role in cellular processes, including molecule transport, cell adhesion and cell cycle signal transduction (Sunaga *et al*., 2004). Caveolin-1 (CAV1) functionally regulates the activity of many signaling molecules, such as G-proteins, Src family kinases, H-Ras, protein kinase C, epidermal growth factor receptor, endothelial nitric oxide synthase and integrins, which are potentially involved in the development of human cancer, by generating signaling complexes (Phillips and Birnby, 2004; Li *et al*., 2010). Thus, CAV1 could be a key molecule for growth-related signaling and cancer development. In human studies, CAV1 gene has been considered as both a tumor suppressor gene in most in vitro studies and an oncogene (Yoo *et al*., 2003). Immunohistochemical studies showed that non-neoplastic bronchial and alveolar epithelium, endothelial cells, fibroblasts and smooth muscle cells were positive for caveolin-1 (membranous and cytoplasmic) (Zhang *et al*., 2008). On the other hand, in NSCLC, high expression of Cav-1 protein is related to the aggressive clinical behavior and advanced tumor stage and indicates malignant progression and high invasion features (Zhang *et al*., 2008; Chen *et al*., 2011).

In order to explore the role of fascin and caveolin-1 in the development of NSCLC, both protein expressions in 78 samples of NSCLC were investigated by immunohistochemistry and the relationship of fascin and caveolin-1 expression with clinical, pathological and radiological characteristics was assessed.

MATERIALS AND METHODS

Seventy eight patients (49 males and 29 females) were examined at the Department of Internal Medicine, Thoracic Surgery and Radiology, El-Minia University Hospital between 2010 and 2012 (Table 1). The patients' age ranged from 46 to 71 years (Mean±SD: 59.92±7.3 years; median: 59 years). The Mean±SD of body metabolic index (kg m^{-2}) was 23±1.7, the Mean±SD of systolic blood pressure was 115±8 mm Hg^{-1} and the Mean±SD of diastoic blood pressure was 75±5 mm Hg^{-1}. Most of the patients were smokers (64.2%) and 23% cases were passive smokers (Table 1). This study was conducted in accordance with the 1975 Declaration of Helsinki. The study protocol was approved by the Ethics committee of Minia University, Egypt, before inclusion of patients in this study; written informed consent was obtained.

Table 1: Clinical and demographic data of the study group

Description		
Total No. 78 (100%)	No.	%
Sex		
Male	49	62.8
Female	29	37.2
Smoking status		
Smoker	50	64.2
Passive smoker	18	23.0
Non smoker	10	12.8
Tumor size		
<5 cm	54	69.2
≥5 cm	24	0.8
Tumor location		
Central	42	53.8
Peripheral	36	46.2
Tumor consistency		
Hard	50	64.2
Degenrated	28	35.8
Tumor margins		
Ill-defined	56	71.8
Well-defined	22	28.2
Pleura involvement		
Involved	22	28.2

All patients were subjected to the following: Full history taking and clinical examination with special emphasis to certain symptoms include: Dyspnea, hemoptysis, chronic coughing with change in coughing pattern, wheezing, cachexia, fatigue, loss of appetite, recurrent pneumonia, bone pain, fever and weight loss. Plain chest x-ray is the first step to patients. If there are no radiographic findings but the suspicion is high (such as a heavy smoker with blood-tinged sputum), sputum cytology was done to patients (done to 28 patients) by collecting the samples first thing in the morning, over a period of three days in receptacles containing a small amount of Saccomanno's fixative (50% ethyl alcohol and 2% carbowax) to preserve the samples then stained by Papanicolaou (PAP) stain. A sputum sample was considered representative if at least five alveolar macrophages or bronchial epithelial cells were present (Thunnissen, 2003). Then Multi-detector computed tomography guided (MDCT) biopsy was used to identify the tumor type.

MDCT protocol: All patients were examined in the radiology center of El-Minia University Hospital using a 16 detectors CT scanner GE bright speed with no contraindications was present to contrast media. The whole thorax was scanned form the lung apices down to the level of the supra-renal gland (Fig. 1 represents CT findings of a patient with solid mass at right lung apex).

Patient preparation: Explain the procedure, obtain consent, previous films and notes should be obtained. Premeditations like sedatives in children and uncooperative patients.

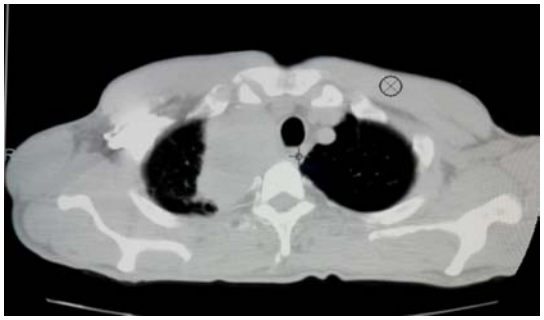

Fig. 1: Axial MDCT chest examination using IV contrast of one of cases studied revealed: Evidence of solid mass lesion involving the apico-anterior segment of the right lung of irregular margin, no calcification, no evidence of bone erosion and no hilar or mediastinal nodal enlargement

Patient position: The patient lies supine, the arm are raised and placed behind the patient's head, out of the scan plane. Head put firstly on the scanner table, with the median sagittal plane perpendicular to the table. Technique: The patient was scanned during inspiration, to avoid the motion artifact.

Multi-detector CT. technical parameters: Postro-anterior (PA) scan projection radiograph is obtained, starting just above the lung apices and ending just below the costophrenic angles. Slice thickness was10 mm contiguous sections, the field of view was 20 cm, matrix 256, radiation factor KV 120-140 and m Amp: 200, Lung window: 1200-600, Mediastinum window: 200-30, Bone window: 1500-250 (in suspected bone lesions). Scanning continues through the adrenal glands to check for metastasis.

After care: Examine the patient for any side effects of drugs used.

CT guided biopsy: The patient had prothrombin time and prothrombin concentration suitable for doing the invasive procedure; the lesions should be accessible by CT, no closely related to major vessels. The needle used was 18 g. Two intact cores of 1.5-2 cm length are enough. Follow up of the patient after the procedure to excluded complications, like pneumothorax.

Forty seven cases were CT guided biopsies, 6 cases were segmental resection of part of lung, 15 cases were lobectomy specimens and 10 cases were pneumenectomy specimens. Gross features of tumors were studied with special regard to tumor size (69.2% were less than 5 cm in diameter), tumor location (53.8% were peripheral), tumor consistency (64.2% were hard), tumor margin (71.8% were ill-defined) and 28.2% of cases had pleural involvement (Table 1).

Biopsies prepared and put into paraffin blocks, all paraffin blocks were retrieved and original haematoxylin and eosin-stained sections were reviewed. There were 42 (53.8%) squamous cell carcinomas, 30 (38.5%) adenocarcinomas, 6 (7.7%) large cell carcinomas. Ten (12.8%) carcinomas were well differentiated, grade I (6 adenocarcinomas and 4 squamous cell), 36 (46.2%) moderately differentiated, grade II (22 squamous cell, 14 adenocarcinomas) and 32 (41%) poorly differentiated, grade III (16 squamous cell, 10 adenocarcinomas and 6 large cell carcinomas) (Table 2). Tumor clinicopathologic stages were determined according to the Standard Tumor Node

Table 2: Relationship between fascin, caveolin-1 expression and clinico-pathological features

		Fascin				Caveolin-1			
	Total (%)	Negative (%)	Low (%)	High (%)		Negative (%)	Low (%)	High (%)	
Variables	78	18 (23.1)	7 (9)	53 (67.9)	p-value	16 (20.5)	20 (25.6)	42 (53.8)	p-value
Age									
<65	51 (65.4)	14 (27.5)	6 (11.8)	31 (60.8)	0.090	13 (25.5)	11 (21.6)	27 (52.9)	0.300
≥65	27 (34.6)	4 (14.8)	1 (3.7)	22 (81.5)		3 (11.1)	9 (33.3)	15 (55.6)	
Sex									
Male	49 (62.8)	14 (28.6)	3 (6.1)	32 (65.3)	0.200	13 (26.5)	15 (30.6)	21 (42.9)	0.030
Female	29 (37.2)	4 (13.8)	4 (13.8)	21 (72.4)		3 (10.3)	5 (17.2)	21 (72.4)	
Smoking									
Smoker	50 (64.2)	13 (26.0)	7 (14.0)	30 (60.0)	0.100	14 (28.0)	12 (24.0)	24 (48.0)	0.100
Passive	18 (23.0)	5 (27.7)	6 (33.3)	7 (39.0)		6 (33.3)	7 (39.0)	7 (39.0)	
Nonsmoker	10 (12.8)	6 (60.0)	3 (30.0)	1 (10.0)		5 (50.0)	3 (30.0)	2 (20.0)	
Histology									
SCC	42 (53.8)	8 (19.0)	2 (4.8)	32 (76.2)	0.200	7 (16.7)	10 (23.8)	25 (59.5)	0.600
AC	30 (38.5)	9 (30.0)	5 (16.7)	16 (53.3)		8 (26.7)	10 (33.3)	12 (40.0)	
LCC	6 (7.7)	1 (16.7)	0 (0.0)	5 (83.3)		1 (16.7)	0 (0.0)	5 (83.3)	
Grade									
1	10 (12.8)	7 (70.0)	1 (10.0)	2 (20.0)	0.001	6 (60.0)	3 (30.0)	1 (10.0)	0.005
2	36 (46.2)	7 (19.4)	5 (13.9)	24 (66.7)		6 (16.7)	11 (30.6)	19 (52.8)	
3	32 (41.0)	4 (12.5)	1 (3.1)	27 (84.4)		4 (12.5)	6 (18.8)	22 (68.8)	
Lymph node									
Negative	50 (64.1)	15 (30.0)	6 (12.0)	29 (58.0)	0.010	14 (28.0)	14 (28.0)	22 (44.0)	0.010
Positive	28 (35.9)	3 (10.7)	1 (3.6)	24 (85.7)		2 (7.1)	6 (21.4)	20 (71.4)	
Stage									
1	49 (62.8)	15 (30.6)	6 (12.2)	28 (57.1)	0.010	13 (26.5)	15 (30.6)	21 (42.9)	0.010
2	17 (21.8)	2 (11.8)	1 (5.9)	14 (82.4)		2 (11.8)	3 (17.6)	12 (70.6)	
3	12 (15.4)	1 (8.3)	0 (0.0)	11 (91.7)		1 (8.3)	2 (16.7)	9 (75.0)	

SCC: Squamous cell carcinoma, Ac: Adenocarcinoma, LCC: Large cell carcinoma, Grade 1: Well differentiated, Grade 2: Moderately differentiated, Grade 3: Poorly differentiated, Values are significant at $p<0.05$

and Metastases (TNM) classification system (Sobin and Wittekind, 1997). Most cases were in stage I (62.8%). Mediastinal lymph node metastasis was detected in 28 cases (Table 2). Whereas distant metastases were almost equally distributed between the two tumor types (13 out of 42 squamous cell carcinomas vs 7 out of 30 adenocarcinomas), respectively: $x^2=1.804$, $p = 0.406$).

Immunohistochemistry: Formalin-fixed and paraffin-embedded tissue samples were subjected for sectioning at 4 μm thickness. Sections were then deparaffinized in xylene and rehydrated through a graded series of ethanol concentrations. Endogenous peroxidase activity was blocked by 10 min incubation with 3% hydrogen peroxide and rinsed in water. Antigen retrieval was achieved with 1 mM EDTA pH 8 (Sigma Chemical Co., St Louis, MO, USA) in a microwave oven at 850 W for 8 min. Following two washes in Phosphate-buffered Saline (PBS), sections incubated in 10% normal goat serum for 15 min. Samples were then incubated overnight using two antibodies, fascin monoclonal antibody clone I M20 (1: 300 dilution, Novocastra Laboratories, Newcastle upon Tyne, UK) and anti caveolin-1 rabbit polyclonal antibody (1: 400 dilution, Santa Cruz Biotechnology, Santa Cruz, CA). After three additional washes, sections were incubated for 30 min with a polyvalent biotinylated goat anti-rabbit antibody at room temperature. Following three additional

washes in PBS, samples were incubated with streptavidin-conjugated peroxidase for 30 min. The reaction product was visualized by incubation for approximately 10 min with 3, 3-diaminobenzidine tetrahydrochloride, followed by washing in distilled water. Sections were counterstained in hematoxylin. Stained slides were dehydrated in ascending grades of alcohol, cleared in xylene and mounted. Normal endothelial cells of microvessels within the tumor section were used as positive internal controls for caveolin-1 and fascin while infiltrating lymphocytes, served as a negative internal control for caveolin-1 and tissue macrophages for fascin.

Fascin and caveolin-1 immunoreactivities of tumor cells were evaluated independently and blindly, without knowledge of the patients' identity or clinical data. Results were expressed semiquantitatively: as negative immunoreactivity, if staining was either completely absent or observed in less than 5% of cells; as low immunoreactivity if staining was observed in 5-25% of tumor cells; and as high immunoreactivity if staining was recognized in more than 25% of tumor cells. Also, the intensity of fascin immunostaining was simultaneously graded as low if a faint to distinct granular labelling was detectable throughout the cytoplasm, but less intense than seen in the normal internal controls, or strong if it was of the same or greater intensity than the latter (Pelosi *et al*., 2003; Kato *et al*., 2004). For each sample, the percentage of positively stained tumor cells and the intensity of staining were recorded. Immunohistochemical staining score was classified into three groups: Negative was represented when less than 5% of the cells were positive, low when there was weak homogeneous staining in 5-25% and high when high intense staining corresponding to the staining of mesenchymal and peripheral lung tissue was present in more than 25% of tumor cells (Wikman *et al*., 2004).

Statistical analysis: The Chi square test was used to analyze the correlations between fascin or caveolin-1 expression and patient parameters, including the histopathological findings. Values of P less than 0.05 were considered to be statistically significant. The spearman correlation coefficients were used to study the correlation between fascin and caveolin-1 expression. All analyses were performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL).

RESULTS

Malignant cells were present in the sputum of 7 of the 28 lung cancer cases detected (21.4%), all were squamous cell carcinomas (Fig. 2a-b). Fascin1 protein exhibited diffuse cytoplasmic cellular localization by immunostaining. The fascin immunoreactive tumors sometimes showed heterogeneity in the percentage of labeled cells and the intensity of immunostaining. Some tumors, especially for the mixed type, showed distinct fascin positivity in the invasive components and fascin negativity in the bronchioloalveolar components. Overall, fascin immunoreactivity was highly detected in 53 out of 78 (67.9%) NSCLC, including 32 out of 42 (76.2%) squamous cell carcinomas (SCC, Fig. 2c), 16 out of 30 (53.3%) adenocarcinomas (Fig. 2d) and 5 out of 6 (83.3%) large cell carcinomas investigated (p-value = 0.2, Fig. 2e). Poorly differentiated tumors (grade I) more often (27 out of 32 cases) exhibited high diffuse fascin immunoreactivity in more than 80% neoplastic cells whereas well differentiated NSCLC (grade I) considered as a whole were more commonly (7 out of 10 cases) fascin-negative, (p-value = 0.001).

The correlation between fascin expression and various clinicopathological characteristics was studied. Fascin expression was correlated with regional lymph node metastasis with high expression in 85.7% cases studied and TNM stage, whereas high expression predominated in 57.1% of stage I, 82.4% in stage II and 91.7% in stage III (p-value = 0.01 for both variables). There was no

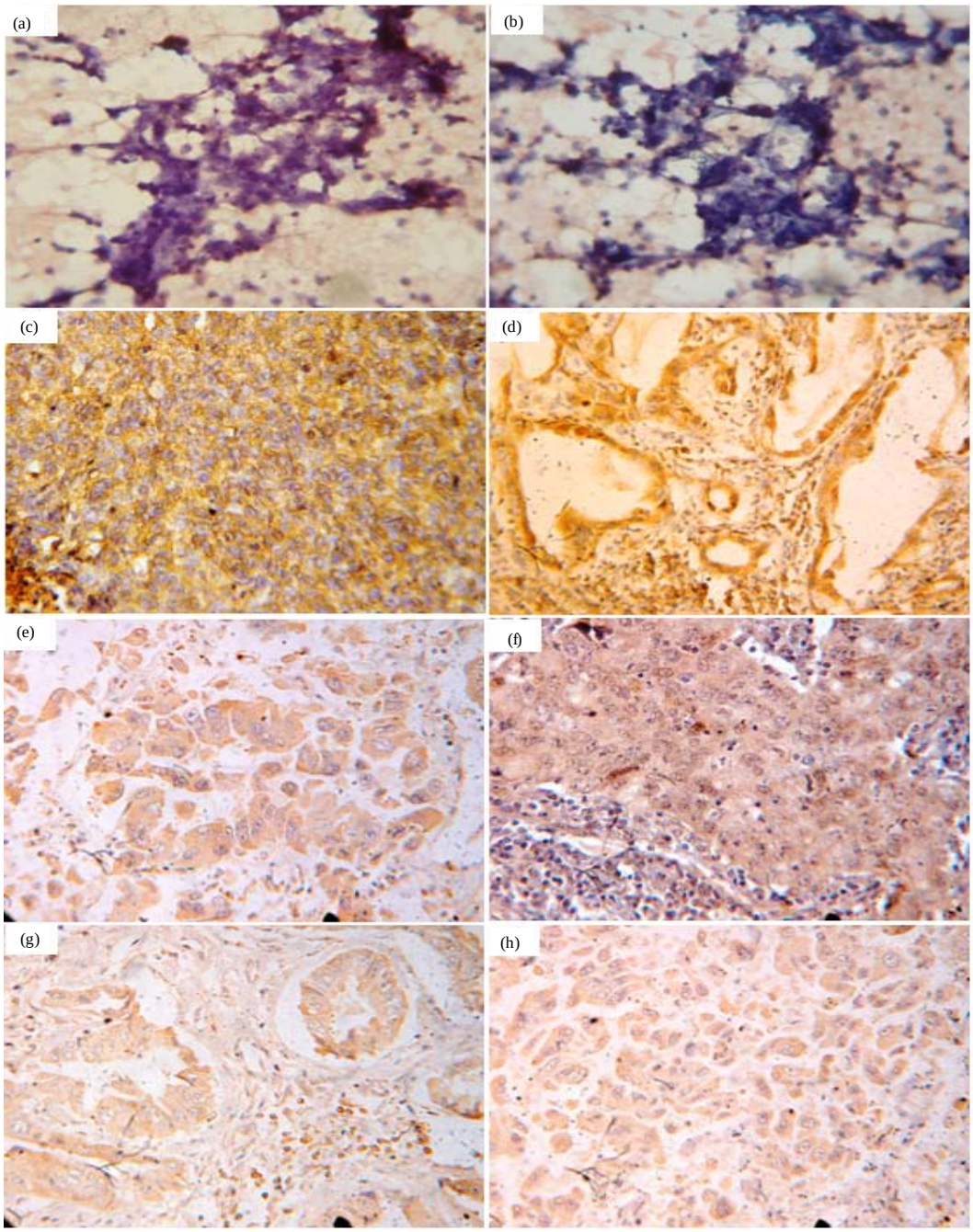

Fig. 2(a-h): (a-b) PAP stained smears of sputum cytology were positive for malignant squamous cells, (c) Fascin expression in squamous cell carcinoma, (d) Strong cytoplasmic expression of Fascin in adenocarcinoma, grade 2, (e) Fascin expression in large cell carcinoma, (f) Caveolin-1 expression in squamous cell carcinoma, (g) Diffuse cytoplasmic expression of caveolin-1 in adenocarcinoma and (h) Caveolin-1 expression in large cell carcinoma, (In immunostaining, DAB used as the chromogen and haematoxylin as counterstain, original magnification×200)

Table 3: Relationship between Fascin and Caveolin-1 in the study group

			Caveolin-1 (%)		
	Total (%)		Negative	Low	High
Fascin					
Negative	18.0	Count	8.0	6.0	4.0
		Within fascin (%)	44.4	33.3	22.2
Low	7.0	Count	4.0	0.0	3.0
		Within fascin (%)	57.1	0.0	42.9
High	53.0	Count	4.0	14.0	35.0
		Within fascin (%)	7.5	26.4	66.0
Total	78.0		16.0	20.0	42.0
	100.0		20.5	25.6	53.8

Spearman correlation was +0.439, p-value was <0.001 (values are significant at p<0.05)

difference between fascin expression and gender, age, smoking status or histological subtypes (p>0.05). However, there was a trend towards high fascin expression in smokers (30 cases) than passive or non smoker (Table 2).

Twenty five out of 42 (59.5%) of squamous cell carcinoma samples exhibited high expressing of caveolin-1 (Fig. 2f). In SCC cases expressing low levels of caveolin-1, immunohistochemical staining was observed mainly in the peripheral cancer nests. High caveolin-1 immunoreactivity was observed in 12 out of 30 (40%) cases of adenocarcinoma samples (Fig. 2g). In high grade large cell carcinoma caveolin-1 expression was detected in 5 out of 6 cases (Fig. 2h).

A gradual increase of caveolin-1 expression with decreasing differentiation was observed; the weak staining present in the well differentiated tumors (60%) was significantly higher than that seen in moderately (16.7%) to poorly differentiated (12.5%) tumors (p-value = 0.005). The expression of caveolin-1 was much higher in patients with lymph node metastasis (71.4%) compared with (44%) in lymph node-negative group (p-value = 0.01). Concerning tumor stage caveolin-1 expression was also higher in stage II to III (70.6 and 75%, respectively) compared with stage I tumors (42.9%) (p-value = 0.01). There was no significant association could be found for caveolin-1 expression in either histopathological subtypes or age. But a significant difference was detected with gender as low expression was detected more in male tissue specimens (30.6%) compared to female ones (17.2%, p-value = 0.03) (Table 2).

A significant positive strong correlation was noted between fascin and caveolin-1 immunostaining. High fascin expression was slightly at the same level (53 of 78 cases) as caveolin-1 expression (42/78; p-value<0.001) (Table 3).

DISCUSSION

Lung cancer is one of the most common causes of cancer deaths in the world (Parkin *et al*., 2005). Biomolecular marker screening techniques for the early detection of lung cancer are still under study. Biomarker screening limits patient exposure to potentially damaging constituents such as radiation (Bach *et al*., 2007). At present, histopathologic evaluation of a tumor and tumor staging are the mainstays for guiding therapeutic interventions and predicting outcomes. Despite this, tumors with identical histopathologies may progress differently, respond differently to therapy and may be associated with different clinical prognosis, suggesting that additional parameters should be identified to predict disease outcomes (Breuer *et al*., 2005). Gene

expression profiling of certain cancers may be able to serve as a complementary tool providing useful information (Amos *et al*., 2008). CT screening is capable of detecting very small, early stage cancers so that their shape and growth can be observed noninvasively. Previous research had demonstrated that LDCT detect very small lung nodules with higher accuracy approximately three times more than chest X-ray, the overwhelming majority are cancer stage I (Jett and Midthun, 2004).

The current study detected fascin expression in 67.9% of NSCLC cases and the expression was diffuse in SCC with a positive rate of 76.2%, 53.3% in adenocarcinomas and 83.3% in large cell carcinomas and the difference between fascin expression in SCC and in other histological types was not statistically significant. This result was lower than that found by Pelosi *et al*. (2003) who documented that fascin is upregulated in most (89%) NSCLC with variable fascin immunoreactivity was detected in 98% of squamous cell carcinomas, in 78% of adenocarcinomas, in 83% of large cell carcinomas. In another study the expression was diffuse in 90.0% of lung SCC and in 38.0% of lung adenocarcinoma (Xue *et al*., 2010). This suggests that different levels of the protein in the same histological subtypes may affect the biological behavior of tumors.

In the present study the fascin expression correlated with higher tumor grade, higher TNM stage and regional lymph node metastasis, similar to that found by other studies (Pelosi *et al*., 2003; Choi *et al*., 2006) which suggested that the increasing accumulation of fascin in squamous cell carcinomas of the lung may be implicated not only in the epithelial morphogenesis needed to tumor growth and stromal invasion, but also in the development of metastases. In another study, fascin immunoreactivity was detectable in 31% of 55 adenocarcinomas and 89% of 55 squamous cell carcinomas, correlating significantly with high tumor grade in the former and low tumor grade in the latter. No relation was noted, however, with tumor stage or patient survival (Del Rosario *et al*., 2001). The comparison of the data is hampered by the lack of details on the evaluation of immunostaining and on the stage distribution of the patients in the previous series. Noguchi *et al*. (1995) documented that fascin upregulation is a late event in the pathogenesis of NSCLC. However, the molecular mechanisms leading to fascin upregulation in these tumors are presently unknown. Some studies have suggested that Her-2/Neu overexpression may increase fascin mRNA and protein levels in human breast cancer cell lines (Grothey *et al*., 2000). Functional interactions with extracellular matrix molecules provide further mechanisms for the cellular regulation of fascin, these molecules alter fascin subcellular distribution, either inducing or stabilising bundling of actin by fascin, or not supporting fascin protrusions (Kureishy *et al*., 2002). On the contrary, some studies have reported that the down regulations of thrombospondin-1 (Yamaguchi *et al*., 2002) or tenascin-C (Cai *et al*., 2002), two molecules of extracellular matrix support the formation of fascin microspikes at cell margins (Adams, 1995; Fischer *et al*., 1997; Adams and Schwartz, 2000; Anilkumar *et al*., 2002).

Previous studies have reported that CAV1 is expressed in 21-36% of primary NSCLCs and that CAV1 expression is associated with poor prognosis of squamous cell lung cancer (Heighway *et al*., 2002; Yoo *et al*., 2003). In these studies, no reasons for expression or nonexpression of CAV1 were provided and no functional correlations were made. The present study therefore focused on the expression of CAV1 expression in NSCLC and its clinicopathologic correlations. In this study CAV1 expression was detected in 59.5% of squamous cell carcinomas, 40% of adenocarcinomas and 83.3% of large cell carcinomas and significantly correlated with higher tumor grade ($p<0.005$), higher TNM stage ($p<0.01$) and presence of nodal metastasis ($p<0.01$). These results were in agreement with CAV-1-positive reported rates in well to moderately differentiated tumors and poorly

differentiated tumors were 56.8 and 75.7%, respectively (Li *et al.*, 2010), higher CAV1 expression in patients with lymph node metastasis (Yu *et al.*, 2006; Liu *et al.*, 2008; Li *et al.*, 2010) and in stage III to IV than in stage I to II disease (75.4%, compared with 58.2%) (Yoo *et al.*, 2003; Zhang *et al.*, 2008; Li *et al.*, 2010; Chen *et al.*, 2011). Some authors suggest a paradoxical role of CAV1 in the development of cancer and regarded it as a conditional tumor suppressor protein (Quest *et al.*, 2008). In addition, they considered caveolin-1 together with different protein partners as an integrated functional unit, but not caveolin-1 alone (Burgermeister *et al.*, 2008). As a result, the identity of the interaction partner determines the physiological impact of caveolin-1 on the phenotype of a given cell and tissue in normal and pathophysiological conditions. In the present study, a statistical correlation was observed between the level of caveolin-1 and histopathological grade and lymph node metastasis in NSCLC, which was in agreement with these previous reports, suggesting that CAV1 expression may be required as a late event in the progression of NSCLC and may be involved in lymph node metastasis. At the same time, the expression level of caveolin1 was higher in squamous cell carcinoma than in adenocarcinoma, suggesting that caveolin-1 could promote lymphatic metastasis more easily in squamous cell carcinoma than in adenocarcinoma. Furthermore, the expression rate of caveolin-1 was higher in T3 than T1-2 tumors, indicating that caveolin-1 may promote tumor growth in NSCLC. In addition, strong caveolin-1 staining of peripheral cells in squamous cell carcinoma nests was detected in our study, which would suggest that caveolin-1 may facilitate tumor spread by the peripheral cells. Our results are consistent with the reported dual functions of caveolin-1both as a tumor suppressor gene and as a metastasis-promoting gene (Ho *et al.*, 2002). In the well differentiated and non metastasizing NSCLC, caveolin-1 expression is absent or low, which is consistent with the fact that downregulated caveolin-1 expression facilitates cell transformation. The upregulation of CAV-1 is also observed positively associated with the metastases and poor prognoses in breast cancer (Yang *et al.*, 1998), NSCLC lung cancer (Ho *et al.*, 2002), prostate cancer (Mouraviev *et al.*, 2002), esophageal carcinoma (Ando *et al.*, 2007), bladder cancer (Cho *et al.*, 2008) and pancreatic carcinoma (Witkiewicz *et al.*, 2008). Such a paradoxical functions in tumorigenesis were also observed for other molecules such as CD44 and granulocyte/macrophage-colony stimulation factor (GM-CSF) (Herrlich *et al.*, 2000; Mann *et al.*, 2001). However, no evidence for mutations, deletions, or epigenetic alterations caused by promoter methylation in the CAV1gene has been reported in lung cancer (Heighway *et al.*, 2002; Ho *et al.*, 2002). The only reported aberrant methylation of CpG islands in the CAV1 gene was associated with transcriptional inactivation in SCLC and involvement in FAK phosphorylation and RalA expression in NSCLC (Sunaga *et al.*, 2004).

CONCLUSION

In conclusion, the high prevalence of fascin and caveolin-1 immunoreactivity in NSCLC and their significant relation with tumor grade, stage and nodal metastasis emphasizes the possible involvement of this protein in the development and progression of NSCLC. Fascin immunoreactivity may thus be considered a more reliable tool for better assessing the biological aggressiveness and clinical course of NSCLC. This conclusion is supported by that documenmted by Choi *et al.* (2006) who suggested that the evaluation of fascin immunoreactivity on the preoperative biopsy sample could be a novel therapeutic strategy for selecting the most appropriate therapy for small-size pulmonary adenocarcinomas.

An interesting aspect of the study was the relation between fascin immunoreactivity and caveolin-1 immunoreactivity of tumor cells. Tumors with high (66%) fascin immunoreactivity showed significantly high caveolin-1 expression ($p<0.001$). This association may refer a closer positive relation was found between both proteins as components of cell membrane and their role in cell motility and combining both proteins could be a novel therapeutic strategy for selecting the most appropriate therapy for NSCLC cases.

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