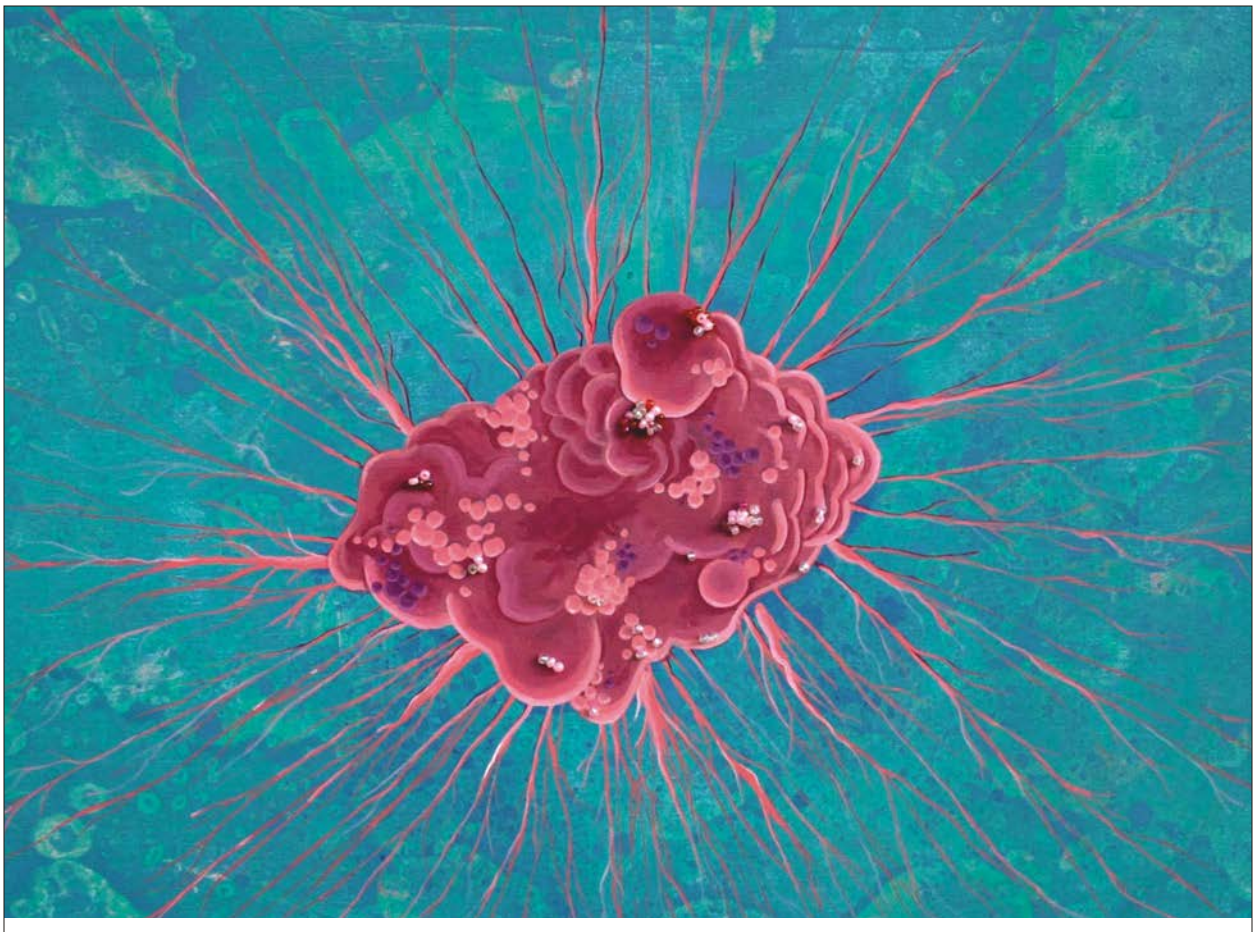

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Cortactin and EGFR Proteins Expression in Egyptian Patients with Non-small Cell Lung Carcinoma

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ABSTRACT

Cortactin is an important prognostic biomarker in several malignancies but its role in Non-small Cell Lung Carcinoma (NSCLC) is still poorly defined. Cortactin and Epidermal Growth Factor (EGFR) are involved in tumor cell motility, invasion and metastasis. The present study was designed to investigate the correlation between cortactin and EGFR expression and their relationship to clinicopathological parameters in surgically resected (stage I-III) NSCLC. Tumor tissue sections from 55 surgically resected NSCLC were evaluated for cortactin and EGFR proteins expression by immunohistochemistry. Cortactin protein expression was detected in 52.7% of NSCLC patients and was significantly associated with the degree of differentiation ($p = 0.023$), pathological lymph node metastasis ($p = 0.005$) and pathological tumor stage ($p = 0.016$). EGFR protein expression was observed in 58.2% of NSCLC, more frequently in Squamous Cell Carcinoma (SCC) than non-SCC (78.3% versus 43.7%, $p = 0.042$). A significantly positive relationship was detected between cortactin and EGFR proteins expression in NSCLC under investigation ($p = 0.004$). A statistically significant association was observed between cortactin and EGFR expression in NSCLC patients. Cortactin expression is significantly associated with clinicopathological parameters of more advanced disease and could serve as a prognostic biomarker in NSCLC.

Key words: Cortactin, epidermal growth factor receptor, non-small cell lung carcinoma, squamous cell carcinoma, non squamous cell lung carcinoma, immunohistochemistry, clinicopathological parameters

INTRODUCTION

Lung cancer is the most common cancer and the leading cause of cancer-related mortality in both sexes worldwide, representing 12.6% of newly diagnosed cancer cases and 17.8% of cancer deaths annually (Travis *et al*., 2004). Non-small cell lung cancer (NSCLC) accounts for 75-80% of newly diagnosed lung cancer worldwide (Hansen, 2002). Despite major advances in diagnostic tools, surgical techniques and cancer chemotherapy, the prognosis of NSCLC remains relatively poor with 5-15% overall 5-year survival rate after initial diagnosis (Dacic *et al*., 2006). This may be attributed to advanced loco-regional or metastatic disease in the majority of patients at the time of presentation (Hansen, 2002).

An important mechanism underlying tumor cell migration, invasion and metastasis is enhancement of tumor cell motility which has been proposed to be largely related to reorganization

of actin cytoskeleton (Yamaguchi and Condeelis, 2007). Cortactin is an actin-binding protein which is involved in the dynamic regulation of actin cytoskeleton through binding and activation of actin-related protein 2/3 (Arp2/3) complex as well as Neuronal Wiskott-Aldrich Syndrome Proteins (N-WASp), thereby promoting cell motility (Van Rossum *et al*., 2006). Cortactin is encoded by the *CTTN* gene (formerly designated *EMSI*) that maps to chromosome 11q13, a region that is frequently amplified in several human malignancies (Schuuring, 1995). Many reports demonstrated prognostic significance of *CTTN* amplification and/or cortactin protein overexpression in some carcinomas, such as head/neck and esophageal squamous cell carcinoma and breast carcinomas (Luo *et al*., 2006; Ormandy *et al*., 2003; Rodrigo *et al*., 2009). However, to our knowledge, cortactin protein expression and its relationship to clinicopathological variables are not yet well defined in NSCLC.

Epidermal growth factor receptor (EGFR, erbB-1) is another important factor that belongs to the erbB family of tyrosine kinase cell surface receptors. These receptors play an important role in tumor cell survival, proliferation and differentiation (Yarden and Sliwkowski, 2001). EGFR is overexpressed in a wide variety of solid human tumors, including NSCLC (Meert *et al*., 2002). However, the prognostic significance of EGFR overexpression in NSCLC remains a controversial issue (Dacic *et al*., 2006; Hirsch *et al*., 2003; Niemiec *et al*., 2005). A potential link has been proposed between cortactin and EGFR based on recent studies in cultured cells (Lynch *et al*., 2003; Timpson *et al*., 2005).

The present study was designed to investigate the immunohistochemical expression of cortactin and EGFR proteins in NSCLC, their association with clinicopathological parameters as well as their relationship to each other.

MATERIALS AND METHODS

Study design: The present study was carried out on paraffin blocks of formalin-fixed tissue sections of 55 primary NSCLC cases that were retrieved consecutively from the archives of Histopathology Lab of Department of Pathology, Minia Faculty of Medicine as well as that of Minia Oncology Centre during the period from 2004-2009. These cases represent surgical resection specimens of operable 55 primary NSCLC distributed from stage I up to stage IIIA. All cases did not receive preoperative chemotherapy or radiotherapy. The study cohort consisted of 44 males and 11 females with a male to female ratio 4:1. Patient's age ranged from 38-70 years and the mean age for males and females combined was 57.1±8.4 years. The histopathologic diagnosis and grade of differentiation were revised in hematoxylin and eosin-stained sections by M. F. Gayyed and A.I. Fawzy according to World Health Organization (WHO) criteria (Travis *et al*., 2004). The investigated NSCLC included 23 Squamous Cell Carcinomas (SCC), 28 adenocarcinomas (ADC) and 4 Large Cell Carcinomas (LCC). These tumors were classified into well (n = 10), moderately (n = 28) and poorly (n = 17) differentiated carcinomas. Staging of investigated tumors was done according to pathologic Tumor Node Metastasis (TNM) classification system considering American Joint Committee on Cancer (AJCC) (Greene *et al*., 2002). The patient and tumor characteristics of the analyzed cases are shown in Table 1.

Immunohistochemical staining: Immunohistochemical staining was performed on 4 μm thick sections. These sections were deparaffinized in xylene, rehydrated in descending grades of alcohol and washed in Phosphate Buffered Saline (PBS) (pH 7.2). Sections were incubated with 3% hydrogen peroxide in methanol for 15 min after heat-induced antigen retrieval (five 3-min

Table 1: Characteristics of tumorous patients (n = 55)

Characteristics	Frequency	
	No.	%
Age at diagnosis		
Range	38-70 years	
Mean	57.1±8.4 years	
Gender		
Male	44	80.0
Female	11	20.0
Histological type		
Squamous cell carcinoma	23	41.8
Adenocarcinoma	28	50.9
Large cell carcinoma	4	7.3
Tumor differentiation		
Well differentiated	10	18.2
Moderately differentiated	28	50.9
Poorly differentiated	17	30.9
pT status		
T1	19	34.5
T2	27	49.1
T3	9	16.4
pN status		
N0	13	23.6
N1	27	49.1
N2	15	27.3
pTNM stage		
I	12	21.8
II	25	45.5
III	18	32.7

pT: Tumor size, pN: Lymph node status, M: Distant metastasis, pTNM: Tumor node metastasis

microwave oven passages at 750 W in 0.01 mol L^{-1} citrate buffer, pH 6.0). Then, sections were incubated for 60 min at room temperature with primary antibodies including rabbit polyclonal antibody to human cortactin (dilution 1:100 in PBS; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and a mouse monoclonal anti-human EGFR antibody (clone E30, dilution 1:25 in PBS, DAKO, Carpentaria, CA, USA). After washing in PBS, sections were incubated with biotin-labeled secondary antibody and then with streptavidin-horseradish peroxidase using the DAKO universal LSAB2/HRP kit (DAKO, Glostrup, Denmark) at room temperature for 30 min for each step. DAB (3,3-diaminobenzidine) was used as chromogen and hematoxylin as the nuclear counterstain. Negative control was performed by omission of primary antibodies and replacement by PBS. Positive controls were lung carcinoma previously known positive for EGFR immunostaining and head and neck SCC previously known positive for cortactin.

Interpretation of immunohistochemistry (IHC) results: Sections were considered as positive when at least 10% of tumor cells were stained. Positive immunostaining was cytoplasmic±membranous for cortactin and membranous±cytoplasmic for EGFR. Immunostaining results were classified into high, moderate, or low expression scores depending upon the proportion of positive tumor cells on one hand and their dominant staining intensity on the other.

Immunostaining in 10-25% of tumor cells was categorized as 1, staining in 26-50% of tumor cells as 2 and staining in >50% as 3. The staining intensity in each specimen was categorized as 1, 2, or 3 (weak, moderate, or strong, respectively). The total immunostaining score consisted of the product of multiplication of the category for the proportion of positive tumor cells (1-3) and the category for staining intensity (1-3), resulting in a score of 1-9. This total score was divided into low expression scores (1-3), moderate scores (4 and 6) and high expression score (9).

Statistical analysis: The statistical analyses were performed using SPSS for windows, version 12.0 (SPSS Inc., Chicago, IL). Chi-square and Fisher's exact tests were used when appropriate to assess the relationships between biomarkers and clinicopathological variables of included tumors. Two-sided p-values of <0.05 were considered significant.

RESULTS

Cortactin protein expression in NSCLC: Cortactin positive immunostaining (>10% of tumor cells) was detected in 29 out of 55 NSCLC cases (52.7%). Our study demonstrated a significant positive association between cortactin immunostaining and higher histological grades ($p = 0.023$) since the rate of cortactin immunostaining as well as the rate of moderate and high expression scores was higher in moderate/poorly differentiated tumors when compared with well differentiated counterpart. Among 10 well-differentiated tumors, only 2 cases (20%) were positive for cortactin staining distributed as one case (10%) showing low expression and another (10%) showing moderate expression but none showed high expression. Meanwhile, moderately and poorly differentiated tumors were more frequently positive as 27 out of 45 tumors (60%) were positively immunostained for cortactin distributed as 8 cases (17.8%) showing low expression score, 10 cases (22.2%) showing moderate score and 9 cases (20%) showing high score of expression (Fig. 1a and c). Also, cortactin immunostaining was highly significantly correlated or associated with lymph node metastasis ($p = 0.005$). Among 13 tumors without nodal metastasis, only 4 (30.8%) were positive for cortactin expression distributed as 3 cases (23.1%) showing low score and one case (7.7%) showing moderate expression score but none showed high score. On the contrary, tumors with lymph node metastasis showed higher rate of cortactin positivity as well as higher expression scores. Thirteen out of 27 tumors with N1 nodal metastasis (48.1%) were positively immunostained for cortactin distributed as 5 cases (18.5%) showing low expression score, 6 cases (22.2%) showing moderate score and 2 cases (7.4%) showing high score of expression. In N2 tumors, 12 out of 15 cases (80%) showed cortactin immunostaining distributed as one case (6.7%) showing low expression score, 4 cases (26.7%) showing moderate score and 7 cases (46.7%) showing high score of expression. The present study also demonstrated positive association between increased cortactin expression and advanced pTNM staging system ($p = 0.016$). Out of 12 tumors classified as stage I, three (25%) were positive for cortactin expression distributed as 2 cases (16.7%) showing low score and one case (8.3%) showing moderate expression score but none showed high score. In stage II tumors, 13 out of 25 cases (52%) were positive for cortactin immunostaining distributed as 6 cases (24%), 5 cases (20%), 2 cases (8%) showing low, moderate and high expression scores, respectively. In stage III tumors, 13 out of 18 cases (72.2%) were positive for cortactin immunostaining distributed as one case (5.6%) exhibiting low expression score, 5 cases (27.8%) moderate score and 7 cases (38.9%) showing high expression score. No significant relationship was found between cortactin expression and sex ($p = 0.424$), age ($p = 0.397$), histological type ($p = 0.205$) and pT status ($p = 0.980$) (Table 2).

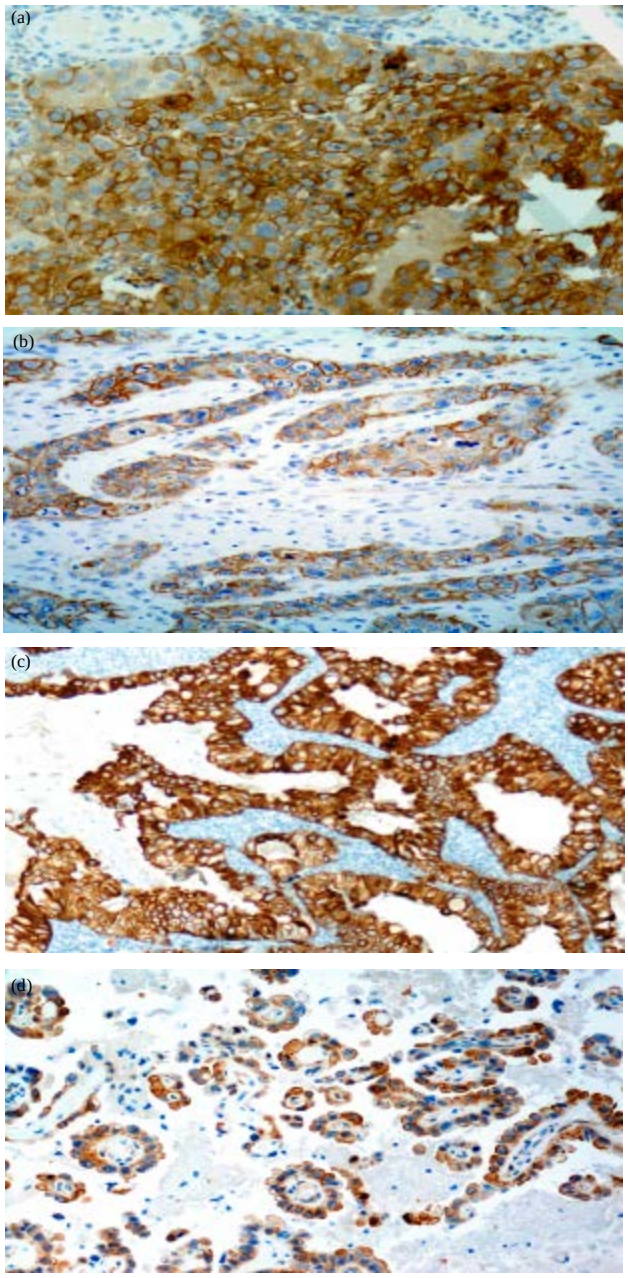

Fig. 1(a-d): Representative results of immunohistochemical staining of cortactin and EGFR protein expressions in NSCLC, (a) Squamous cell carcinoma demonstrating cytoplasmic±membranous staining for cortactin (high expression score), (b) Squamous cell carcinoma demonstrating membranous±cytoplasmic EGFR staining (moderate expression score), (c) Adenocarcinoma demonstrating cytoplasmic±membranous cortactin expression (high immunostaining score) and (d) Adenocarcinoma demonstrating membranous±cytoplasmic EGFR expression (moderate immunostaining score)

Table 2: Cortactin immunostaining and clinicopathological variables in NSCLC

			Positive staining cases expression score No. (%)			
	No.	Negative staining cases No. (%)	Low	Moderate	High	p-value
Sex						
Male	44	20 (45.5)	9 (20.4)	8 (18.2)	7 (15.9)	0.424
Female	11	6 (54.5)	0	3 (27.3)	2 (18.2)	
Age (years)						
Range		38-68	42-69	44 -70	43 -67	0.397
(Mean±SD)		(55.1±8.6)	(59.0±7.6)	(59.6±7.9)	(57.7±9.1)	
Histological types						
SCC	23	8 (34.8)	3 (13)	6 (26.1)	6 (26.1)	0.205
Non-SCC	32	18 (56.2)	6 (18.8)	5 (15.6)	3 (9.4)	
(ADC + LCC)						
Tumor differentiation						
Well	10	8 (80)	1 (10)	1 (10)	0	0.023
Moderate/ poor 45	18 (40)	8 (17.8)	10 (22.2)	9 (20)		
pT						
T-1	19	10 (52.6)	3 (15.8)	4 (21.1)	2 (10.5)	0.980
T-2	27	12 (44.4)	5 (18.5)	5 (18.5)	5 (18.5)	
T-3	9	4 (44.4)	1 (11.1)	2 (22.2)	2 (22.2)	
pN						
N 0	13	9 (69.2)	3 (23.1)	1 (7.7)	0	0.005
N 1	27	14 (51.9)	5 (18.5)	6 (22.2)	2 (7.4)	
N 2	15	3 (20)	1 (6.7)	4 (26.7)	7 (46.7)	
pTNM stage						
I	12	9 (75)	2 (16.7)	1 (8.3)	0	0.016
II	25	12 (48)	6 (24)	5 (20)	2 (8)	
III	18	5 (27.8)	1 (5.6)	5 (27.8)	7 (38.9)	

SD: Standard deviation, SCC: Squamous cell carcinoma, $p<0.05$ Non-SCC: Non squamous cell carcinoma, ADC: Adenocarcinoma; LCC: Large cell carcinoma, *Values are significant at $p<0.05$

EGFR protein expression in NSCLC: EGFR protein expression was detected in 32 out of 55 cases (58.2%). Higher rates of EGFR protein expression as well as higher expression scores were significantly correlated with SCC histology when compared with tumors of non-squamous histology ($p = 0.042$). EGFR positive immunostaining was detected in 18 out of 23 SCCs (78.3%) distributed as 4 cases (17.4%), 8 cases (34.8%) and 6 cases (26.1%) showing low, moderate and high expression scores, respectively. In tumors of non-squamous histology, EGFR protein expression was detected in 14 out of 32 (43.7%) distributed as 6 cases (18.8%) exhibiting low expression score, 5 cases (15.6%) showing moderate score and 3 cases (9.4%) showing high expression score (Fig. 1b and d). No correlation was found between EGFR expression and other clinicopathological parameters including sex ($p = 0.977$), age ($p = 0.992$), grade ($p = 0.734$), pT status ($p = 0.504$), pN status ($p = 0.415$) and pTNM stage ($p = 0.540$) (Table 3).

Correlation between cortactin and EGFR protein expression: A significantly positive relationship was detected between cortactin and EGFR proteins expression in NSCLC under investigation ($p= 0.004$) (Table 4).

Table 3: EGFR immunostaining and clinicopathological variables in NSCLC

			Positive staining cases expression score No. (%)			
		Negative staining cases				
	No.	No. (%)	Low	Moderate	High	p-value
Sex						
Male	44	19 (43.2)	8 (18.2)	10 (22.7)	7 (15.9)	0.977
Female	11	4 (36.4)	2 (18.2)	3 (27.3)	2 (18.2)	
Age (years)						
Range		38-69	43-70	43-67	42-66	0.992
(Mean±SD)		(56.7±9.4)	(57.5±9.1)	(57.4±7.8)	(57.1±6.8)	
Histological types						
SCC	23	5 (21.7)	4 (17.4)	8 (34.8)	6 (26.1)	0.042
Non-SCC	32	18 (56.3)	6 (18.8)	5 (15.6)	3 (9.4)	
(ADC+LCC)						
Tumor differentiation						
Well	10	5 (50)	2 (20)	1 (10)	2 (20)	0.816
Moderate	28	13 (46.4)	5 (17.9)	6 (21.4)	4 (14.3)	
Poor	17	5 (29.4)	3 (17.6)	6 (35.3)	3 (17.6)	
pT						
T-1	19	6 (31.6)	6 (31.6)	4 (21.1)	3 (15.8)	0.504
T-2	27	14 (51.9)	2 (7.4)	7 (25.9)	4 (14.8)	
T-3	9	3 (33.3)	2 (22.2)	2 (22.2)	2 (22.2)	
pN						
N 0	13	8 (61.5)	3 (23.1)	1 (7.7)	1 (7.7)	0.415
N 1	27	11 (40.7)	5 (18.5)	7 (25.9)	4 (14.8)	
N 2	15	4 (26.7)	2 (13.3)	5 (33.3)	4 (26.7)	
pTNM stage						
I	12	7 (58.3)	3 (25)	1 (8.3)	1 (8.3)	0.540
II	25	11 (44)	3 (12)	7 (28)	4 (16)	
III	18	5 (27.8)	4 (22.2)	5 (27.8)	4 (22.2)	

SD: Standard deviation, SCC: Squamous cell carcinoma, $p<0.05$ Non-SCC: Non squamous cell carcinoma, ADC: Adenocarcinoma; LCC: Large cell carcinoma. *Values are significant at $p<0.05$

Table 4: Correlation between cortactin and EGFR immunohistochemical expression

	Cortactin				
EGFR	0.00	1.00	2.00	3.00	p* value
0.00	17	3	3	0	0.004
1.00	6	2	1	1	
2.00	2	1	5	5	
3.00	1	3	2	3	

00: Negative immunostaining, 1.00: Low expression score, 2.00: Moderate expression score, 3.00: High expression score. *Values are significant at $p<0.05$

DISCUSSION

Regulation of actin cytoskeleton is an important prerequisite for tumor cell motility responsible for tumor cell migration, invasion and metastasis. Motility is initiated by the formation of polarized lamellipodia resulting from protrusion of the leading edge of the cell towards the direction of movement (Small *et al*., 2002; Yamaguchi and Condeelis, 2007). Cortactin is a Src kinase substrate

and filamentous (F)-actin- binding protein that is enriched in lamellipodia. It plays a fundamental role in remodeling of actin cytoskeleton through binding and activation of Arp2/3 complex and (N)-WASp, stabilization of Arp2/3-F-actin networks and promotion of actin polymerization within tumor cell lamellipodia resulting in their persistence (Rothschild *et al*., 2006; Van Rossum *et al*., 2006). Concurrent with its role in tumor cell motility, cortactin is required for invadopodia formation and is involved in the recruitment of membrane type 1 matrix metalloproteinase responsible for extracellular matrix degradation, thereby promoting tumor cell invasion and dissemination (Artym *et al*., 2006). Consistent with these functional properties, cortactin overexpression in cancer cell lines has been shown to increase the cellular motility and ability to migrate whereas its down-regulation by RNA interference leads to impaired tumor cell migration and dissemination (Chuma *et al*., 2004; Luo *et al*., 2006; Rothschild *et al*., 2006). Ever since, many investigators have reported cortactin protein expression with or without associated *CTTN* amplification to be associated with clinicopathological variables of advanced disease and hence, its prognostic significance in several human carcinomas including esophageal and head/neck squamous cell carcinomas (Hofman *et al*., 2008; Luo *et al*., 2006), breast carcinoma (Ormandy *et al*., 2003), ovary adenocarcinoma (Lin *et al*., 2009), gastric adenocarcinoma (Tsai *et al*., 2007), laryngeal carcinoma (Gibcus *et al*., 2008), renal cell carcinoma (Wang *et al*., 2009) and malignant melanoma (Xu *et al*., 2010). Nevertheless, limited data is available in the literature about cortactin expression in NSCLC. Therefore, the present study was designed to investigate immunohistochemical cortactin expression in NSCLC and surprisingly, more than half of included tumors showed expression of this biomarker. Moreover, in concordance with previous reports in other carcinomas just mentioned before, our findings indicate that cortactin could serve as a prognostic biomarker in NSCLC since higher expression scores of cortactin were significantly associated with clinicopathological parameters of aggressive disease including higher histological grades, nodal metastasis and higher pN status and with advanced pTNM stages.

Epidermal Growth Factor Receptor (EGFR) is a 170 kDa transmembrane glycoprotein with an intracellular domain that exhibits tyrosine kinase activity. Autophosphorylation of EGFR intracellular domain results in activation of several downstream cytoplasmic signaling pathways including the Ras/Raf/mitogen-activated protein kinase, JAK-STAT and (PI3K)/Akt pathways, thereby influencing several biologic processes as cell proliferation, invasion, metastasis and angiogenesis, while inhibiting apoptosis (Schlessinger, 2002; Yarden and Sliwkowski, 2001). EGFR expression has been observed in many types of human malignancies, including NSCLCs, breast, prostate, gastric, colorectal, ovarian, head and neck, bladder cancers and glioblastoma (Meert *et al*., 2002). In our study, EGFR expression rate was 58.2% and this rate lies within the reported range (27-83%) in NSCLC (Jeon *et al*., 2006). In agreement with previous reports, the rate of EGFR expression in the present work was significantly higher in NSCLC of squamous histology than those of non-squamous histology (Hirsch *et al*., 2003; Jeon *et al*., 2006; Ludovini *et al*., 2009). No significant association between EGFR immunohistochemical expression and clinicopathological variables of prognostic value in NSCLC included in the present study. Our results concur with the majority of previous reports in the literature since in two systemic reviews with meta-analysis conducted by Meert *et al*. (2002) and Nicholson *et al*. (2001), no correlation was found between EGFR expression and clinical or pathologic indicators of prognosis in NSCLC in 70% and 75% of reports included in these reviews, respectively. Also, several more recent studies have confirmed that EGFR IHC is not a prognostic marker for NSCLC (Dacic *et al*., 2006; Hirsch *et al*., 2003; Ludovini *et al*., 2009). Meanwhile, one report demonstrated a prognostic significance

(Niemiec *et al.*, 2005) and in another study by Cappuzzo *et al.* (2005), EGFR immunohistochemistry (IHC) has been shown to be beneficial as predictive marker for the therapeutic response as well as survival in patients with advanced NSCLC treated by EGFR inhibitors gefitinib. These conflicting data regarding the prognostic significance of EGFR expression may be attributed to differences in study design, patient selection criteria and immunostaining protocols in addition to variations in sensitivity and specificity of available antibodies. Another important factor is the lack of standardized interpretation criteria manifested by variations in preferential location of positive immunostaining, cut-off points and scoring methods of EGFR expression (Dacic *et al.*, 2006; Jeon *et al.*, 2006; Meert *et al.*, 2002).

Our results also demonstrated a highly significant positive association between cortactin and EGFR expression in NSCLC under investigation. This finding is concordant with a proposed cross talk between EGFR and Src/cortactin pathways in the literature where c-Src pathway was recognized as an upstream activator and downstream mediator or target of the epidermal growth factor (Timpson *et al.*, 2005; Yang *et al.*, 2004). Recent studies in Head and Neck Squamous Cell Carcinoma (HNSCC) cell lines demonstrated that cortactin could participate in receptor-mediated endocytosis of the EGFR so that cortactin overexpression inhibits ligand-induced down-regulation of EGFR and leads to sustained activation of EGFR signalling. Moreover, cortactin works as a key link between the EGFR mediated endocytic complex and F-actin cytoskeleton, through binding to CD2-Associated Protein (CD2AP) and actin-related protein 2/3 (Arp2/3) complex, respectively (Lynch *et al.*, 2003; Timpson *et al.*, 2005). Alternatively, EGFR signalling pathway is involved in Src activation, cortactin tyrosine phosphorylation and hence, cortactin overexpression (Rothschild *et al.*, 2006). Supporting this functional association between EGFR and cortactin, some investigators reported resistance to the EGFR kinase inhibitor gefitinib in response to cortactin overexpression (Timpson *et al.*, 2007). Meanwhile, others reported down-regulation of cortactin tyrosine phosphorylation in HNSCC cells treated with EGFR inhibitors (Rothschild *et al.*, 2006). Fortunately, one recent study by Hashimoto *et al.* (2006) highlighted the role of cortactin as a potential therapeutic target in mammary carcinoma cell line since a cell-permeable peptide as well as a small-molecule compound were found to block binding of cortactin SH3 domain with AMAP1 (a GTP-Arf6 effector overexpressed in invasive mammary tumors) and therefore, inhibit tumor cell invasion *in vitro* and lung metastasis *in vivo*. Therefore, therapeutic targeting Src/cortactin pathway could be promising in treatment of NSCLC. Actually, new drugs with anti-Src activity are under clinical trial investigation in NSCLC (Weiss and Kingsley, 2008).

CONCLUSION

In conclusion, the present work identifies cortactin as a potential prognostic biomarker in NSCLC since higher levels of cortactin protein expression were closely correlated with clinicopathological variables of aggressive disease. Also, the present study supports the proposed relationship between cortactin and EGFR expression and demonstrates it in NSCLC. Therapeutic targeting of Src/cortactin pathway could be promising in treatment of NSCLC and multiple hits of EGFR/Src/cortactin axis at different levels may improve therapeutic response and patient outcome.

REFERENCES

Artym, V.V., Y. Zhang, F. Seillier-Moiseiwitsch, K.M. Yamada and S.C. Mueller, 2006. Dynamic interactions of cortactin and membrane type 1 matrix metalloproteinase at invadopodia: Defining the stages of invadopodia formation and function. Cancer Res., 66: 3034-3043.

Cappuzzo, F., F.R. Hirsch, E. Rossi, S. Bartolini and G.L. Ceresoli *et al.*, 2005. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J. Natl. Cancer Inst., 97: 643-655.

Chuma, M., M. Sakamoto, J. Yasuda, G. Fujii and K. Nakanishi *et al.*, 2004. Overexpression of cortactin is involved in motility and metastasis of hepatocellular carcinoma. J. Hepatol., 41: 629-636.

Dacic, S., M. Flanagan, K. Cieply, S. Ramalingam, J. Luketich, C. Belan and S.A. Yousem, 2006. Significance of EGFR protein expression and gene amplification in NSCLC. Am. J. Clin. Pathol., 125: 860-865.

Gibcus, J.H., M.F. Mastik, L. Menkema, G.H. de Bock, P.M. Kluin, E. Schuuring and J.E. van der Wal, 2008. Cortactin expression predicts poor survival in laryngeal carcinoma. Br. J. Cancer, 98: 950-955.

Greene, F.L., D.L. Page, I.D. Fleming, A.G. Fritz, C.M. Balch, D.G. Haller and M. Morrow, 2002. AJCC Cancer Staging Manual. 6th Edn., Springer, New York, USA.

Hansen, H.H., 2002. Treatment of advanced non-small cell lung cancer. Br. Med. J., 325: 452-453.

Hashimoto, S., M. Hirose, A. Hashimoto, M. Morishige and A. Yamada *et al.*, 2006. Targeting AMAP1 and cortactin binding bearing an atypical Src homology 3/proline interface for prevention of breast cancer invasion and metastasis. Proc. Natl. Acad. Sci. USA., 103: 7036-7041.

Hirsch, F.R., M. Varella-Garcia, P.A. Bunn Jr., V. Di Maria and R. Veve *et al.*, 2003. Epidermal growth factor receptor in non-small-cell lung carcinomas: Correlation between gene copy number and protein expression and impact on prognosis. J. Clin. Oncol., 21: 3798-3807.

Hofman, P., C. Butori, K. Havet, V. Hofman and E. Selva *et al.*, 2008. Prognostic significance of cortactin levels in head and neck squamous cell carcinoma: Comparison with epidermal growth factor receptor status. Br. J. Cancer, 98: 956-964.

Jeon, Y.K., S.W. Sung, J.H. Chung, W.S. Park, J.W. Seo, C.W. Kim and D.H. Chung, 2006. Clinicopathologic features and prognostic implications of Epidermal Growth Factor Receptor (EGFR) gene copy number and protein expression in non-small cell lung cancer. Lung Cancer, 54: 387-398.

Lin, C.K., T.K. Chao, C.P. Yu, M.H. Yu and J.S. Jin, 2009. The expression of six biomarkers in the four most common ovarian cancers: Correlation with clinicopathological parameters. Acta Pathol. Microbiol. Immunol. Scand., 117: 162-175.

Ludovini, V., G. Bellezza, L. Pistola, F. Bianconi and L. Di Carlo *et al.*, 2009. High coexpression of both Insulin-like Growth Factor Receptor-1 (IGFR-1) and Epidermal Growth Factor Receptor (EGFR) is associated with shorter disease-free survival in resected non-small-cell lung cancer patients. Ann. Oncol., 20: 842-849.

Luo, M.L., X.M. Shen, Y. Zhang, F. Wei and X. Xu *et al.*, 2006. Amplification and overexpression of CTTN (EMS1) contribute to the metastasis of esophageal squamous cell carcinoma by promoting cell migration and anoikis resistance. Cancer Res., 66: 11690-11699.

Lynch, D.K., S.C. Winata, R.J. Lyons, W.E. Hughes and G.M. Lehrbach *et al.*, 2003. A Cortactin-CD2-Associated Protein (CD2AP) complex provides a novel link between epidermal growth factor receptor endocytosis and the actin cytoskeleton. J. Biol. Chem., 278: 21805-21813.

Meert, A.P., B. Martin, P. Delmotte, T. Berghmans and J.J. Lafitte *et al.*, 2002. The role of EGF-R expression on patient survival in lung cancer: A systematic review with meta-analysis. Eur. Respir. J., 20: 975-981.

Nicholson, R.I., J.M.W. Gee and M.E. Harper, 2001. EGFR and cancer prognosis. Eur. J. Can., 37: 9-15.

Niemiec, J., L. Kolodziejski and S. Dyczek, 2005. EGFR LI and Ki-67 LI are independent prognostic parameters influencing survivals of surgically treated squamous cell lung cancer patients. Neoplasm, 52: 231-237.

Ormandy, C.J., E.A. Musgrove, R. Hui, R.J. Daly and R.L. Sutherland, 2003. Cyclin D1, EMS1 and 11q13 amplification in breast cancer. Breast Cancer Res. Treat, 78: 323-335.

Rodrigo, J.P., D. Garcia-Carracedo, L.A. Garcýa, S.T. Menendez and E. Allonca *et al.*, 2009. Distinctive clinicopathological associations of amplification of the cortactin gene at 11q13 in head and neck squamous cell carcinomas. J. Pathol., 217: 516-523.

Rothschild, B.L., A.H. Shim, A.G. Ammer, L.C. Kelley and K.B. Irby *et al.*, 2006. Cortactin overexpression regulates actin-related protein 2/3 complex activity, motility, and invasion in carcinomas with chromosome 11q13 amplification. Cancer Res., 66: 8017-8025.

Schlessinger, J., 2002. Ligand-induced, receptor-mediated dimerization and activation of EGF receptor. Cell, 110: 669-672.

Schuuring, E., 1995. The involvement of the chromosome 11q13 region in human malignancies: Cyclin D1 and EMS1 are two new candidate oncogenes-a review. Gene, 159: 83-96.

Small, J.V., T. Stradal, E. Vignal and K. Rottner, 2002. The lamellipodium: Where motilitybegins. Trends Cell Biol., 12: 112-120.

Timpson, P., A.S. Wilson, G.M. Lehrbach, R.L. Sutherland, E.A. Musgrove and R.J. Daly, 2007. Aberrant expression of cortactin in head and neck squamous cell carcinoma cells is associated with enhanced cell proliferation and resistance to the epidermal growth factor receptor inhibitor gefitinib. Cancer Res., 67: 9304-9314.

Timpson, P., D.K. Lynch, D. Schramek, F. Walker and R.J. Daly, 2005. Cortactin overexpression inhibits ligand-induced down-regulation of the epidermal growth factor receptor. Cancer Res., 65: 3273-3280.

Travis, W.D., E. Brambilla, H.K. Muller-Hermelink and C.C. Harris, 2004. Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart: World Health Organization Classification of Tumours. IARC Press, Lyon, France.

Tsai, W.C., J.S. Jin, W.K. Chang, D.C. Chan and M.K. Yeh *et al.*, 2007. Association of cortactin and fascin-1 expression in gastric adenocarcinoma: Correlation with clinicopathological parameters. J. Histochem. Cytochem., 55: 955-962.

Van Rossum, A.G., W.H. Moolenaar and E. Schuuring, 2006. Cortactin affects cell migration by regulating intercellular adhesion and cell spreading. Exp. Cell Res., 312: 1658-1670.

Wang, G.C., P.S. Hsieh, H.H. Hsu, G.H. Sun, S. Nieh, C.P. Yu and J.S. Jin, 2009. Expression of cortactin and survivin in renal cell carcinoma associated with tumor aggressiveness. World J. Urol., 27: 557-563.

Weiss, G.J. and C. Kingsley, 2008. Pathway targets to explore in the treatment of non-small cell lung cancer. J. Thoracic Oncol., 11: 1342-1352.

Xu, X.Z., M.V. Garcia, T.Y. Li, L.Y. Khor and R.S. Gajapathy *et al.*, 2010. Cytoskeleton alterations in melanoma: Aberrant expression of cortactin, an actin-binding adapter protein, correlates with melanocytic tumor progression. Mod. Pathol., 23: 187-196.

Yamaguchi, H. and J. Condeelis, 2007. Regulation of the actin cytoskeleton in cancer cell migration and invasion. Biochim. Biophys. Acta (BBA)-Mol. Cell Res., 1773: 642-652.

Yang, Z., R. Bagheri-Yarmand, R.A. Wang, L. Adam and V.V. Papadimitrakopoulou *et al.*, 2004. The epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 (Iressa) suppresses c-Src and Pak1 pathways and invasiveness of human cancer cells. Clin. Cancer Res., 10: 658-667.

Yarden, Y. and M.X. Sliwkowski, 2001. Untangling the ErbB signalling network. Nat. Rev. Mol. Cell Biol., 2: 127-137.