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Prognostic Implications of Tumoral Expression of Insulin-like Growth Factor (IGF)-1 and -2 in Patients with Non-small Cell Lung Cancer

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Abstract

Introduction/Background—The currently available systemic therapies for non-small cell lung cancer (NSCLC) have limited efficacy. Our previous report indicate the association of elevated insulin-like growth factor (IGF)-1 receptor (IGF-1R) and insulin receptor (IR) expression levels with poor survival in NSCLC patients. To better understand the molecular biomarkers involved in the IGF signaling pathway in NSCLC, we characterize the expression levels of IGF-1 and IGF-2 and to evaluate their association with IGF-1R and phosphorylated IGF-1R (pIGF-1R) expression in NSCLC.

Methods—A total of 352 patients who underwent NSCLC resection with curative intent were studied. The expression patterns of the IGF-1, IGF-2, IGF-1R and pIGF-1R proteins were assessed immunohistochemically using tissue microarrays.

Results—The IGF-1 expression was higher in patients with adenocarcinoma (ADC) than in those with squamous cell carcinoma (SCC), whereas the IGF-2 score was higher in patients with SCC than those with ADC. Likewise, the IGF-1 score was higher in patients with mutated EGFR (mtEGFR) than in those with wild type EGFR (wtEGFR), while the IGF-2 score was higher in

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The authors declare no conflict of interest for this study.

Statement of author contributions

Jin-Soo Kim was involved in the data analysis, interpretation and drafting the article. Luisa Solis, Carmen Behrens and Ignacio I. Wistuba were involved in the acquisition of data. Diane Liu and J. Jack Lee were involved in the data analysis and interpretation. Scott M. Lippman, Edward S. Kim, Waun Ki Hong and Ho-Young Lee were involved in conception and design. All authors were involved in writing or critical review of the draft report and all approved the final version. The authors had full access to the study data and had final responsibility for the decision to submit for publication.

patients with wtEGFR than in those with mtEGFR. Patients with low levels of IGF-1 expression had longer overall survival (OS) than those with high IGF-1 expression, and subgroup analyses revealed a significant difference in OS in ADC patients only.

Conclusions—The overexpression of IGF-1 predicts poor survival among patients with NSCLC, especially those with ADC. These results might serve as a future guide for clinical trials involving IGR-1R-targeting agents.

Keywords

Carcinoma; Non-Small-Cell Lung; IGF Type 1; Prognosis; and Survival

Introduction

Lung cancer accounts for 13% (1.6 million) of all cancer cases and 18% (1.4 million) of the cancer deaths in 2008 worldwide [1]. The two major forms of lung cancer are non-small-cell lung cancer (NSCLC, approximately 85% of all lung cancers) and small-cell lung cancer (SCLC, approximately 15% of lung cancers). NSCLCs are further divided into three major types, squamous cell carcinoma (SCC), adenocarcinoma (ADC) and large cell carcinoma. Despite recent advances in treatments for the disease, the currently available systemic therapies for non-small cell lung cancer (NSCLC) have limited efficacy, indicating the need for innovative treatment strategies [2].

The insulin-like growth factor 1 receptor (IGF-1R) signaling pathway has important roles in tumorigenesis, metastasis, and resistance to existing forms of anti-cancer therapy. The main components of the IGF axis include receptors [IGF-1R and insulin receptor (IR)], ligands (IGF-1 and -2), and at least six high-affinity IGF-binding proteins (IGFBP1-6) that regulate the availability of the IGF-1R ligands. Targeting the IGF signaling pathway is one evolving anticancer therapy with potential efficacy; however, the outcomes of recent phase III clinical trials with a monoclonal antibody against IGF-1R (IGF-1R mAb) have been unsatisfactory [3]. IR has been proposed to induce intrinsic and/or acquired resistance to IGF-1R-targeted antibodies [4]. In this respect, co-targeting IGF-1R and IR might be more effective in blocking the IGF-1R/IR pathway in patients with NSCLC. Nonetheless, a better understanding of the molecular biomarkers involved in the IGF-1R signaling pathway is necessary to develop effective therapeutic strategies and to identify the target population for anticancer remedies with IGF-1R inhibitors.

In a recent report, we showed that elevated IGF-1R and insulin receptor (IR) expression levels in NSCLC were associated with poor survival in NSCLC patients [5]. However, the impact of IGFs on lung cancer risk and/or prognosis remains controversial. Recent prospective studies have suggested that IGF-1 levels are related to the risk of some epithelial cancers [6]. A nested case-control study from the Beta-Carotene and Retinol Efficacy Trial in heavy smokers showed a modest association between serum IGF-1 level and lung cancer risk [7]. Conversely, IGF-1 level was not associated with elevated lung cancer risk in the Alpha-Tocopherol/Beta-Carotene Cancer Prevention Study [8]. Serum levels of IGFs were higher in lung cancer patients than in normal subjects [9]. More recently, high plasma levels

of IGFs and IGFBP-3 were associated with good prognosis in patients with advanced NSCLC in a phase II study with chemotherapy [10].

All of the aforementioned studies have evaluated serum or plasma IGF levels, which fail to reflect the impact of these ligands from autocrine or paracrine production on aggressive cancers [11]. This study was conducted to assess the impact of tumoral expression of IGF-1 on survival of patients with NSCLC.

Materials and Methods

Patient Characteristics

The baseline characteristics of the patients represented in the tissue microarray (TMA) are described in Table 1. Detailed clinical and pathologic information was available for most of these cases and included patient demographic data, smoking history (never smokers or ever smokers, patients who had smoked at least 100 cigarettes in their lifetime), pathologic tumor-node-metastasis (TNM) staging, overall survival (OS) time, and recurrence-free survival (RFS) time. The TMA included similar numbers of samples from men and women, and nearly 2/3 of the samples were from patients with adenocarcinoma (ADC). Most patients had early stage lung cancer and were former or current smokers. The tissue banking and research conduct protocols were approved by the M. D. Anderson Cancer Center Institutional Review Board. All patients provided informed consent, and all patient identifying information was removed from the database.

Case Selection and Tissue Microarray (TMA) Construction

For this study, we obtained archived formalin-fixed and paraffin-embedded (FFPE) samples from previously described tissue banks at the University of Texas M. D. Anderson Cancer Center (Houston, TX) [12]. The tissue specimens had originally been collected between 1997 and 2005 and classified using the 2004 World Health Organization (WHO) classification system [13]. The TMA comprises NSCLC specimens obtained from patients who underwent surgery at the M.D. Anderson Cancer Center from 1997–2003. Among these patients, only those with available staging information were included in the analysis ($N = 352$). After a histological examination of the NSCLC specimens, the NSCLC TMAs were constructed by obtaining three 1-mm in diameter cores of each tumor at three different sites (periphery, intermediate and central tumor sites). The TMAs were prepared with a manual tissue arrayer (Advanced Tissue Arrayer ATA100, Chemicon International, Temecula, CA).

Immunohistochemistry and Analysis

The expression of IGF-1, IGF-2, pIGF-1R/IR and IGF-1R was determined by immunohistochemical (IHC) analysis. Primary IGF-1R and p-IGF-1R/IR antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). The antibody against IGF-1 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The antibody against IGF-2 was purchased from Upstate/Millipore (Billerica, MA, USA). Details of the IHC protocol were previously described elsewhere [5,14] The protein expression was quantified by two independent observers (C.B and I.I.W). The results of three cores from each patient were averaged. The results for each observer were averaged to obtain the final

IHC score. Cytoplasmic, membranous, and nuclear expression were quantified using a four-value intensity score (0, 1+, 2+, and 3+) and a percentage (0% to 100%) indicating the extent of reactivity. Next, the expression score was obtained by multiplying the intensity and reactivity extension values (range, 0–300). EGFR exons 18 through 21 and the K-Ras mutational hot spot codons 12, 13, and 61 were amplified as described in a previous report from our group [14].

Statistical Analysis

The Wilcoxon rank sum test or Kruskal-Wallis test was used to evaluate the difference in marker expression between/among categorical variable levels. The continuous markers were dichotomized by either negative vs positive on the basis of median. Spearman rank correlation coefficients were calculated to determine the correlations among biomarkers. The OS and RFS durations in each subgroup of patients were then determined using the Kaplan-Meier method and compared using the log-rank test. Cox proportional hazard models were used for the multivariate analysis. All statistical tests were two-sided, and *P* values of less than 0.05 were considered statistically significant.

Results

IGF-1, IGF-2, IGF-1R and pIGF-1R/IR expression in lung cancer specimens

We performed IHC staining of IGF-1 and IGF-2 in tissue specimens obtained from patients with NSCLC. We observed quantifiable IGF-1 and IGF-2 expression mainly in the cytoplasm in most of the NSCLCs (Fig 1). The levels of biomarker expression were not obviously different between tumors of different stages. The expression of IGF-1 was higher in adenocarcinomas (ADCs) than in squamous cell carcinomas (SCCs) and higher in tumors harboring mutant EGFR (mtEGFR) than in tumors with wild type EGFR (wtEGFR) (Fig 2A and 2C, *P* = 3.7 E-4 and 9.8 E-4, respectively). In contrast, the IGF-2 expression was higher in SCCs than in ADCs and higher in tumors with wtEGFR than in tumors with mtEGFR (Fig 2B and 2D, *P* = 6.8 E-4 and 0.019, respectively). K-ras mutation was not significantly correlated with the levels of IGF-1 or IGF-2 expression in the tumors (Fig 2E and 2F). The differential expression pattern of IGF-1 and IGF-2 suggested that IGF-1 and IGF-2 may have a distinct role in NSCLC.

Recurrence-free survival and overall survival

We assessed the prognostic impact of IGF expression in NSCLC. The median follow-up duration of the censored observations was 3.93 years (data cut-off: September 2010). The OS durations in patients with higher IGF-1 expression (equal or greater than median) levels were significantly shorter than those in patients with lower IGF-1 expression levels (median: 8.9 years vs. 5.1 years, *P* = 0.022 [log-rank test]) (Fig 3A), while the RFS durations in these groups were similar (Fig 3B). IGF-1 expression in SCC patients was not associated with a statistically significant difference in OS (Fig 3C) or RFS (Fig. 3D). In contrast, ADC patients with higher IGF-1 expression had shorter OS durations than those with lower IGF-1 expression (Fig 3E), while ADC patients with different IGF-1 expression levels did not show a detectable difference in RFS (Fig 3F). Using the median as a cut-off, the level of cytoplasmic IGF-2 expression did not impact OS (Fig 4A, C, E) or RFS (Fig. 4B, D, F) the

survival of NSCLC patients regardless of the histological subtype. We also analyzed IGF-1R and pIGF-1R/IR expression in the same tissue specimens. We found that neither IGF-1R nor pIGF-1R/IR level had an impact on RFS and OS of these patients in univariate analyses (Table 2). In a multivariate analysis, IGF-1 expression was an independent prognostic marker for overall survival in NSCLC patients (Table 3).

Discussion

Recent evidence has implied that the IGF-1R signaling pathway plays a major role in the development and progression of various human cancers, including NSCLC. The prognostic impacts of IGF-1R, IR, and activated IGF-1R/IR have been evaluated in patients with NSCLC [3,15–17]. However, robust tissue-derived ligand expression data in primary human cancers are extremely limited. Herein, we have provided the first demonstration that: 1) IGF-1 expression was higher in patients with ADC or mtEGFR tumors, whereas IGF-2 expression was higher in patients with SCC or wtEGFR tumors; 2) the tumor expression of IGF-1, but not IGF-2, IGF-1R, or pIGF-1R/IR (an indicator of activated IGF-1R/IR), is an independent prognostic factor in patients with NSCLC. Our data suggest that IGF-1 could be a potential target for future cancer therapies, in which patients with NSCLC should be stratified based on IGF-1 expression.

The IGF-1R pathway is frequently deregulated in a variety of human cancers, including NSCLC, and components of the IGF axis have been variably associated with tumor grade and prognosis [5,18]. Different from ERBB2 and EGFR, there are rare activating mutations and gene amplifications in the IGF-1R physiology. We have demonstrated that the down-regulation of IGF-binding protein 3 occurs frequently in patients with NSCLC [18,19]. We have also provided evidence from a multivariate analysis that tumor expression of IR is an independent prognostic factor in patients with NSCLC [5]. In the same study, patients with high expression levels of both IR and IGF-1R had shorter RFS and OS compared to patients with low levels of IR and/or IGF-1R expression. These findings suggest that the expression of receptors and ligand-binding proteins of the IGF-1R/IR pathway have a prognostic impact in patients with NSCLC. However, the role of IGFs in lung cancer has been controversial in prior epidemiological studies [7,20,21]. Serum levels of IGF-1 and IGF-2 were higher in lung cancer patients than in normal subjects [9], and high levels of circulating IGF-1 have been associated with an increased risk of lung cancer. However, existing data regarding the significance of serum IGF levels in terms of clinical outcome are inconclusive. This ambiguity could be due to the complexity of IGF physiology at least in part: Activity of IGF-2 can be influenced by the level of IGF-2R. Furthermore, there are at least 7 IGF binding proteins in human, which provide an additional level of control of IGF biological activity. Also, abnormal autocrine or paracrine expression of ligands, particularly IGF-2, is common in many malignancies [3]. We believe serum or plasma IGF-2 levels are not good markers for this auto- or paracrine expression of IGFs.

Prior studies suggest that serum IGF level serves as a prognostic marker for survival of patients with NSCLC and a predictive marker for response to cancer therapy. The serum level of IGF-1 has been proposed to serve as a predictive biomarker for the outcome of anti-IGF-1R mAb treatment. A variety of IGF-1R-targeting anticancer drugs, mainly anti-

IGF-1R mAb and small molecular tyrosine kinase inhibitors (TKIs), are in preclinical or clinical stages of development. IGF-1R overexpression and high gene copy number were proposed to predict patient response to anti-IGF-1R Ab. From a phase II study of figitumumab in patients with advanced NSCLC [22], a follow-up biomarker study suggested that higher levels of free serum IGF-1 were partially associated with poor PFS [23]. Unfortunately, the original trial and the biomarker analysis have been withdrawn because the response rate and PFS were reported incorrectly. Furthermore, two phase III trials of figitumumab (CP-751,871), the anti-IGF-1R mAb, were discontinued in 2010 because neither trial seemed to meet its primary endpoint in unselected patients. The disappointing clinical data for figitumumab suggest that further clinical studies may benefit from the use of appropriate predictive biomarkers to identify probable responders and the use of rational combination therapies.

The IGF axis has also been implicated in anticancer drug resistance, including EGFR-targeting agents, and IGF-1R-targeting treatments have been proposed to overcome resistance to treatment with EGFR-TKIs. A positive serum IGF-1 level is predictive of shorter overall survival [24], and a negative response to gefitinib therapy in NSCLC patients, independent of EGFR mutational status [24]. However, a previous study with 61 patients samples showed that patients with low serum levels of IGF-1 (<20%) did not seem to benefit from EGFR-TKIs [25]. Furthermore, in a phase I/II study of erlotinib and cixutumumab in patients with NSCLC, a trend toward better PFS with higher free baseline IGF-1 levels was observed [26]. In a previous study, the plasma IGF-1 level also failed to retain its importance for overall survival in the multivariate analysis. These results may suggest that locally produced IGFs play a major role in biological function during lung cancer progression.

In the current study, we focused on the prognostic impact of tumor expression of IGFs on survival of lung cancer patients. IGF-1 and IGF-2 produce very similar biological responses; however, their pattern of expression could be significantly different. In mammals, We noted: 1) tumoral IGF-1 level was not significantly associated with IGF-2; and 2) higher tumor expression of IGF-1 in patients with ADC or those harboring mtEGFR than in those with SCC or those with wtEGFR, while the expression of IGF-2 showed the opposite pattern. The significant correlation between tumor IGF-1 expression and histologic grouping (i.e., ADC) is consistent with a previous report that showed significantly lower levels of baseline plasma IGF-1 levels in patients with SCC than in patients with ADC (23.4% vs 76.6%, $P=0.0019$) [27]. The correlation between IGF expression patterns with EGFR mutational status could be informative for future studies with specific ligand antibodies, even though these observations need to be validated in independent studies. As might be predicted from the pattern of expression, the biological function of these two ligands could be different. Indeed, tissue-derived IGF-1, but not IGF-2, served as an independent predictive marker for survival of NSCLC in our study. We also noted that IGF-1R expression was not associated with EGFR parameters or clinical outcomes, consistent with a previous report [28].

We have observed that the IGF-1 expression was predictive of OS, not RFS in the present study. This observation could be just associated with the impact of IGF-1 on patients' survival. But, whether the level of IGF-1 alter longevity has yet to be established in human,

even though that is an acceptable marker for ageing and longevity in mouse [29]. Also, we do not have both serum and tumoral IGF-1 should be utilized to address this issue. Moreover, the definition of recurrence is not always clear in clinical practice and research. We might not be able to confirm the recurrence by biopsy if the suspicious lesion is very small or located in an unapproachable area. Meanwhile, the definition of overall survival is usually very clear and not affected by clinical judgments. Another weak point of our study is that the impact of IGF-1 might not be interpolated in stage IV lung cancer patients, who are mostly candidates for systemic treatment. Because we built the TMAs with the resected lung tissues, we only have 3.4% of those patients in the present study. Even though the expression of IGFs was not different among the clinical stages in the present study, it would be great if we could examine the expression in stage IV lung cancer patients.

In conclusion, we provide the first evidence that supports the importance of tumor expression of IGF-1, but not IGF-2, as a prognostic biomarker in NSCLC. We also show that the tumoral expression of IGF-1 is distinctly correlated with EGFR mutational status. These findings suggest that IGF-1 and IGF-2 exert different biological function through distinct pathways in addition to the IGF-1R/IR pathway, as suggested in a previous study [30]. The clinical trials with IGF-1R antibodies have been disappointing, and newer strategies targeting IGF-mediated pathways, including ligand-neutralizing antibodies [31], are still worth investigating. We believe that the present study will help guide the patient selection in future trials of molecular targeting agents blocking the IGF signaling pathway. Future biomarker studies of serum and tissue specimens for analyzing circulating and tumor levels of IGF-1 and IGF-2 expressions are warranted to assess the role of locally produced IGFs in lung cancer progression.

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Figure 1. Representative photographs of the IHC staining of IGF-1 and IGF-2 in lung tumor samples from patients with primary NSCLCs
Expression of IGF-1 (A, C) and IGF-2 (B, D) in adenocarcinoma (ADC)(A, B) and squamous cell carcinoma (SCC) (C, D).

Figure 2. Comparison of IGF-1 and IGF-2 expression according to histology and mutational status

The level of IGF-1 expression was significantly higher in patients with ADC (A) or mtEGFR (C) than in patients with SCC or wtEGFR. In contrast, the level of IGF-2 expression was significantly higher in patients with SCC (B) or wtEGFR (D) than in patients with SCC or with wtEGFR. The levels of IGF-1 and IGF-2 expression were not associated with K-Ras mutational status (E and F).

Figure 3. Kaplan-Meier OS and RFS curves for all patients, SCC, and ADC patients according to IGF-1 expression

Kaplan-Meier curves depicting recurrence-free survival and overall survival according to the expression levels of IGF-1 in all patients (A and B), squamous cell carcinoma patients (C and D) and adenocarcinoma patients (E and F). The patient groups were compared using the log-rank test.

Figure 4. Kaplan-Meier RFS curves for all patients, SCC, and patients according to IGF-2 expression

Kaplan-Meier curves depicting recurrence-free survival and overall survival according to the expression levels of IGF-2 in all patients (A and B), squamous cell carcinoma patients (C and D) and adenocarcinoma patients (E and F). The patient groups were compared using the log-rank test.

Table 1

Summary of clinicopathologic characteristics of patients and tumors

Feature	NSCLC histologic type*		
	Adenocarcinoma (n = 231)	Squamous carcinoma (n=121)	Total (n=352)
Mean age (range), y	65 (34–87)	69 (42–90)	66 (34–90)
Sex			
Male	88	72	189
Female	142	47	160
Smoking status [†]			
Never	57	6	63
Former	105	69	174
Current	70	44	114
Race			
Caucasian	203	110	313
Others	28	11	39
EGFR mutation			
Positive	38	0	38
Negative	159	48	207
K-ras mutation			
Positive	32	0	32
Negative	162	46	208
T category			
1	100	30	130
2	112	73	185
3	6	12	18
4	14	6	20
N category			
0	172	82	254
1	28	32	60
2	32	7	39
M category			
0	223	119	342
1	9	2	11
TNM stage			
I	160	66	226
II	28	37	65
III	36	14	50
IV	10	2	12

* Values are number of cases unless otherwise indicated

[†] Smoking status was not available in 2 patients with SCC

Table 2

Univariate analyses of clinical prognostic factors and biomarkers

Factors	Category	Disease free survival		Overall survival	
		RR of relapse and 95% CI	P-value	RR of dying and 95% CI	P-value
Histology	Adenocarcinoma	1		1	
	Squamous	.946 (.626–1.430)	.792	1.442 (1.068–1.949)	.017
Gender	Male	1		1	
	Female	.813 (.549–1.203)	.300	1.324 (0.981–1.786)	.066
Age	<60	1		1	
	60 – 69	1.377 (.838–2.262)	.206	2.156 (1.353–3.434)	.0012
	70	.971 (.584–1.617)	.911	2.974 (1.904–4.645)	1.7 E-6
Smoking status	Never	1		1	
	Ever	.669 (.418–1.072)	.095	1.136 (.754–1.712)	.542
Race	White	1		1	
	Others	2.123 (1.261–3.574)	.005	1.043 (.640–1.699)	.867
Stage	I or II	1		1	
	III or IV	3.973 (2.623–6.018)	7.4 E-11	1.936 (1.357–2.764)	2.7 E-4
IGF-1R	<20	1		1	
	20	1.300 (0.861–1.963)	.212	1.201 (0.877 – 1.644)	.253
pIGF-1R	<0	1		1	
	0	1.440 (0.858 – 2.418)	.167	0.897 (0.622 – 1.295)	.563
IGF-1	<70	1		1	
	70	1.150 (.774–1.707)	.489	1.455 (1.053–2.012)	.023
IGF-2	<100	1		1	
	100	.930 (.615–1.4006)	.732	.992 (.715–1.377)	.964

Abbreviations: RR = Relative risk, CI = Confidence interval

Note: If the relative risk is greater than 1, the relative risk can be thought as the average increased risk of relapse or dying compared to the reference group. The group with the ratio equal to 1 is the reference group. Univariate p-values are based on Cox Proportional Hazard Model.

Table 3

Multivariate analyses of clinical prognostic factors and biomarkers

Factors	Category	Disease free survival			Overall survival		
		RR of relapse and 95% CI	P-value		RR of dying and 95% CI	P-value	
Histology	ADC vs. SCC	1.071 (0.685 – 1.673)	0.764		1.361 (0.961 – 1.928)	0.083	
Sex	Male vs. Female	0.737 (0.487 – 1.115)	0.148		1.239 (0.891 – 1.725)	0.203	
Age		1.001 (0.982 – 1.020)	0.929		1.043 (1.025 – 1.060)	8.7 E-7	
Stage	I/II vs. III/IV	4.349 (2.826 – 6.694)	2.3 E-11		7.435 (3.238 – 17.072)	2.1 E-5	
IGF1	< 70 vs. ≥ 70	1.137 (0.755 – 1.713)	0.538		1.607 (1.144 – 2.257)	0.006	