RESEARCH ARTICLE

Lack of any Prognostic Role of Insulin-Like Growth Factor-1 Receptor in Non-Small Cell Lung Cancer

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Abstract

Background: The purpose of this study is to determine whether the IGF1R expression has a prognostic role in non-small cell lung cancer. Materials and Methods: Forty-seven patients histopathologically diagnosed with small cell lung cancer upon bronchoscopic biopsy or resection materials were included in the study. IGF1R expression was examined via immunohistochemical methods. In samples, >10% staining were assessed as positive and ≤10% as negative. Information about demographic datas and treatments was obtained by retrospective searches of patient files. Results: IGF1R expression was determined as positive in 38 (80.9%) and as negative in 9 (19.1%) patients. There was no significant relation between IGF1R expression and histological sub-type, local invasion, lymph node and metastasis status (p=0.842, p=0.437, 0.064, 0.447, respectively). There was also no correlation with IGF1R expression and survival (p=0.141). Conclusions: There are conflicting results between IGF1R and its prognostic effects in the various studies. It has been claimed in some studies it is not related to prognosis as in our study, and in some studies it has been claimed that it is a good prognostic factor whereas in some studies it has been claimed as being a factor for worse prognosis. We think that IGF1R expression in non-small cell lung carcinoma patients deserves further analysis, because of its potential prognostic and predictive roles.

Keywords: IGF1R - insulin-like growth factor-1 receptor - lung cancer - prognosis

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Introduction

Lung cancer is still the most common cancer worldwide despite all the primary prevention studies (Siegel et al., 2012; Luqman et al., 2014). The prognosis of lung cancer patients may vary depending on the histologic type, stage, and molecular characteristics such as Epidermal Growth Factor Receptor (EGFR) and Anaplastic Lymphoma Kinase (ALK) expression of the tumour and the overall performance of the patient (Kulesza et al., 2011; Mandal et al., 2013; Unal et al., 2013; Tantraworasin et al., 2014).

In different stages of lung cancer, determination of mutations in different oncogenes and tumor suppressor genes in different histologic subtypes demonstrates that accumulation of different genetic factors plays a role in the progression of disease. A better understanding of lung cancer biology led to the development of drugs targeting oncogenesis, specifically tumor suppressor genes, growth factor receptors, and angiogenesis (Yildirim et al., 2013).

Insulin-like growth factor (IGF) is a mitogenic polypeptide with a structure similar to that of proinsulin. It is secreted from the liver primarily and plays a role in embryogenesis and childhood development (Casa et al., 2008). In the adulthood period, it has protective functions against proliferation and apoptotic stimulations, besides sustaining normal cell metabolism. Abnormal functions of IGF may contribute to the development of malignant proliferation and progression (Pollak, 2000; Suzuki et al., 2009; Tong et al., 2010).

IGF shows both autocrine and paracrine effects. It binds to insulin-like growth factor 1 receptor (IGF1R) and insulin-like growth factor 2 receptor (IGF2R) in tissues and in the circulatory system, where 99% of it is bound to IGF-binding protein 3 (IGFBP-3) (Frasca et al, 2008). Its biological functions are primarily mediated by IGF1R, which is a kind of mitogenic tyrosine kinase receptor (O'Brien et al., 2001). It has been demonstrated that IGF1R plays an important role in the development and progression of many cancers such as prostate, breast, liver, and colorectal cancers and brain tumors (Resnik et al.,1998; O'Brien et al., 2001; Alexia et al., 2004; Samani et al., 2007; Xiong et al., 2012; Li et al., 2013). Even though studies performed to assess the role of IGF1R in lung cancer have given conflicting results, the

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common outcome of the studies is that IGF1R may be an autocrine growth factor for lung cancer (Ding et al., 2013; Vincent et al., 2013). The purpose of this study was to determine whether IGF1R expression evaluated by an immunohistochemical method would have a prognostic role in non-small cell lung cancer (NSCLC).

Materials and Methods

Patient selection

The study included 47 cases histopathologically diagnosed with NSCLC by bronchoscopic biopsy or resection materials and followed up at Antalya Education and Research Hospital between 2008 and 2010. Patients whose imaging and clinical staging were completed were staged according to the 7th edition of the American Joint Committee on Cancer (AJCC) staging system. Demographic data such as age, gender, stage of the disease, and treatments were obtained retrospectively, by searching patient files.

Immunohistochemical examination

Tumor samples obtained by surgery or bronchoscopic biopsy were fixed in 10% formaldehyde right after the procedure. After fixation, the tumor samples were embedded in paraffin. Histological sections of 4 μm thickness were obtained from paraffin blocks and were initially stained with hemotoxylin-eosin for initial assessment. Afterwards, they were kept in xylol for 20 minutes and deparaffinized. Then the slides were rehydrated by passing through an alcohol series starting from 96% alcohol till 70% alcohol. After the slides were washed with a hydroxymethyl-amino methane (TRIS) solution (pH 7.2) for 5 minutes, they were boiled in a citric acid solution in special vessels in a microwave oven for 20 minutes for antigen exposure. After cooling, they were washed with TRIS again and the edges of the sections were marked with a bounding pen. Endogenous peroxidase activity was blocked by keeping the slides in 3% hydrogen peroxide (H_2O_2) for 5 minutes. Slides were washed with TRIS again and were treated with a blocking solution (nonimmune serum) (Zyned Histostatin-Plus, 01062420, California) for 10 minutes. Immunohistochemical staining was performed using a primary monoclonal antibody against IGF1R (rabbit polyclonal, clone SC-9038, dilution 1:100, Santa Cruz, Texas, USA), which was dripped onto the slides. The slides were kept at room temperature for 60 minutes, then washed in TRIS solution for 5 minutes. Secondary antibody was dripped on the slides, and after incubation for 10 minutes, they were washed in TRIS solution for 5 minutes. Finally, a streptavidin peroxidase solution was dripped on the slides, which were incubated for 10 minutes. The slides were washed in TRIS solution for 5 minutes and incubated for 5 minutes in Mayer's hematoxylin in order to provide contrast staining. The slides were washed under tap water and dehydrated by being passed through 70% ethyl alcohol up to 96% alcohol and isopropyl alcohol. They were kept in xylol for 20 minutes to provide transparency and then they were covered with Entellan mounting medium (Merck®). Histological sections of examined lung cancer patients with positive immunohistochemical staining for IGF1R were used as positive control.

Evaluation of immunohistochemically stained sections

All samples were evaluated and scored twice by two pathologists (DA and AA) without knowledge of clinical and pathological characteristics and follow-up results. In the samples, >10% staining was accepted as positive and ≤10% staining was considered negative (Figure 1). The staining pattern was mainly membranous and cytoplasmic in all positive cases.

Statistical analysis

Statistical analyses were performed using SPSS for Windows 15.0 software. Normal distribution of variables was examined by visual (histogram and probability graphics) and analytical methods (Kolmogorov-Smirnov and Shapiro-Wilk tests). In Kolmogorov-Simirnov test, data where the p value was above 0.05 were considered as normal distribution. Differences between groups were examined by chi-square and Mann-Witney U tests. The relationship of each positive and negative immunohistochemical result with survival was assessed by Kaplan-Meier survival analysis. Statistical differences were validated with the log-rank test. A p value of <0.05 was considered significant.

Results

A total of 47 patients, 10 (21.3%) female and 37 (78.7%) male, were included in the study. The mean age of patients was 62±11.8 (range 41-88) years. Fourteen (29.8%) patients had comorbid disease and the most frequent disease was hypertension, determined in 4 (8.5%) patients. Other comorbid diseases were atherosclerotic heart disease, in 3 (6.4%) patients, hypertension and atherosclerotic heart disease, in 3 (6.4%) patients, diabetes mellitus, in 1 (2.1%) patient, and a history of lung tuberculosis, in 1 (2.1%) patient.

The first symptom was cough in 38 (80.9%) patients, dyspnea in 22 (46.8%) patients, weight loss in 17 (36.2%) patients, loss of appetite in 10 (21.3%) patients, hemoptysis in 7 (14.9%) patients, and hoarseness in 3 (6.4%) patients.

The most frequently determined NSCLC subtype was squamous cell carcinoma, in 22 (46.8%) patients, and

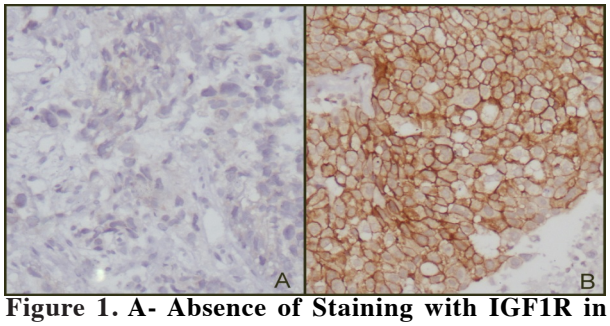

Figure 1. A- Absence of Staining with IGF1R in Tumor Cells (IGF1R negative), B- Membranous Staining in All of the Tumor Cells (IGF1R Positive) (Magnifications x100)

adenocarcinoma, in 20 (42.6%) patients. Other subtypes were bronchogenic cancer, undifferentiated cancer, adenocarcinoma with neuroendocrine differentiation, in 1 (2.1%) patient each, and large cell carcinoma, in 2 (4.3%) patients.

When patients were evaluated in terms of stage, 4 (8.5%) had stage IB, 3 (6.4%) had stage IIA, 2 (4.3%) had stage IIB, 8 (17%) had stage IIIA, 9 (19.1%) had stage IIIB, and 21 (44.7%) had stage IV disease. Local invasion and lymph node involvement of the tumor were evaluated histopathologically in patients who underwent surgery and with imaging methods in patients who had only biopsy. The most frequently determined local invasion was T2A, in 16 (34%) patients. This was followed by T3, in 15 (31.9%) patients, T4, in 11 (23.4%) patients, T2B and T1B, in 2 (4.3%) patients each, and T1A, in 1 (2.1%) patient. Lymph node involvement was not determined in 18 (38.3%) patients, N1 was determined in 7 (14.9%) patients, N2 was determined in 19 (40.4%) patients, and N3 was determined in 3 (6.4%) patients.

The most frequent metastasis location was the pleura, with malignant pleural effusion in 8 (17%) patients. Other metastasis locations were bone, in 6 (12.8%) patients, brain, in 4 (8.5%) patients, opposite lung retention, in 2 (4.3%) patients, and liver, in 1 (2.1%) patient.

Twenty-one (44.7%) patients underwent surgery. Eight (17%) of the patients who underwent surgery had wedge resection, 6 (12.8%) patients had lobectomy, 1 (2.1%) patient had pneumonectomy, and 6 (12.8%) patients had palliative surgery. The most frequently applied first-line treatment was chemotherapy. Taxane-based chemotherapy combinations were applied in 14 (29.8%) patients. Other chemotherapy combinations applied were gemcitabine-cisplatin, in 13 (27.7%) patients, cisplatin-etoposide, in 13 (27.7%) patients, and navelbine-cisplatin, in 2 (4.3%) patients. Five patients did not come for follow-up after diagnosis. Eleven (23.4%) patients received palliative radiotherapy.

IGF1R expression was positive in 38 (80.9%) patients and negative in 9 (19.1%) patients. There was no relationship between IGF1R expression and gender and age (p=0.939 and p=0.144, respectively). When the relationship between initial symptoms and IGF1R was assessed, hoarseness was more often seen in IGF1R-negative patients and weight loss was more often seen in IGF1R-positive patients. There was no significant relationship between IGFR1 expression and histological subtype, local invasion, lymph node status, and metastasis status (p=0.842, 0.437, 0.064, and 0.447, repectively) (Table 1).

The median survival of patients was 11.9±1.2 months (95% confidence interval 9.5-14.2). Analysis of survival by univariant analyses found no relationship between survival and comorbid disease, age, gender, smoking history, the presence of dyspnea, cough, loss of appetite, weight loss, and lumph node status (p=0.522, 0.954, 0.918, 0.677, 0.508, 0.908, 0.863, and 0.350, respectively). In univariant analyses, hoarseness, history of hemoptysis, histological subtype, and T stage were found to be related to survival (p=0.001, 0014, 0.014, and 0.011, respectively).

Table 1. The Relationship between Expression of IGF1R and Demographic Characteristics, Symptoms of the First Presentation and Stage

		IGF1R (-) N (%)	IGF1R (+) N (%)	p value
Gender	Female	2 (22.2)	8 (21.1)	0.939
	Male	7 (77.8)	30 (78.9)	
Age		67.2±14.5	60.7±10.9	0.144
Smoking	Yes	7 (77.8)	35 (92.1)	0.21
	No	2 (22.2)	3 (7.9)	
Hoarseness	Yes	2 (22.2)	1 (2.6)	0.031*
	No	7 (77.8)	37 (97.4)	
Hemoptysis	Yes	1 (11.1)	6 (15.8)	0.596
	No	8 (88.9)	32 (84.2)	
Dyspnea	Yes	3 (33.3)	19 (50)	0.368
	No	6 (66.7)	19 (50)	
Cough	Yes	6 (66.7)	32 (84.2)	0.229
	No	3 (33.3)	6 (15.8)	
Anorexia	Yes	0	10 (26.3)	0.083
	No	9 (100)	28 (73.7)	
Weight loss	Yes	0	17 (44.7)	0.012*
	No	9 (100)	21 (55.3)	
T	T1A	0	1 (2.6)	0.437
	T1B	0	2 (5.3)	
	T2A	3 (33.3)	13 (34.2)	
	T2B	2 (22.2)	0	
	T3	0	15 (39.5)	
	T4	4 (44.4)	7 (18.4)	
N	N0	6 (66.7)	12 (31.6)	0.064
	N1	1 (11.1)	6 (15.8)	
	N2	1 (11.1)	18 (47.4)	
	N3	1 (11.1)	2 (5.3)	
M	M0	3 (33.3)	23 (60.5)	0.144
	M1	6 (66.7)	15 (39.5)	
Stage	Stage 1B	1 (11.1)	3 (7.9)	0.54
	Stage 2A	1 (11.1)	2 (5.3)	
	Stage 2B	0	2 (5.3)	
	Stage 3A	0	8 (21.1)	
	Stage 3B	2 (22.2)	7 (18.4)	
	Stage 4	0	16 (42.1)	

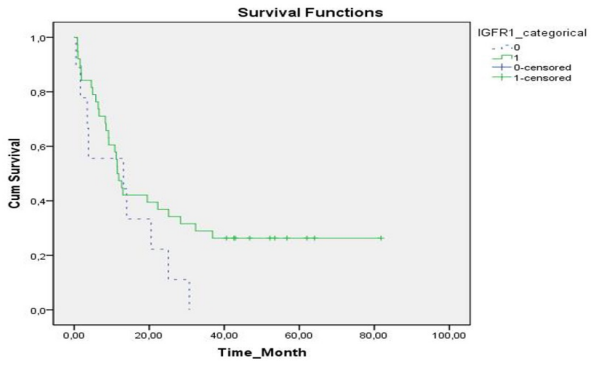

Figure 2. This Shows Kaplan-Meier Estimates of Survival among the Study Population, according to (IGF-1R) Negative or Positive Expression

There was no significant relationship between IGF1R expression and survival (p=0.141; Figure 2). The median survival was 13.1±13.9 months (95% confidence interval 0-40.4) in patients with negative IGF1R expression and 11.5±1.1 months (95% confidence interval 9.3-13.7) in IGF1R-positive patients.

Discussion

In our study, no prognostic relationship was found between IGF1R evaluated by an immunohistochemical method and NSCLC. There was no relationship between local invasion, lymph node involvement, and metastatic behavior of the tumor and IGF1R expression.

Cappuzzo et al. (2010) found that IGF1R was more often expressed in patients with NSCLC of squamous histology than that patients with NSCLC of non-squamous histology. However, they did not find any survival difference between the IGF1R-expressing group and the group in which no expression was found. Similar to our study, they claimed that IGF1R expression was not a prognostic factor (Cappuzzo et al., 2010). Similar results were demonstrated in the study by Dziadziuszko et al. (2010) on operated patients with locally advanced NSCLC. In that study, IGF1R expression was at a higher level and it was claimed that IGF1R expression did not have a prognostic role.

In contrast, Kim et al. (2012) demonstrated that high IGF1R expression was related to shorter survival and was a worse prognostic factor. Also, there are studies claiming that IGF1R expression is a worse prognostic factor in the adenocarcinoma subtype of NSCLC. Kikuchi et al. (2012) demonstrated that IGF1R expression was at a lower level in the poorly differentiated group among adenocarcinoma patients, and that the low expression of IGF1R was a worse prognostic factor.

Merrick et al. (2007) found a significant relationship between IGF1R expression and prognosis only in stage 1 NSCLC patients. They claimed that IGF1R expression was a worse prognostic factor in these patients. In contrast, Lee et al. (2008) found no relationship between IGF1R expression and prognosis in stage 1 patients.

There was no relationship between lymph node involvement status and IGF1R expression in our study. However, Yamamoto et al. (2012) claimed that IGF1R overexpression was related to lymph node metastasis and tumor recurrence. Also, Sun et al. (2012) claimed that IGF1R expression played a role in the development of resistance to cisplatin and radiotherapy in NSCLC.

Studies about IGF1R expression in NSCLC place emphasis on anti-IGF1R treatments. It was demonstrated that addition of anti-IGF1R treatment to paclitaxel-carboplatin combination therapy in NSCLC improved the response to treatment (Gualberto and Karp, 2009; Karp et al., 2009; Goto et al., 2012; Chen et al., 2013). Studies have shown that there could be a correlation between K-Ras status and IGF1R expression. For example, K-Ras-mutated patients showed resistance to anti-IGF1R agents (Kim et al., 2012).

In conclusion, there are conflicting study findings on IGF1R and its prognostic role. We think that IGF1R expression in NSCLC deserves further analysis, because of its potential prognostic and predictive roles.

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