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Comparison of the dysadherin and E-cadherin expression in primary lung cancer and metastatic sites

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Summary. Dysadherin, a cancer associated cell membrane glycoprotein, has been reported to downregulate E-cadherin. Aberrant expression of E-cadherin has been associated with the development of metastases in patients with cancer. Even though the expression of dysadherin and E-cadherin has been studied in primary non-small cell lung carcinoma, little is known about its expression at the distant metastases sites. We investigate by immunohistochemistry the relationship between E-cadherin and dysadherin in 111 cases of primary lung carcinomas (53 squamous cell carcinomas, 21 adenocarcinomas, 13 large cell carcinomas, and 24 small cell carcinomas), and their distant metastases. The intensity, the expression pattern and the percentage of neoplastic cell staining were recorded and the results were correlated with clinicopathological findings of the subjects. Dysadherin immunostain was expressed in 61 (54.95%) of the cases, and increased dysadherin expression was significantly correlated with tumour size (p=0.003), distant metastases (p=0.0034), and metastasis size (p=0.0008). Reduced E-cadherin expression was noted in 46 (41.45%) of the cases, and was correlated with high-grade tumour (p=0.02), infiltrative growth pattern (p=0.042), and advanced stage (p=0.032). Although the correlation between the expression of dysadherin and E-cadherin was not significant, a group of patients showed reduced E-cadherin expression with dysadherin overexpression. In lung carcinomas dysadherin expression seems to reflect tumour aggressiveness and may be considered a positive marker of poor prognosis when considered alone or/and in combination with down-regulation of E-cadherin.

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Introduction

Lung cancer is one of the most important avoidable causes of death around the world; it is the most widespread carcinoma with a very poor prognosis, and it is leading cause of cancer death in both developed and developing countries. Lung cancer is estimated to have accounted for 1.35 million new cases, representing 12.4% of all new cancers, and 1.18 million deaths (17.6%) in 2002 (Parkin et al., 2005). The majority of patients with lung cancer present with advanced disease at the time of diagnosis, and death can be attributed to distant multiorgan metastases. Metastasis is a multistep process involving disruption of the cell-matrix adhesion, dissolution of the extracellular matrix, angiogenesis, and invasion in the blood vessel wall, extravasation and establishment of a secondary growth (Wittekind and Neid, 2005). Nowadays, a large number of biochemical and cell biological studies have indicated the important role of extracellular matrix adhesion molecules, proteinases and angiogenic factors in the dissemination of cancer (Huang et al., 2005). Adhesion molecules belong to five known families: integrins, cadherins, immunoglobulin supergene family (IgSF), selectins and CD44 (Takeichi et al., 1991; Charalabopoulos et al., 2002). Cadherins represent the most important subclass of adhesion molecules (Gumbiner and Yamada, 1995).

Cadherins are members of a large family of transmembrane glycoproteins that mediate calcium-dependent, homophilic cell-cell adhesion and play an important role in the maintenance of normal tissue architecture (Shimada et al., 1990; Nagar et al., 1996;

Charalabopoulos et al., 2002). Cadherins are connected indirectly to the actin cytoskeleton by means of a group of proteins known as the catenins (Takeichi, 1991). E-cadherin is present in most epithelial cells, and numerous studies have demonstrated the importance of the E-cadherin/catenin complex and the initiation and progression of human tumours (Takeichi, 1991). Aberrant E-cadherin expression has been detected in a variety of well and poorly differentiated, invasive, and metastatic carcinomas, including lobular breast carcinoma (Parker et al., 2001), lung cancer (Charalabopoulos et al., 2004, 2006), colorectal carcinoma (Ghadmi et al., 1999), head and neck carcinoma (Schipper et al., 1991), prostate adenocarcinoma (Koksal et al., 2002), bladder (Lipponen and Eskelinen, 1995), renal cancer (Katarigi et al., 1995), and thyroid carcinomas (Wiseman et al., 2006; Mitselou et al., 2007). In lung cancer downregulation of E-cadherin has been associated with loss of differentiation, increased likelihood of metastases, and poor prognosis (Bohm et al., 1994, Charalabopoulos et al., 2006). Few studies have specifically looked at the expression pattern of E-cadherin in lung primaries and patient matched nodal metastasis and/or distant metastasis. Despite a large body of literature on the prognostic value of E-cadherin in lung cancer, in particular non-small cell lung cancer (NSCLC), the role of E-cadherin as a prognostic marker is at best ambiguous and needs clarification.

Dysadherin is a novel cancer-associated cell membrane glycoprotein (Ino et al., 2002). Its cDNA encodes 178 aminoacids, which include a putative signal sequence, a potential O-glycosylated extracellular domain, a single trans–membrane domain, and a short cytoplasmic tail. Dysadherin immunoreactivity to the monoclonal antibody NCC-3G10 has been detected in a wide variety of carcinoma cells but in only a limited number of normal cells, including lymphocytes, endothelial cells, and the basal cells of stratified squamous epithelium (Ino et al., 2002; Tsuiji et al., 2003). Transfection of dysadherin cDNA into the liver carcinoma cell lines PLC/PRF/5 reduces cell-cell adhesiveness, as assessed by morphology and by a Ca^{++}-dependent cell aggregation assay. In these transfected cells, levels of E-cadherin protein were markedly decreased in inverse proportion to the expression level of dysadherin, and the expression of E-cadherin mRNA remained unaffected. Moreover, when metastatic potential was examined by injecting dysadherin-transfected PLC/PRF/5 cells into the spleens of mice with severe combined immunodeficiency, the transfectants formed many more nodules than controls. Thus, dysadherin appears to be involved in the downregulation of E-cadherin by a post-trancriptional mechanism and play an important role in tumour development and metastasis (Ino et al., 2002). This novel cancer-associated protein has been detected in head and neck, gastric, testis, pancreatic, esophageal, thyroid, lung, and colorectal carcinomas (Haruhiro et al., 2003; Shimamura et al., 2003; Shimada et al., 2004; Taratani et al., 2004; Batistatou et al., 2005, 2006; Tamura et al., 2005; Kyzas et al., 2006).

From a clinical standpoint, lung carcinomas are broadly divided into non-small cell carcinomas (NSCLC) and small-cell carcinoma (SCLC) for treatment purposes and providing the basis of epidemiological studies, as well as the diagnosis and finally the sub-classification of lung cancer must be simple and practical to every surgical laboratory.

In the present study, we examined the dysadherin and E-cadherin expression patterns in primary lung carcinomas versus metastases in both lymph nodes and distant organs, and their relationship to various clinicopathological factors.

Materials and methods

Study population

Epirus is located in northwest Greece. It is an economically and technologically isolated region and is considered to be one of the least developed prefectures of the European Community. The urban and semi-urban population of the region represented 33% of the total, while the remaining 67% was rural. The Department of Forensic Medicine and Toxicology was established in March 1998 (Vougiouklakis et al., 2005). It is legally authorised to perform all medico-legal autopsies, in the four prefectures of the region, including: deaths that are unexpected; death unnatural or violent or resulting directly or indirectly from accident or injury; death in persons whose identity is unknown; those for which a death certificate has not been completed, and deaths of people held in care. This report focuses on individuals with lung cancer who died from natural causes and deaths from accidental injury, homicide, suicide, or unexpected sudden death. For the purposes of this report "incidental lung cancers" are defined as those that were not diagnosed or reported to be symptomatic prior to death and were found not to have contributed to death at autopsy.

Tissue specimens

Formalin-fixed and paraffin-embedded tissue specimens from 111 subjects diagnosed with lung cancer, from 1998 to 2008, were retrieved from the archival material of the Forensic Pathology Department of the University Hospital of Ioannina, Greece. The cause of death and percentages are shown in Table 1. At autopsy the lungs were first examined in situ, including pleural surfaces. The lungs and other major internal organs were then removed from the body and examined. The trachea, major bronchi and minor segmental branches were all opened and examined. The pulmonary arteries and branches were also examined. Each lung was then sectioned from the apex to the base in the coronal plane and examined visually and by palpation

for pathology. In the case of tumour, sections were routinely taken for histological examination. The organs with malignant involvement were noted. The number of metastases to the specific organs was not recorded, but the particular system involved, e.g. liver, was regarded as one site and measurement of the metastasis size (minimum and maximum diameter) was made. The frequency of intrathoracic and extrathoracic malignant involvement was also determined. All cases were re-evaluated and graded, as well as histologically subtyped according to the standard WHO criteria (Travis et al., 1999). Primary carcinomas and metastases were further categorized according to the tumour-node-metastasis (TNM) classification of malignant tumours of the International Union Against Cancer (IUAC) (Greene et al., 2002). Demographic, clinical, and histopathologic variables are shown in Table 2.

Immunohistochemistry

For each specimen, a 10% formalin-fixed, paraffin-embedded block containing both the carcinoma and its adjacent non-neoplastic lung tissue, involved lymph nodes, as well as tumour metastases from the involved organs were prepared. Consecutive sections of 4μm thick tissue from each block were mounted on adhesive coated slides, deparaffinized, and hydrated with xylen and ethanol. Then, they were heated in a microwave oven for two cycles of 15 min each at 300 W, in citrate buffer, for antigen retrieval. Endogenous peroxidase activity was blocked with H_2O_2 solution in methanol (0.01 M), for 30 min, the primary antibodies, NCC-M53 (monoclonal antibody against dysadherin, dilution 1:1,000, Biocare Medical) and CM170B (monoclonal antibody against E-cadherin, dilution 1:50, Biocare Medical), were applied for incubation, 30 min at room temperature and overnight at 40°C, respectively. Then, the slides were washed for 10 min with PBS and were visualized with the EnVision system (DAKO) using diaminobenzidine tetrahydrochloride as a chromogen.

Table 1. Autopsy findings in 111 cases with lung cancer.

Autopsy results	Number of cases (n)	Percentage (%)
Metastasis	**14**	**12.61%**
Cardiovascular	**36**	**32.43%**
Myocardium infarction	18	16.21%
Coronary disease	9	8.10%
Coronary thrombosis	4	3.60%
Cardiomyopathy	1	1.11%
Disecant aneurysm	4	3.60%
Pulmonary	**13**	**11.71%**
Pulmonary embolism	6	5.40%
Pneumonia	4	3.60%
Brochopneumonia	1	1.11%
Interstitial pneumonia	2	1.80%
Gastrointestinal		
Ischemic necrosis bowel	3	2.70%
Cerebral		
Stroke	2	1.80%
Kidney disease		
Glomerulus disease	1	1.11%
Liver		
Cirrhosis	2	1.80%
Violent death	**40**	**36.03%**
Car crash accident	23	20.72%
Suicide	6	5.40%
Others accidents	**11**	**9.90%**

Table 2. Patient characteristics.

	No patients	Percentage (%)
Total	**111**	
Sex		
Male	64	57.65
Female	47	42.35
Age (years)		
<49	12	10.81
50-59	17	15.31
60-69	21	18.91
>70	61	54.95
Pathologic tumour status		
pT1	19	17.12
pT2	23	20.72
pT3	34	30.63
pT4	35	31.53
Pathologic node status		
pN0	40	30.01
pN1	23	20.72
pN2	28	25.22
PN3	20	18.01
Pathologic metastasis status		
pM0	49	44.14
pM1	62	55.86
Tumour grade		
G I	56	51.35
G II	41	37.84
G III	14	10.81
Histological type		
Squamous cell Ca	53	47.75
Adenocarcinoma	21	18.92
Small cell cancer	24	21.62
Large cell cancer	13	11.71
Stage		
I	19	17.12
II	9	8.11
IIIa	10	9.90
IIIb	10	9.01
IV	62	58.85
Tumor size		
<2 cm	4	3.60
2-4 cm	51	45.95
>4cm	56	50.45
Metastasis size		
<2 cm	7	11.29
2-4 cm	27	43.55
>4 cm	28	45.16

Finally, all sections were counterstained with hematoxylin. Positive staining of endothelial cells and lymphocytes was used as an internal positive control for dysadherin. As an internal positive control for E-cadherin, positive staining of normal bronchia epithelial cells was used. As a negative control, the first antibody was substituted with normal mouse immunoglobulin of the same class.

Evaluation of the staining

Two pathologists (AM and AB) performed independently semiquantitative evaluation of the staining, without knowledge of the patient's information. When disagreement arose, slides were reviewed together and a consensus view was obtained. For each sample, at least 1000 neoplastic cells were counted, and the percentage of the cancer cells with positive membranous immunostaining and the staining intensity were recorded. Cytoplasmic staining without associated membrane staining was reported as negative. The possible association with dysadherin was examined by using a standardized scale of 0 to 4 (0, none; +, 1-25%; ++, 26-50%; +++, >51%). The percentage of tumour cells of primary lung cancer that stained positively for dysadherin was 47.2±16.7% (mean ± SD). Therefore, we set the cut-off value for dysadherin immunopositivity at 50%. For E-cadherin evaluation, the localization of the staining, and the percentage of tumour cells were semiquantitativelly estimated by using a standardized scale of 0 to 4 (0: no staining; +, 1-25%; ++, 26-50%; +++, >51%). The percentage of tumor cells that stained positively for E-cadherin was 52.4±15.1%. Therefore, we set the cut-off value for E-cadherin immunopositivity at less than 50%. Staining for E-cadherin was more heterogeneous, since in many cases only cytoplasmic staining was noted, while in others a weak incomplete membranous staining was observed. Such stains were considered aberrant.

Statistical analysis

All analyses were performed by the statistical package STATA (v.80). Two tails statistical significance was set at 5%. Data are presented as mean ± standard error of the mean (S.E.M.). The Shapiro-Wilk W test for normal data was used to detect the normality of distribution ($p<0.05$). All comparisons were based on t-test, χ^2 test and the ANOVA (analysis of variance and covariance). Each group showing dysadherin and E-cadherin expression was analyzed separately.

Results

Patients demographics

Of the 111 patients included in this study, 64 were men (57.65%), and 47 (42.35%) were women. Their ages range from 45 to 95 years, with a mean age of 68.83 (SD ± 12.73). All patients underwent complete autopsy with both primary tumour and metastases resection. The underlying cause of death in 12.61% of these 111 patients was directly related to malignancy. Only a few records were selected from patients who received chemotherapy and/or radiotherapy at the time of diagnosis. Thirty-one (27.92%) cases of primary lung cancer were classified as "incidental", and the majority was either stage I or II and did not contribute to death. In 36.03% of cases, the most common immediate cause of death was accident, injury, and suicide. Deaths attributed to cardiovascular disease (32.43%) included such diagnoses as myocardial infarction, myocardial ischemia, coronary occlusion, and disecant aneurysm. The other leading cause of death, other than malignancy, was pulmonary disease, including diagnoses of pulmonary embolism, pneumonia and broncho-pneumonia (Table 1). There were 53 squamous cell carcinomas, 21 adenocarcinomas, 13 large cell carcinomas, and 24 small cell carcinomas. The tumour diameter ranged from 1.2 cm to 18.5 cm (mean 6.9, SD ± 3.69). 19 had stage I disease, 9 had stage II disease, 10 had stage IIIa disease, 10 had stage IIIb disease, and 62 had stage IV disease. 19 had T1 disease, 23 had T2 disease, 34 had T3 disease, and 35 had T4 disease. 40 had N0 disease, 23 had N1 disease, 28 had N2 disease, and 20 had N3 disease. All the histopathologic variables are shown in Table 2. Frequencies of organs with malignant involvement at autopsy are presented in Table 3. The most commonly involved organs were: primary lung 100%, contra lateral lung 27.92%, lymph nodes 63.96%, pleura 23.42%, liver 27.92%, pericardium 9.90%, thyroid 10.81%, heart 7.20%, brain 8.10%, bones 5.40%, and spleen 3.60%. Direct invasion was observed in 21 (18.9%) of the cases, mostly pleura, organs of mediastinum, and pericardium. A significant statistical association between tumour size and metastasis ($p<0.0001$), metastasis size ($p<0.0001$), and tumour differentiation ($p<0.0001$) was found. There was no significant relationship between tumour size, sex, and

Table 3. Organs with more frequent involvement among 111 patients with lung cancer at autopsy.

Organs	n	Percentage (%)
Primary lung	111	100%
Lymph nodes	71	63.96%
Contralateral lung	31	27.92%
Bones	6	5.40%
Brain	9	8.10%
Pericardium	11	9.90%
Heart	8	7.20%
Thyroid	12	10.81%
Liver	31	27.92%
Pleura	26	23.42%
Spleen	4	3.60%

histological type (p>0.05).

Expression of dysadherin in primary and distant metastases

Expression of dysadherin was examined by immunohistochemistry in both tumorous and nontumorous lung tissues. In nontumorous tissues, no dysadherin expression was seen in normal bronchial mucosa and alveoli; dysadherin expression was present at the cell membrane of endothelial cells and lymphocytes. In tumours, dysadherin expression was present at the cell membrane (Fig. 1a-d). Dysadherin expression was detected in almost all cancer cells of infiltrative and poorly differentiated tumour. According to the proportion of dysadherin positive cancer cells, 12 (10.81%) of 111 primary tumours were classified as 0%, 11 (9.90%) into 1-20% expression group, 27 (24.32%) into the 21-50% expression group, and 61 (54.95%) into 51-100% expression group. Of the 111 tumours, 31 (58.5%) squamous cell carcinomas showed preserved dysadherin expression, 10 (47.6%) adenocarcinomas, 7 (53.8%) large cell carcinomas, and 13 (54.2%) small cell carcinomas (Table 4). Although patients with increased dysadherin expression had a tendency to show reduced E-cadherin expression, there was no significant relationship between dysadherin expression and E-cadherin expression. In metastases the staining was noted in fewer cases, 28 out of 62 (45.2%) metastatic tumours expressed dysadherin staining (Figs. 3a, 4a). In all the positive metastatic cases, primary tumours expressed dysadherin as well. Positive cancer cells of tumour emboli, within vessels, were observed in 27% of the cases (Fig. 5a). Increased dysadherin expression was significantly correlated with tumour size (p=0.003), distant metastasis (p=0.0034), and infiltrative type of growth pattern (p=0.01). An inverse correlation between dysadherin expression and histological type (p<0.001) was found. No significant statistical relationship between dysadherin expression and age, sex, and differentiation was found.

An interesting finding in this study was the detection

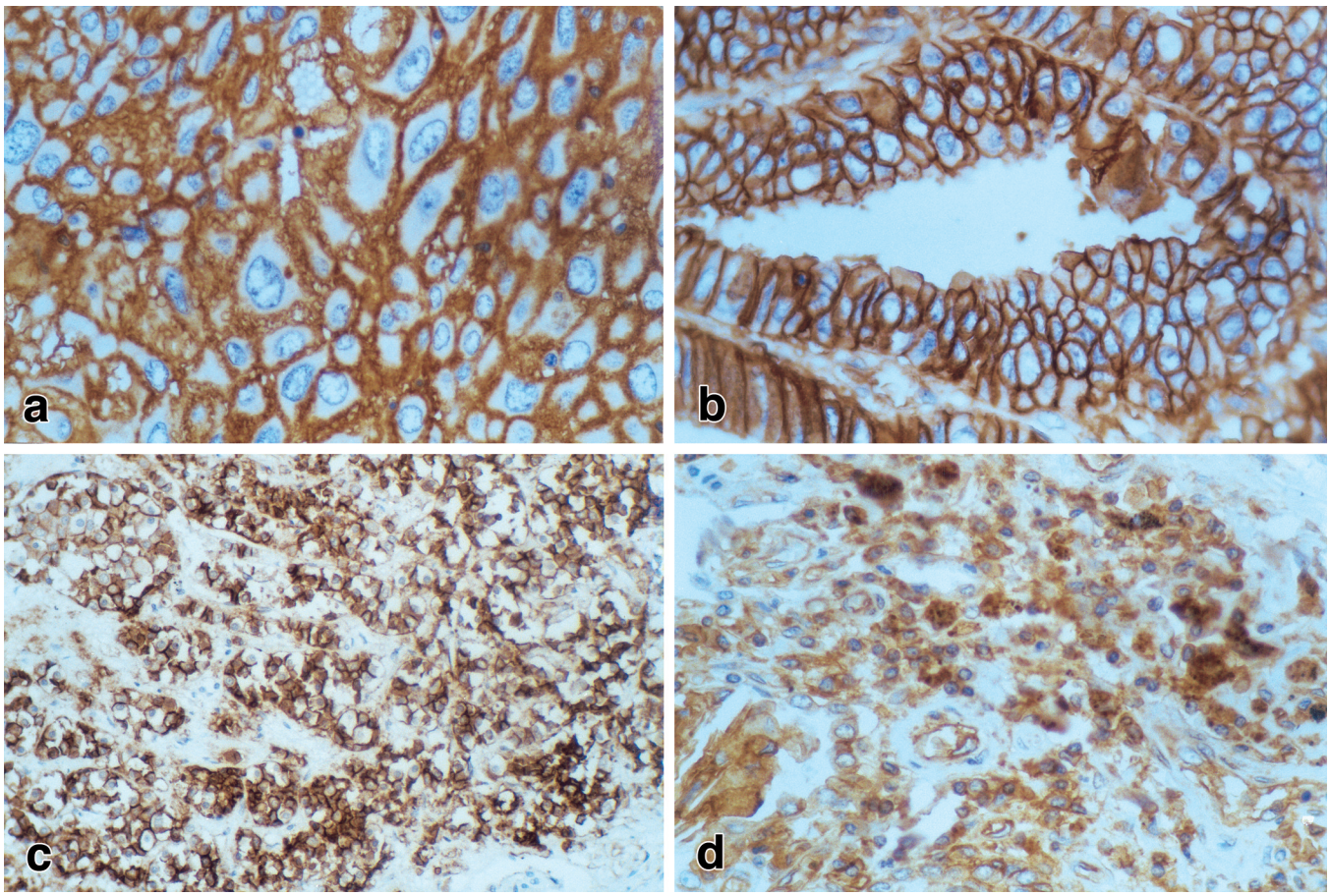

Fig. 1. Intense dysadherin immunostaining in the membranes of the cancer cells, in grade II squamous cell carcinoma of the lung **(a)**, in adenocarcinoma **(b)**, in small cell lung cancer **(c)**, and in undifferentiated cell carcinoma **(d)**. a, b, x 400; c, d, x 200

of dysadherin expression in the tumour cell cytoplasm of 21 (18.92%) cases, and most of them were adenocarcinomas (in the 21-50% expression group), as well as in 48 (23.2%) of metastatic tumours (in the 1-20% expression group).

E-cadherin expression in primary and distant metastases

Immunohistochemistry showed uniform E-cadherin expression along the cell membrane at the normal bronchial mucosa. The expression of E-cadherin was observed mainly on membranes of the tumour cells, and in some cases in the cytoplasm. According to the proportion of E-cadherin positive cancer cells, 3 (2.70%) of 111 primary tumours were classified as 0%, 17 (15.31%) into 1-20% expression group, 26 (23.42%) into the 21-50% expression group, and 65 (58.55%) into 51-100% expression group. Preserved membranous E-cadherin staining was present in 58.5% (65/111) of cases, and reduced/aberrant expression was found in 46 (41.5%) of the cases (Fig. 2a-d). In cancer cells, reduced E-cadherin expression was detected more frequently in poorly differentiated tumour nests than in well-differentiated tumour nests. In squamous cell carcinomas 39 (73.6%) of the cases preserved E-cadherin expression, in adenocarcinomas 13 (61.9%) cases, in large cell carcinomas 4 (30.7%) cases, and small cell carcinomas 9 (37.5%) cases (Table 5). A statistically significant difference between preserved E-cadherin expression and histological type ($p<0.05$), tumour size ($p<0.05$), and metastasis size ($p<0.05$) was observed. Reduced E-cadherin expression was significantly correlated with high grade tumour ($p=0.02$), infiltrative type of growth pattern ($p=0.042$), and advanced stage ($p=0.032$). There was no significant correlation between the reduced expression of E-cadherin and gender, age, histological type, grade, metastases and metastases size. In metastases the staining was less intense and heterogeneous; 27 (43.5%) of the cases were immunopositive in the membrane, 15 (24.2%) of the cases were positive in both membrane and cytoplasm of tumour cells (Figs. 3b, 4b). In tumour emboli immunoreactivity

Table 4. Clinicopathological characteristics of lung cancer patients, according to the expression of dysadherin.

	n	Preserved (%)	Reduced (%)	Significance
Total	**111**	**61 (54.95%)**	**50 (45.05%)**	
Histology				
Squamous Ca	53	31 (58.5%)	22 (41.5%)	P<0.001
Adenocarcinoma	21	10 (47.6%)	11 (52.4%)	
Large cell	13	7 (53.8%)	6 (46.2%)	
Small cell	24	13 (54.25%)	11 (45.8%)	
Tumour differentiation				
Well	56	27 (48.2%)	29 (51.8%)	
Moderate	41	23 (56.1%)	18 (43.9%)	
Poorly	14	11 (78.6%)	3 (21.4%)	P<0.001
T factor				
PT1	19	7 (36.8%)	11 (57.9%)	
PT2	23	11 (47.8%)	12 (52.2%)	
PT3	34	19 (55.9%)	16 (47.1%)	
PT4	35	24 (68.6%)	11 (31.4%)	P=0.01
N factor				
PN0	40	-	-	
PN1	23	9 (39.2%)	12 (52.2%)	
PN2	28	11 (39.3%)	16 (57.2%)	
PN3	20	9 (45.0%)	16 (80.0%)	P=0.003
M factor				
M0	49	-	-	
M1	62	28 (45.2%)	34 (58.2%)	P=0.0034
Tumour size				
<2 cm	4	3 (75.0%)	1 (25.0%)	
2-4 cm	51	29 (56.7%)	14 (27.5%)	
>4 cm	56	31 (55.4%)	24 (42.9%)	P=0.003
Metastasis size				
<2 cm	7	4 (57.2%)	3 (42.8%)	
2-4 cm	27	15 (55.6%)	12 (44.4%)	
>4 cm	28	19 (67.9%)	9 (32.1%)	P=0.0008

Table 5. Clinicopathological characteristics of lung cancer patients according to the expression of E-cadherin.

	n	Preserved (%)	Reduced (%)	Significance
Total	**111**	**65 (58.55%)**	**46 (41.45%)**	
Histology				
Squamous Ca	53	39 (73.6%)	14 (26.4%)	P<0.05
Adenocarcinoma	21	13 (61.9%)	8 (30.1%)	
Large cell	13	4 (30.7%)	9 (69.3%)	
Small cell	24	9 (37.5%)	15 (62.5%)	P=0.2
Tumour differentiation				
Well	56	36 (69.6%)	19 (33.9%)	
Moderate	41	15 (51.2%)	18 (43.9%)	
Poorly	14	2 (35.7%)	9 (64.3%)	P=0.02
T factor				
PT1	19	11 (56.5%)	7 (36.8%)	
PT2	23	13 (55.9%)	10 (47.5%)	
PT3	34	19 (55%)	14 (41.2%)	
PT4	35	22 (62.9%)	15 (42.9%)	P=0.042
N factor				
PN0	40	-		
PN1	23	11 (47.8%)	14 (60.1%)	
PN2	28	15 (53.6%)	16 (57.1%)	
PN3	20	13 (65.1%)	14 (70.1%)	P=0.01
M factor				
M0	49	-		
M1	62	25 (40.3%)	37 (59.7%)	P>0.05
Tumour size				
<2 cm	4	4 (100%)		
2-4 cm	51	27 (52.9%)	24 (47.1%)	
>4 cm	56	34 (60.7%)	22 (39.3%)	P<0.05
Metastasis size				
<2 cm	7	3 (42.8%)	3 (42.8%)	
2-4 cm	27	13 (48.2%)	14 (51.9%)	
>4 cm	28	11 (39.3%)	17 (60.7%)	P<0.05

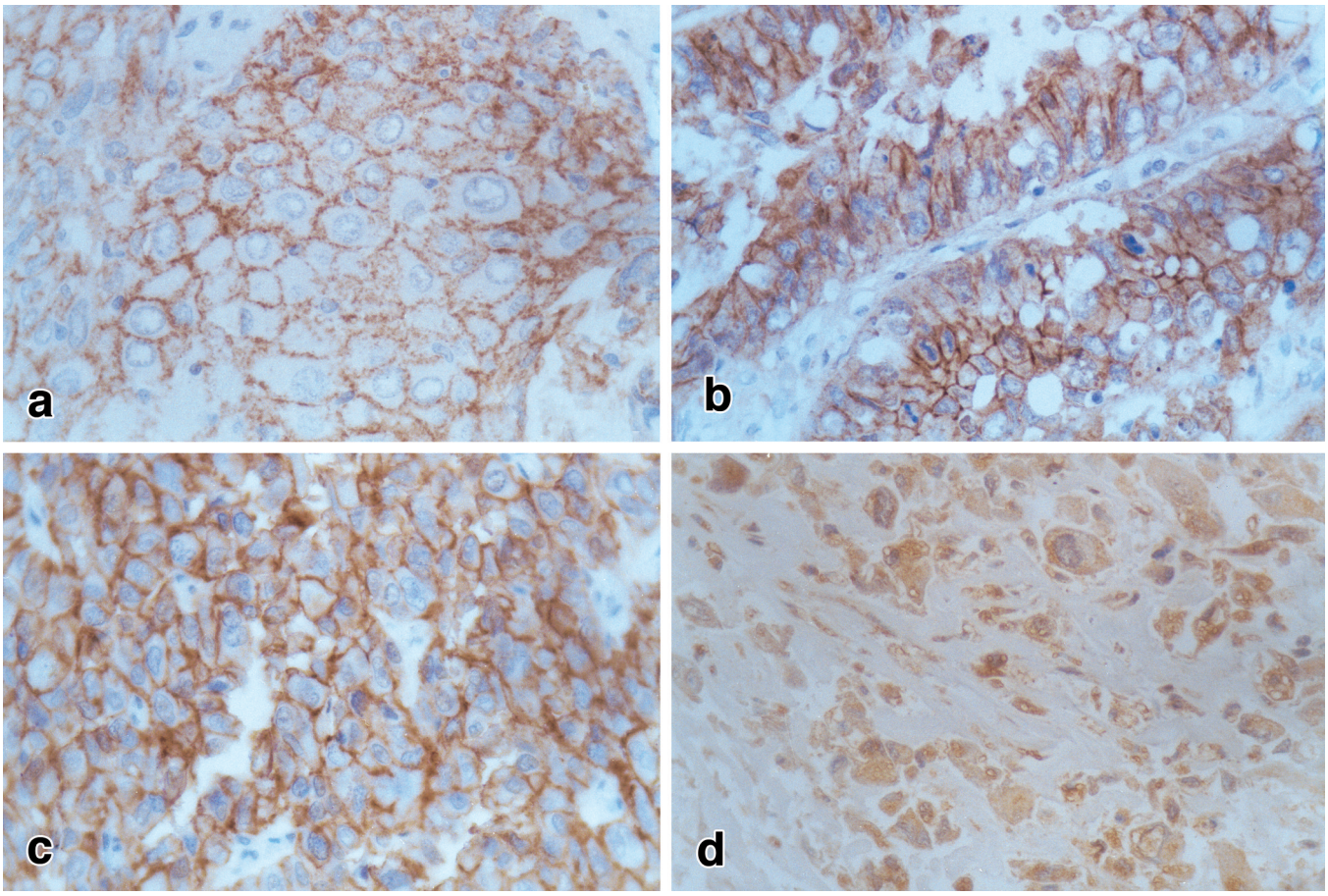

Fig. 2. E-cadherin immunostaining in the membranes of cancer cells, in grade II squamous cell carcinoma of the lung **(a),** in adenocarcinoma **(b)**, in small cell lung cancer **(c)**, and in undifferentiated cell carcinoma **(d)**. x 400

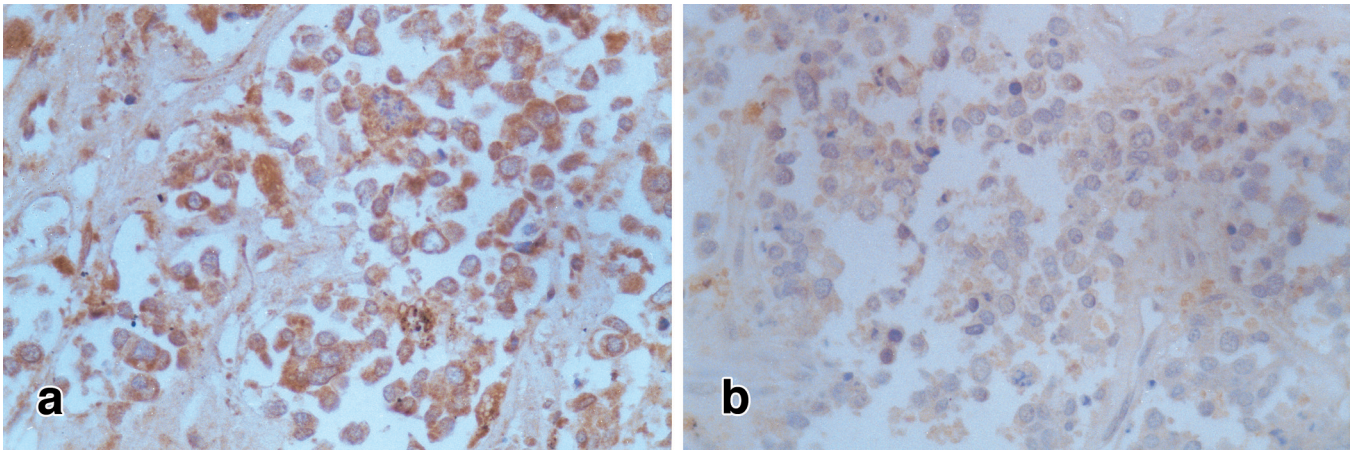

Fig. 3. Intense dysadherin expression in lymph node metastasis **(a)**, moderate E-cadherin expression in lymph node metastasis **(b)**. x 200

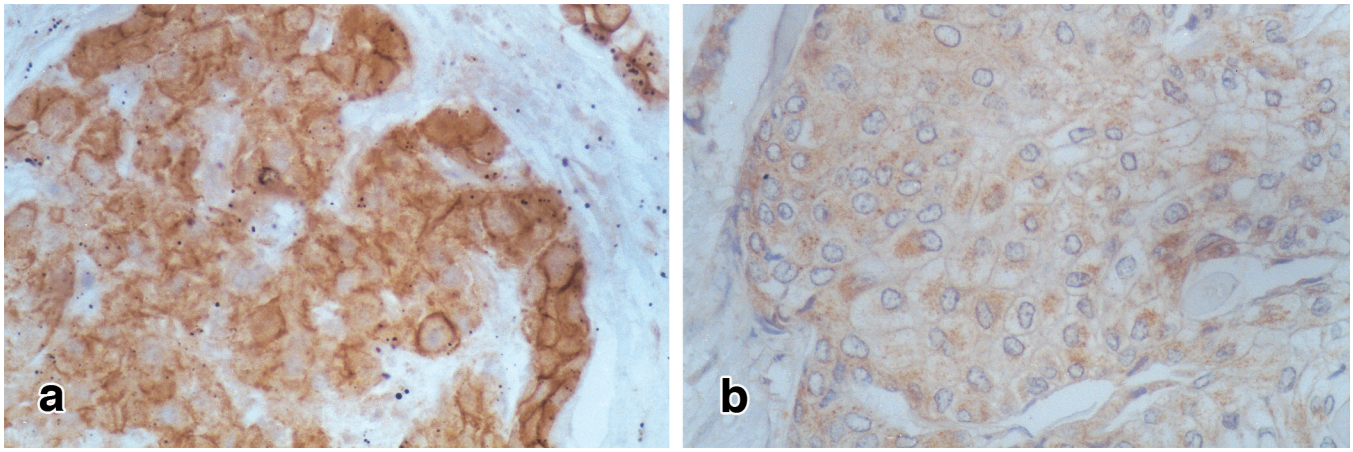

Fig. 4. Dysadherin expression in liver metastasis **(a)**, E-cadherin expression in liver metastasis **(b)**. x 400

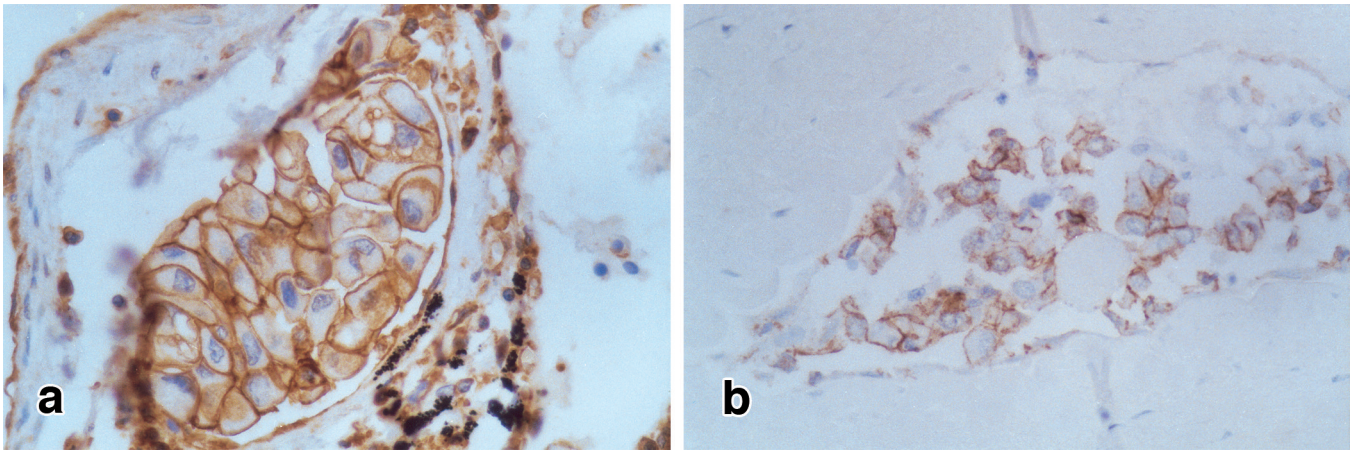

Fig. 5. Dysadherin positive cancer cells in tumour emboli within intratumoural vessel **(a)**, and E-cadherin positive cancer cells in tumour emboli within intratumoural vessel **(b)**. x 400

was observed in 9 (8.1%) cases (Fig. 5b).

Discussion

Lung cancer is one of the leading causes of death worldwide. Surgery is the treatment of choice, but almost 75% of the patients present at the time of diagnosis with non-resectable disease with lymph node metastases and distant organ involvement (Cortes-Funes, 2002). Although incidental lung cancer is uncommon, there are some lung cancers that remain undetected during life and do not contribute to death. In this retrospective review we found 27.92% of lung cancers detected at autopsy were incidental, and the majority of them were either stage I or II.

Lung cancer spreads by direct extension proximally and distally along the bronchus of origin and may reach the trachea at the level of the carina. It also grows into the lung parenchyma, from where it may reach the mediastinum or pleura. Invasion of blood vessels is also common. Lymph node metastases occur first in the hilar region, then in the mediastinal and lower cervical groups. Distant metastases are more common in other areas of lung, contralateral lung, liver, adrenal, bone, bone marrow, kidney, and central nervous system (Stenbygaard et al., 1999; Rosai, 2005).

Metastatic disease is responsible for the majority of cancer-related deaths, either directly due to tumour involvement of critical organs or indirectly due to complications of therapy to control tumour growth and spread. An understanding of the mechanisms of tumour cell invasion and metastasis may be important for devising therapies aimed at preventing tumour cell spread. Many studies have revealed that alterations in the adhesive proprieties of tumour cells correlates with progression to tumour malignancy (Wittekind and Neid,

2005). In particular, tumour invasion and metastasis, both hallmarks of tumour malignancy, frequently coincide with the loss of E-cadherin mediated cell-cell adhesion. Recently, clinical studies have suggested that reduced expression of E-cadherin is associated with dedifferentiation, local invasion, regional metastasis and reduced survival of patients with lung cancer (Shiozaki et al., 1991; Bhom et al., 1994; Charalabopoulos et al., 2006).

In this study, we evaluated immunohistochemically the expression of E-cadherin and dysadherin in formalin-fixed, paraffin-embedded tissue specimens of primary lung carcinoma and metastasis in both lymph nodes and distant organs, and we analysed clinicopathological parameters with the expression of the two molecules. One hundred and eleven cases of primary lung malignancies were studied. Forty-six (41.46%) of tumour cases showed reduced expression of E-cadherin, and 58.5% showed preserved E-cadherin expression. The preserved E-cadherin expression was inversely correlated with the histological type, tumor size, and metastasis size. Our results are in accordance with other studies if we take into account the functional membranous as well as the aberrant cytoplasmic E-cadherin immunostaining. Previous studies have reported that the cytoplasmic expression of E-cadherin reflects the functional disruption of the E-cadherin-catenin system. The complex of E-cadherin and the catenins is necessary for tight cell-cell adhesion in adherence junctions (Ilays and Tomlinson, 1997), thus the E-cadherin reduced tumour with positive cytoplasmic staining could be considered to have a loss of function of the E-cadherin-catenin unit.

Studies of the metastatic pattern in primary lung cancer reveal large variations in frequencies of involvement of various organ systems. The most frequently involved intrathoracic sites are: lungs (24-97%), mediastinal lymph nodes (46-85%), and pleura (15-45%). The most frequently extrathoracic sites are: liver (38-58%), brain (14-45%), bone (20-40%), and adrenals (36-64%). The data from our study are within the same range. In the present study we observed that 40.3% of metastases retaining E-cadherin expression, in contrast with the report of Kremer et al. (2003), who found that all metastases, including lung metastases, were completely negative for E-cadherin expression. The significance and pathogenic mechanisms of this phenomenon are issues under investigation. It has been shown that neoplastic cells with reduced/aberrant E-cadherin expression are far more likely to detach from the tumour mass in response to low shear forces, such as those found in the lymphatic vessels or venules. E-cadherin expression may also enable malignant cells to form a metastatic deposit by facilitating intercellular adhesion (Birchmeier and Weiland, 1994; Shibanuma et al., 1998). The normal expression or re-expression of E-cadherin in cancer metastases appears to be similar in breast (Kowalski et al., 2003), lung cancer (Bongiorno et al., 1995), and prostate cancer (Rubin et al., 2001).

Dysadherin is a recently characterized and denominated cancer-associated cell-membrane glycoprotein, identified by our research group (Ino et al, 2002; Batistatou et al., 2005). In vitro studies have shown that overexpression of dysadherin is reported to facilitate cell motility, and promotes metastatic potential of liver, pancreatic and colorectal metastasis in the animal model (Ino et al., 2002). In vivo, dysadherin expression has been studied in pancreatic (Shimamura et al., 2003), colorectal carcinoma (Aoki et al., 2003), thyroid (Sato et al., 2003), esophageal squamous cell carcinoma (Shimada et al., 2004), tongue cancer (Nakanishi et al., 2004), cervical squamous cell carcinoma (Wu et al., 2004), gastric carcinoma (Shimada et al., 2004), cutaneous malignant melanoma (Nishizawa et al., 2005), testicular tumors (Batistatou et al., 2005), non-small cell lung carcinoma (Tamura et al., 2005), head and neck squamous cell carcinoma (Kyzas et al., 2006), and lymph node metastases of colorectal carcinoma (Batistatou et al., 2006). In all these cancer types a general phenomenon is that dysadherin expression seems to reflect tumour aggressiveness, being furthermore a marker of poor prognosis when considered alone or/and in combination with down-regulation of E-cadherin, the same results included in the present study. To this end, we raise the question of the stability of dysadherin expression during the metastatic process in lung cancer.

The specificity of dysadherin expression to malignant cells also appeared in our primary lung cancer collection and detailed investigation by immunohistochemistry method revealed that dysadherin expression was more frequently found in infiltrative tumour nests than well-differentiated tumour nests. We found that 54.95% of primary lung carcinomas had immunostaining expression in the membrane of cancer cells, in adenocarcinomas 47.6% showed positive dysadherin expression ($p<0.001$), squamous cell carcinomas showed 58.5%, large cell carcinoma 53.8%, and small cell carcinomas 54.25%. Concerning tumour differentiation, well-differentiated cancer has a greater tendency towards low positive dysadherin expression than poorly differentiated cancer ($p=0.03$).

There is evidence that cancer cells may re-express not only E-cadherin protein once they reach distant sites but also dysadherin (Batistatou et al., 2006; Kyzas et al., 2006) and we found that 45.2% of metastatic foci had expression of dysadherin protein in the membrane, and in some cases at the cytoplasm of cancer cells, suggesting a novel mechanism of action. In contrast to the study of Tamura et al., we found statistically significant correlation between dysadherin expression and lymph node involvement ($p=0.0003$), and distant metastasis ($p=0.0034$). We must keep in mind that immunohistochemistry is not a strictly quantitative method, as there is no uniform scoring system and the interpretation of staining intensity is highly subjective. In addition, variations in protocols, such as in fixation procedures, antibodies and storage time of tissue

samples are likely to affect sensitivity of these assays, making comparison of results from different laboratories difficult. So, it is not clear whether the discrepancy between membranous versus cytoplasm expression of both proteins was or not significant.

Our study showed that the expression of dysadherin and E-cadherin in carcinomas was closely correlated to several malignant features, such as distant metastasis, high tumour grade, and infiltrative growth pattern. Tumour invasion and metastases are not a simple diffusion through the stroma towards blood or lymphatic vessels due to the loss of intercellular junctions, but a complex process that involves cell-cell and cell-matrix interactions, proliferation activity, and is closely associated with the mechanisms of angiogenesis. Therefore, the exact manner by which E-cadherin and dysadherin manipulate cancer cell metastasis must be examined throughout the spectra of the metastatic procedure.

In conclusion, the present study provides evidence that primary lung carcinomas that develop distant metastases have aberrant E-cadherin protein expression. E-cadherin is expressed or re-expressed at the distant metastases of lung cancer, supporting the hypothesis that re-expression of E-cadherin may play a role in the establishment of the metastatic cells at distant sites. Dysadherin expression is a good cancer-associated marker and is also a positive indicator for tumour aggressiveness, including invasiveness, lymph node metastases, and distant metastases in lung carcinomas. Additional studies, with more samples, are needed to evaluate metastasis E-cadherin and dysadherin levels in order to identify the aggressiveness of tumours, with the ultimate goal of reserving adjuvant systemic therapy for those with the poorest prognosis of lung carcinomas.

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