EGFR immunoexpression, RAS immunoexpression and their effects on survival in lung adenocarcinoma cases

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Background: The impacts of epidermal growth factor receptor (EGFR) immunoexpression and RAS immunoexpression on the survival and prognosis of lung adenocarcinoma patients are debated in the literature.

Methods: Twenty-six patients, who underwent pulmonary resections between 2002 and 2007 in our clinic, and whose pathologic examinations yielded adenocarcinoma, were included in the study. EGFR and RAS expression levels were examined by immunohistochemical methods. The results were compared with the survival, stage of the disease, nodal involvement, lymphovascular invasion, and pleural invasion. Nonparametric bivariate analyses were used for statistical analyses.

Results: A significant link between EGFR immunoexpression and survival has been identified while RAS immunoexpression and survival have been proven to be irrelevant. Neither EGFR, nor RAS has displayed a significant link with the stage of the disease, nodal involvement, lymphovascular invasion, or pleural invasion.

Conclusions: Positive EGFR immunoexpression affects survival negatively, while RAS immunoexpression has no effect on survival in lung adenocarcinoma patients.

**Keywords:** Lung adenocarcinoma; epidermal growth factor receptor (EGFR); RAS; survival

Introduction

Epidermal growth factor receptor (EGFR), also known as *ErbB1* is an intracellular transmembrane glycoprotein, which has intrinsic tyrosine kinase activity (1,2). When the ligand is bound to the cell, autophosphorylation occurs in the intracytoplasmic segment, which activates intracellular tyrosine kinase (1). As a consequence RAS-RAF-MAPK (mitogen activated protein kinase) signal transduction pathway is activated (3). EGFR overexpression causes tumor cell growth, tumor invasion, angiogenesis and eventually metastasis (2). A relation between EGFR overexpression and cellular adhesion, inhibition of apoptosis and resistance to chemotherapy have been identified (4). *EGFR* gene mutations have been confirmed to play role in development of pulmonary adenocarcinoma (5). EGFR expression can be detected in non-small cell lung cancer (NSCLC) cases with immunohistochemical staining (6). EGFR overexpression has been shown to have negative impacts on prognosis in NSCLC patients by a recent meta-analysis (2,7,8).

RAS is the human analog of a gene, which is coded by a retrovirus and causes sarcoma in rats (9). RAS is activated by binding guanosine triphosphate and facilitates intracellular signal transduction (10). *K-RAS* gene codon 12 mutation has been identified in approximately 40% of lung adenocarcinoma cases. RAS mutation plays an important role in cell growth and inhibition of apoptosis (11). RAS can be detected with immunohistochemical techniques in NSCLC cases (12). A meta-analysis has suggested that RAS has no effect on prognosis in NSCLC cases (13).

This study aims at identifying the presence of EGFR
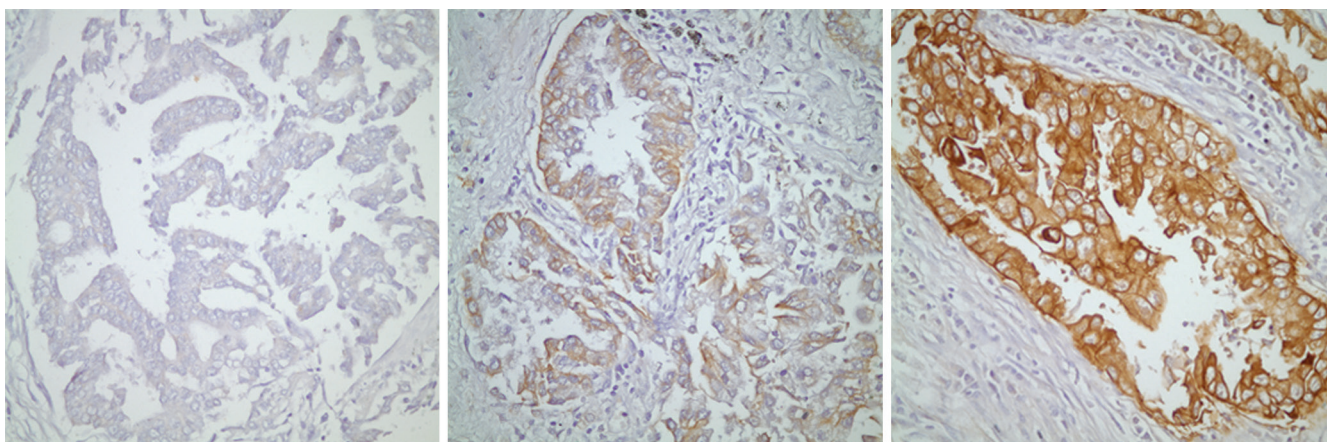
Figure 1 EGFR immunoexpression at tissue level (negative, weak, strong expressions respectively at ×400 magnification). EGFR, epidermal growth factor receptor.

immunoexpression and RAS immunoexpression and their effects on survival in pulmonary adenocarcinoma cases.

Materials and methods

Patients

Twenty-six patients, who underwent complete anatomical resection and mediastinal lymph node dissection due to bronchial adenocarcinoma at Hacettepe University Hospital, Department of Thoracic Surgery between 2002 and 2007, were included in the study. The study was conducted upon approval of local ethics committee of our university. The preoperative diagnostic and metastatic workup was carried out for each patient and the resectable cases were operated on. At least one year follow up survival data was recorded for all patients. Stage of the disease, lymph node involvement, lymphovascular invasion, and pleural invasion were noted for each patient according to the pathological examination reports after the surgery. EGFR and RAS immunoexpressions were examined using the paraffin blocks of the pathological specimens.

Immunohistochemistry

Twenty of the patients that are included in the study had tissue microarray (TMA) of their pathological specimens. TMA of the 20 patients and the routine paraffin blocks of the remaining six patients were used for the preparation of 5 μm slides. Antigen retrieval procedure and avidin-biotin-peroxidase method were used for all prepared sections.

21E1-1 mouse, monoclonal antibody (ImmunoVision Technologies, USA) and RB-1627P rabbit, polyclonal antibody (Neomarkers, Fremont, CA, USA) were used respectively for EGFR and RAS. Immunohistochemical results were evaluated by using Zeiss Axioskop 2. For EGFR, the intensity of the staining was grouped as: absent (score 0), weak (score 1), medium (score 2) and strong (score 3). The percentage of the staining was grouped as: 0-10% (score 1), 11-50% (score 2) and 51-100% (score 3). The degree of immunoexpression was categorized by the multiplication of intensity score by the percentage score. A multiplication score of 0 was considered to be negative; 1, 2 and 3 were considered to be weak; and 4 and 6 were considered to be strong expressions for EGFR (*Figure 1*). The sections were grouped as weak and strong expressions according to the nuclear staining patterns of RAS (*Figure 2*).

Statistical analysis

Since the number of the patients included in the study was less than 30, nonparametric analyses were used. The relationships of EGFR immunoexpression and RAS immunoexpression with survival, stage of the disease, lymphovascular invasion, and pleural invasion were analyzed using Kendall's tau_b ve Spearman's rho tests in the SPSS 11.5 program.

Results

Twenty-six patients included in the study consisted of 18 (69.2%) male and 8 (30.8%) female participants. The
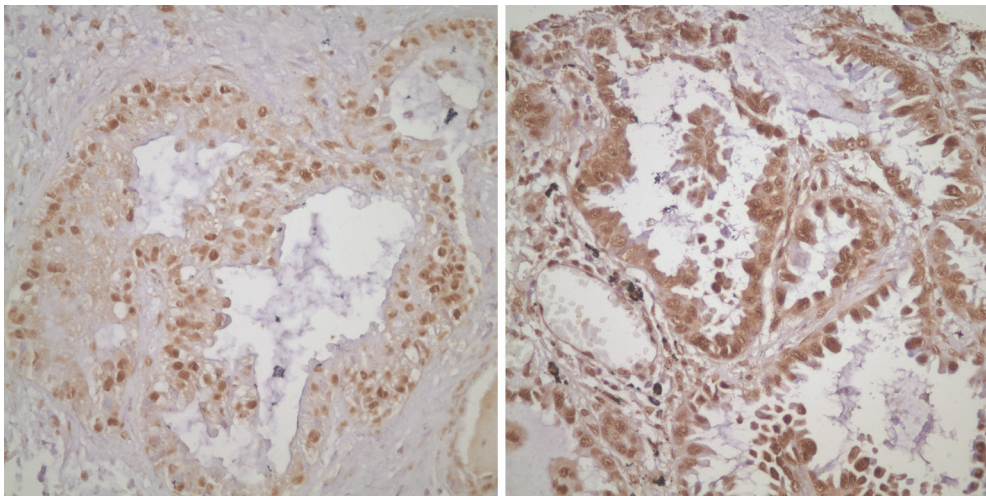
Figure 2 RAS immunoexpression at tissue level (weak and strong expressions respectively at ×400 magnification).

Table 1 EGFR immunoexpression, RAS immunoexpression and the clinicopathological parameters of the patients

		Number of patients				
		EGFR immunoexpression			RAS immunoexpression	
		Absent	Weak	Strong	Weak	Strong
Stage	IA	2	3	1	1	5
	IB	5	4	1	2	8
	IIA	0	0	1	0	1
	IIB	0	1	0	0	1
	IIIA	4	1	2	3	4
	IIIB	1	0	0	0	1
Nodal involvement	N0	7	8	2	3	14
	N1	0	0	1	0	1
	N2	5	1	2	3	5
Lymphovascular involvement	Positive	6	1	1	3	5
	Negative	6	8	4	3	15
Pleural invasion	Positive	7	5	2	4	10
	Negative	5	4	3	2	10
EGFR, epidermal growth factor receptor.						

mean age of patients was 56.3±10.4 [35-74]. At the time of the study, 18 (69.2%) patients were alive and 8 (30.8%) had passed away. According to the staging conducted after the pathological examination of the resected specimens; 6 (23.1%) patients were Stage IA, 10 (30.8%) patients were Stage IB, 1 (3.8%) patient was Stage IIA, 1 (3.8%) patient was Stage IIB, 7 (26.9%) patients were Stage IIIA and 1 (3.8%) patient was Stage IIIB. Seventeen (65.4%) patients had N0, 1 (3.8%) patient had N1 and 8 (30.8%) patients had N2 disease after the tissue evaluation of the surgically dissected lymph nodes. Seventeen (65.4%) patients had lymphovascular invasion and 9 (34.6%) patients did not have lymphovascular invasion. Twelve (46.2%) patients had pleural invasion, while 14 (53.8%) patients did not.

EGFR immunoexpression was negative for 12 (46.2%) patients, weak for 9 (34.6%) patients and strong for 5 (19.2%) patients. RAS immunoexpression was weak for 6 (23.1%) patients and strong for 20 (76.9%) patients. EGFR immunoexpression, RAS immunoexpression and the clinicopathological parameters of the patients are shown in *Table 1*.

Table 2 Relationship between EGFR immunoexpression and survival

			EGFR	Survival
Kendall's tau_b	EGFR	Correlation coefficient	1.000	–0.400*
		Sig. (2-tailed)	–	0.035
		N	26	26
	Survival	Correlation coefficient	–0.400*	1.000
		Sig. (2-tailed)	0.035	–
		N	26	26
Spearman's rho	EGFR	Correlation coefficient	1.000	–0.421*
		Sig. (2-tailed)	–	0.032
		N	26	26
	Survival	Correlation coefficient	–0.421*	1.000
		Sig. (2-tailed)	0.032	–
		N	26	26

EGFR, epidermal growth factor receptor; *, correlation is significant at the 0.05 level (2-tailed).

Table 3 Relationship between RAS immunoexpression and survival

			RAS	Survival
Kendall's tau_b	RAS	Correlation coefficient	1.000	–0.167
		Sig. (2-tailed)	–	0.403
		N	26	26
	Survival	Correlation coefficient	–0.167	1.000
		Sig. (2-tailed)	0.403	–
		N	26	26
Spearman's rho	RAS	Correlation coefficient	1.000	–0.167
		Sig. (2-tailed)	–	0.414
		N	26	26
	Survival	Correlation coefficient	–0.167	1.000
		Sig. (2-tailed)	0.414	–
		N	26	26

Correlation is not significant.

Nonparametric bivariate analyses were used for the evaluation of the statistically significant relationship between the immunoexpression status and survival, stage of the disease, nodal involvement, lymphovascular invasion; and pleural invasion. Strong EGFR immunoexpression had a negative relationship with survival, which is statistically significant at $\alpha =0.05$ level according to Kendall's tau_b and Spearman's rho tests (Kendall's tau_b, r =–0.400; Spearman's rho, r =–0.421; *Table 2*). There was no statistically significant relationship between EGFR immunoexpression and stage of the disease, nodal involvement, lymphovascular invasion, or pleural invasion (Kendall's tau_b, r =–0.075, –0.032, –0.271, –0.037; Spearman's rho, r =–0.088, –0.042, –0.286, –0.039, respectively). RAS immunoexpession had no significant relationship with survival, stage of the disease, nodal involvement, lymphovascular invasion, or pleural invasion. (Kendall's tau_b, r =–0.167, –0.134, –0.194, –0.177, –0.225; Spearman's rho, r =–0.167, –0.147, –0.197, –0.177, –0.225, respectively) (*Table 3*).

Discussion

Lung cancer is one of the leading causes of death in developed countries. The poor prognosis in lung carcinoma

may be due to the patient related factors or the influences associated with the self-nature of the tumor (8). Age, patient status, stage of the disease are among the survival predictors in resectable NSCLC (14). Serum lactate dehydrogenase level, white cell count and neutrophil count are also found to be effective on survival (15). Recently, new techniques to investigate prognosis have been put forward with the advances in molecular biology and cytogenetics (8).

The development and progression of cancer is caused by various factors on the cellular level, such as autonomous growth signals, refraction to growth inhibiting signals, insensitivity to apoptotic signals, unlimited growth potential, angiogenesis, invasion and metastasis (16). Several proto-oncogenes and tumor suppressor genes play a role in these genetic irregularities (17). Some of these genetic irregularities affect the tumor cell behavior much more, and thus can be used as prognostic markers. Molecular prognostic markers reveal themselves as changes in gene copy number, messenger ribonucleic acid (mRNA), and expression levels of proteins (12).

Immunohistochemistry is a quite practical method for detecting the changes in protein expression. It not only shows protein expression in a semi-quantitative manner, but also provides information on the cellular localization of the protein expression. Immunohistochemistry has been used in many different studies as it is involved in literature in this field (12).

EGFR, which has intrinsic tyrosine kinase activity, is a transmembrane protein with an intracellular domain (1). EGFR activates a couple of intracellular signal transduction pathways (7). These pathways cause the cells to transform and grow, inhibit apoptotic signals, and lead to angiogenesis and tumor invasion (18). Kozuki *et al*. have shown mutations of the *EGFR* gene in the development of lung adenocarcinoma (5).

The effect of EGFR expression on survival in NSCLC has been studied by immunohistochemical methods and different results were obtained. In 1997, Rusch *et al*. found EGFR expression to have an effect on survival of NSCLC by using Northern Blot and immunohistochemical methods (19). Volm *et al*. stated that EGFR immunoexpression has a negative effect on survival of squamous cell lung cancer cases (20). In 2000, by conducting an immunohistochemical study, Ohsaki *et al*. have similarly found a negative effect of EGFR expression on survival of NSCLC patients (21). Meert *et al*. conducted a meta-analysis in 2002 by reviewing 16 different studies, which were published between 1989 and 2000. Fourteen of these studies were based on

immunohistochemical methods. EGFR expression was found to be positive in 51% NSCLC cases and 46.2% lung adenocarcinoma cases. In a quantitative meta-analysis of eight studies, EGFR expression was shown to be a poor prognostic sign (8). In the current study, the researchers examined the pathological specimens of the patients who were operated for lung adenocarcinoma and grouped them as negative, weak, and strong according to the immunohistochemically detected EGFR expression. Since the total number of the patients was less than 30, the researchers used nonparametric bivariate analyses for the statistical evaluation. Strong EGFR immunoexpression was found to affect survival in a negative fashion. This result is consistent with that obtained by Meert *et al*. in their meta-analysis. In another meta-analysis carried out by Nicholson *et al*. EGFR expression was found to negatively affect the survival in 10-20% of the studies they reviewed (22). In 2005, Niemiec *et al*. showed a statistically significant relationship between EGFR and prognosis (23). The results of the current study are parallel to some of those in the literature. The different results achieved in various studies are dependent on the immunohistochemical methods used. There is no standard evaluation method for EGFR immunoexpression. The scoring is semi-quantitative. The scoring methods depend on the evaluation of staining intensity, percentage of the stained cells, the location of the staining, and the combination of these three parameters (12). The combination process of the staining intensity and percentage of stained cells also varies in different studies. These factors lead to different immunoexpression levels.

Lung adenocarcinoma cases have been shown to bear 40% *K-RAS* gene codon 12 mutation (11). RAS proteins bind GTP. They play a key role in intracellular molecular events. The intrinsic GTPase activity of RAS is lost due to the mutations in tumor cells, and the intracellular signal transduction pathways are continuously stimulated. This gives rise to uncontrolled cell proliferation (13). RAS mutation is important in cell growth and the inhibition of apoptosis (11). The relationship of RAS with prognosis of the disease has been evaluated in various studies using immunohistochemical methods. In NSCLC patients, Harada *et al*. showed RAS to be important for survival, to be an independent prognostic factor, and to be a good marker for understanding the malignant potential of the tumor (24). Miyamato *et al*. used immunohistochemical methods and found RAS expression to be a survival predictor independent of tumor stage (25). In 2005 Mascaux *et al*. published a meta-analysis in which they evaluated the

relationship between RAS expression and survival in lung cancer patients (13). Both immunohistochemistry and other molecular diagnostic methods were used in the studies included in this meta-analysis. In a couple of these studies, there was a statistically significant negative relationship between RAS expression and survival. Yet in a considerable amount of them, there was no relationship. There were also some papers denoting that RAS expression an effect on the metastatic potential of the tumor. The current study did not find a significant relationship between RAS expression and survival. These results are consistent with some of the literature. The studies conducted on a larger number of patients with the use of molecular diagnostic techniques may lead to better results for the evaluation of relationship between RAS and survival.

Conclusions

Positive EGFR immunoexpression affects survival negatively, while RAS immunoexpression has no effect on survival in lung adenocarcinoma patients.

Acknowledgements

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