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Expression and clinical significance of insulin-like growth factor 1 in lung cancer tissues and perioperative circulation from patients with non-small-cell lung cancer

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ABSTRACT

Objective We explored the role of insulin-like growth factor 1 (IGF-1) in the development of lung cancer.

Methods We used immunohistochemistry to measure the expression of IGF-1 and IGF-1 receptor (IGF-1R) in specimens of tissue and perioperative circulation from 80 patients with primary non-small-cell lung cancer (NSCLC) and from 45 patients with benign pulmonary lesions (BPLs). Correlations of those measurements with clinicopathologic characteristics and clinical follow-up were analyzed. Circulating IGF-1 was measured before and after surgery in all patients.

Results Compared with BPL specimens, NSCLC specimens showed overexpression of IGF-1and IGF-1R ($p < 0.001$). The expression levels of IGF-1 and IGF-1R were significantly associated with advanced-stage disease ($p = 0.034$ and 0.029 respectively) and lymph node metastasis ($p = 0.012$ and 0.017 respectively), and expression of IGF-1 correlated with tumour differentiation and tumour diameter ($p = 0.011$ and 0.021 respectively). Specimens positive for IGF-1 or IGF-1R were significantly correlated with shorter patient survival ($p = 0.0012$ and 0.0016 respectively). After surgery, circulating IGF-1 was significantly elevated in patients with BPL ($p = 0.0346$) and significantly lower in patients with NSCLC ($p = 0.0030$), especially in those with advanced-stage disease, a larger tumour size, regional lymphoid node metastasis, or lesser differentiation ($p = 0.0092$, 0.0051, 0.0131, and $p < 0.001$ respectively).

Conclusions In NSCLC, IGF-1 and IGF-1R are upregulated, and expression of those factors is correlated with tumour progression and prognosis in NSCLC patients. Radical resection of NSCLC can directly influence the serum concentration of IGF-1. Autocrine/paracrine IGF-1 might be playing an important role in the development of lung cancer.

Key Words IGF-1, IGF-1R, non-small-cell lung cancer, immunohistochemistry, ELISA

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INTRODUCTION

The growth hormone insulin-like growth factor 1 (IGF-1) is produced primarily by the liver as an endocrine hormone; it is also produced in target tissues in a paracrine/autocrine fashion[1]. Its primary action is mediated by binding to its receptor, IGF-1 receptor (IGF-1R), which is present in many cell types in various tissues. Several studies have indicated that the IGF system is involved in the pathogenesis and progression of various malignancies[2,3]. In several studies, including earlier work by our group, the IGF-1 signalling pathway was shown to play a critical role in the development of lung cancer[2–7]. Targeting IGF-1 might help to achieve a favourable outcome in lung cancer patients, but few studies have looked at the expression of IGF-1 in serum and tissue in non-small-cell lung cancer (NSCLC) and at the significant role it might play in that disease.

In a previous study, we found that circulating serum IGF-1 was significantly higher in NSCLC patients whose tumours were larger in size, indicating that IGF-1 might exerts

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its effects by the paracrine/autocrine route[8]. In accord with our previous research, immunologic and molecular analyses showed that IGF-1 is produced in human lung parenchyma and lung cancer cell lines[9–12] and that IGF-1 is an autocrine regulator for the brain-metastatic variants of human NSCLC and small-cell lung cancer cell lines[10,11]. The significance of circulating IGF-1 in the development of NSCLC is not clear, and few studies have assessed the circulating level of IGF-1 in patients undergoing surgical treatment for an existing lung cancer[13].

We considered the perioperative alteration of circulating IGF-1 from a new point of view; and to determine whether excision induces a change in serum IGF-1 concentration, we decided to measure circulating levels of IGF-1 before and after surgical removal of primary lung cancer. We also evaluated whether any change in circulating IGF-1 correlates with known tumour characteristics such as size or clinicopathologic parameters. The expression of IGF-1 or IGF-1R in NSCLC specimens was also explored, together with the significance of that expression with respect to clinicopathologic parameters and prognosis.

METHODS

Selection of NSCLC and Control Cases

For this study, we recruited 80 patients with pathologically proven primary NSCLC who underwent a surgical intervention during the period from April 2005 to October 2007 at the Department of Thoracic Surgery of the Affiliated Tongji Hospital of Huazhong University of Science and Technology, Tongji Medical College. None of the patients had received prior chemotherapy or radiotherapy. Tumour stage was classified according to the TNM classification of the International Union Against Cancer[14]. Table I shows the patient characteristics.

As a control group, we recruited 45 patients who received surgical treatment for benign pulmonary lesions (BPLS). The control group included 23 men 52–70 years of age (mean: 59.8 ± 7.9 years) and 22 women 53–66 years of age (mean: 58.2 ± 10.2 years), including 21 with pulmonary tuberculosis, 12 with bronchiectasis, 7 with pulmonary sequestration, and 5 with inflammatory pseudotumour. Exclusion criteria considered were endocrine disorders, major hepatic and renal diseases, or a report of significant weight loss in the preceding 6 months.

Every patient had a performance status exceeding 80%, and before surgery, every patient underwent blood sampling, electrocardiography, chest radiography, abdominal ultrasonography, and computed tomography. All patients had normal hepatic and renal function and no abnormality of the endocrine system. In addition, they had not received steroids in the past. Each patient received radical surgery, intended to achieve a cure.

Ethics approval for our investigation was obtained from the Research Ethics Committee, Tongji Medical College (no. 20111201).

Sample Collection

For tissue microarray preparation (Shanghai Outdo Biotech, Shanghai, P.R.C.), samples were fixed in 10% formaldehyde, embedded in paraffin, and sectioned; sections were diagnosed and confirmed by at least 2 lung cancer pathologists. Preoperative serum samples were collected before surgery, typically on the morning of the day of the surgery, after an overnight fast. Postoperative plasma samples were collected on the 7th day after surgery. Blood was collected into Vacutainer CPT 8 mL tubes (Becton Dickinson, Franklin Lakes, NJ, U.S.A.) containing 0.1 mL of 1 mol/L sodium citrate anticoagulant and was centrifuged at room temperature for 20 minutes at 1500*g*. The top layer (corresponding to plasma) was decanted using sterile transfer pipettes and immediately frozen and stored at –80°C in polypropylene cryopreservation vials (Nalge Nunc International, Rochester, NY, U.S.A.).

Materials

Insulin-like growth factor 1 [500 ng/mL IGF-1 prepared in phosphate-buffered saline (PBS)] was obtained from Invitrogen (Carlsbad, CA, U.S.A.); goat anti-human IGF-1 polyclonal antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.); and rabbit anti-human IGF-1R polyclonal antibody was obtained from Invitrogen. Protein lysis buffer (Beyotime Biotechnology, Jiangsu, P.R.C.), fetal calf serum (HyClone Laboratories, Logan, UT, U.S.A.), RPMI-1640 medium (Gibco BRL, Grand Island, NY, U.S.A.), and a 3,3′-diaminobenzidine substrate kit (Wuhan Life Technologies, Wuhan, P.R.C.) were also obtained for the present study.

Immunohistochemistry

Sections were deparaffinized, hydrated, and immersed in 0.01 mol/L citrate buffer (pH 6.0). Antigen retrieval was performed by boiling the tissue sections for 5 minutes in a high-pressure cooker. The sections were further treated with 3% hydrogen peroxide for 10 minutes to inactivate any endogenous peroxidase activity and blocked in 5% bovine serum albumin for 20 minutes. The sections were subsequently incubated overnight at 4°C with primary antibodies against IGF-1 and IGF-1R (1:200). Control incubations were performed by omitting the primary antibody and by pre-absorbing the antibody using a quintuple molar excess of the immunizing peptide or the respective recombinant protein. After a wash in PBS, the sections were incubated with the secondary antibody at room temperature for 20 minutes. After another wash in PBS, the sections were treated with streptavidin–biotin complex for 20 minutes at room temperature. After a third wash in PBS, the nuclei were visualized with 3,3′-diaminobenzidine and then counterstained with hematoxylin, before being dehydrated, transparentized, and mounted. Standardization of the incubation conditions allowed for an accurate comparison of expression levels in all cases. In addition, for each parameter, staining was repeated for at least 20 different sections to confirm the initial results.

Immunohistochemistry Evaluation

Sections were observed and evaluated pathologically in a double-blind manner under light microscopy. Staining for IGF-1 and IGF-1R was evaluated on light microscopy and on digitally scanned images by 2 observers. The observers had minor disagreements in about 8% of the cases. Those

TABLE I Relationship between the perioperative expression of serum insulin-like growth factor 1 (IGF-1) and clinical pathologic characteristics of non-small-cell lung cancer (NSCLC)

Characteristic	Pts (*n*)	IGF-1 (ng/mL)		*p* Value[a]
		Preoperative	At 7 days after surgery	
Sex				
Men	43	23.09±6.54	19.25±5.76	0.0660
Women	37	20.33±4.56	15.99±7.70	0.0568
Age group				
<55 Years	26	23.29±5.68	18.62±5.44	0.0546
≥55 Years	54	19.76±6.95	15.33±8.43	0.0894
Smoking index				
<400	23	24.34±4.21	14.21±5.40	0.0773
≥400	57	20.93±6,41	19.26±6.80	0.0634
Benign pulmonary lesions	45	12.37±4.51	18.60±6.53	
Histologic NSCLC type				
Squamous cell carcinoma	46	24.80±7.80	19.9±11.72	0.0760
Adenocarcinoma	34	20.65±6.30	17.1±11.96	0.0890
Lymph node metastasis				
Yes	53	25.90±7.58	16.57±7.11	**0.0131**
No	27	16.59±5.73	18.92±7.74	0.0877
TNM staging				
I and II	34	17.61±6.70	17.26±6.77	0.9576
III and IV	46	23.69±8.83	18.75±7.09	**0.0092**
Differentiation				
Moderate to well	33	20.78±7.49	19.50±6.37	0.0769
Poorly	47	22.28±5.75	13.85±6.58	**<0.0001**
Tumour diameter				
<3 cm	26	12.08±4.22	20.44±7.53	0.1477
≥3 cm	54	25.45±8.02	14.87±7.59	**0.0051**

[a] Significant values appear in boldface type.

samples were re-evaluated by both observers to reach a consensus. Cells positive for IGF-1 or IGF-1R appeared yellow or yellowish brown in the nucleus or cytoplasm, or contained yellowish brown granules.

In the pathology examination, 5 randomly selected fields were examined at 400× and 200× magnification as previously described[15]. In each field, 200 cancer cells were counted (10,000 cells total), and the proportion of positive cells was calculated. Semi-quantitation was performed as previously reported[16]. In brief, quantitation was based on the staining intensity and the proportion of positive cells. Cell positivity was scored on a scale of 1–4, indicating 20% or fewer positive cells, 21%–50% positive cells, 51%–75% positive cells, or more than 75% positive cells respectively. Staining intensity was also scored on a scale of 1–4, meaning negative, weakly positive, moderately positive, or strongly positive respectively. The measurements from staining intensity and proportion of positive cells were multiplied to yield a score ranging from 1 to 16, which was interpreted as follows: 4 or less, negative; 5–8, mildly positive (+); 9–12, moderately positive (++); and 13–16, strongly positive (+++).

ELISA

Total plasma concentration of IGF-1 was assayed by ELISA, using reagents from Diagnostic Systems Laboratories (Webster, TX, U.S.A.). The IGF-1 assay had a sensitivity of 3.9 ng/mL, with no cross-reactivity to insulin and growth hormone (GH) and only 0.7% cross-reactivity to IGF-2. Every sample was run in duplicate, with the mean value being used in the analysis. If the difference between duplicate results for the same sample was more than 5%, the assay was repeated.

Statistical Analysis

The statistical analysis was performed using the SPSS software application (version 15.0: SPSS, Chicago, IL, U.S.A.). Differences in the tissue expression of IGF-1 and IGF-1R in NSCLC and BPL were examined using the chi-square test. Two-sample comparisons of mean serum IGF-1 were analyzed by t-test. Correlation analyses of the expression levels of IGF-1 and IGF-1R in lung cancer were performed. The mean method was used to compare the means of measured data between the various groups. Eta and eta-squared were used to measure the correlations of preoperative serum IGF-1

with IGF-1 expression intensity in lung tissues from patients with NSCLC. Survival durations were calculated using the Kaplan–Meier method and were analyzed by log-rank test to compare cumulative survival duration in the patient groups. The Cox proportional hazards model was used for the univariate and multivariate analyses. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Expression of IGF-1 and IGF-1R in NSCLC and BPL

In NSCLC, IGF-1–positive granules were yellow or yellowish brown and found predominantly in cytoplasm and on the cell membrane. The membrane was strongly positive for IGF-1 [Figure 1(A)]. In BPL, cells were mildly-to-moderately positive for IGF-1. In the NSCLC specimens, 68.75% of cells were positive for IGF-1—a proportion that was significantly higher than the 17.78% seen in BPL specimens ($p < 0.001$, Table II). In NSCLC, IGF-1R–positive granules were yellowish and found mainly in cytoplasm or on the cell membrane. The cell membrane was strongly positive for IGF-1R [Figure 1(B)]. In BPL specimens, only a few cells (<20%) were mildly-to-moderately positive for IGF-1R. In addition, 82.50% of NSCLC specimens were positive for IGF-1R—a proportion that was significantly higher than the 22.22% seen in BPL specimens ($p < 0.001$, Table II).

Expression of IGF-1 and IGF-1R, and Clinicopathologic Characteristics of NSCLC

The intensity of IGF-1 expression was significantly associated with tumour stage ($p = 0.034$), lymph node metastasis ($p = 0.012$), differentiation ($p = 0.011$), and tumour diameter ($p = 0.021$); it was not associated with sex, age, smoking index, or histologic type (Table III). Expression of IGF-1R was markedly higher in samples from patients with advanced-stage disease and lymph node metastasis than in samples from patients with early NSCLC or disease with no lymph node metastasis ($p = 0.029$ and 0.017 respectively), but it had no significant association with sex, age, smoking index, histologic type, differentiation, or tumour size (Table III).

Expression of IGF-1 and IGF-1R in NSCLC Tissue and Survival Rates

Kaplan–Meier survival analyses showed significantly poorer overall survival in the IGF-1–positive group than in the IGF-1–negative group [$p = 0.0012$, Figure 2(A)]. Moreover, prognosis was significantly poorer in patients who were IGF-1R–positive than in those who were IGF-1R–negative [$p = 0.0016$, Figure 2(B)]. On univariate analysis, 3 factors—IGF-1R overexpression, pathologic stage, and lymph node metastasis—were significantly associated with worse overall survival. Multivariate survival analysis indicated that pathologic stage and lymph node metastasis tended to be independent prognostic indicators. Positivity for IGF-1 and IGF-1R were not independent predictors of poor prognosis.

Change in Serum IGF-1 After Surgery in NSCLC and BPL

Figure 3 shows mean levels of IGF-1 and albumin before surgery and at 7 days after surgery in the groups with benign and malignant disease. Compared with the preoperative level of serum IGF-1 (21.59 ± 9.04 ng/mL), postoperative levels in the primary NSCLC group were significantly lower (16.80 ± 5.66 ng/mL, $p = 0.0030$). However, in the BPL group, serum IGF-1 was significantly higher postoperatively (18.60 ± 6.53 ng/mL) than preoperatively (12.37 ± 4.51 ng/mL, $p = 0.0346$). Figure 3 contrasts the mean percentage preoperative–postoperative differences in serum IGF-1 between the groups with benign and malignant disease. Serum IGF-1 fell by about 20.80% at the 7th day after surgery in the NSCLC patients; it rose in patients who underwent surgical removal of benign lung lesions.

TABLE II Expression of insulin-like growth factor 1 (IGF-1) and its receptor (IGF-1R) in patients with non-small-cell lung cancer (NSCLC) and benign pulmonary lesions (BPLs)

Expression level	IGF-1		IGF-1R	
	NSCLC	BPL	NSCLC	BPL
Negative	25	37	14	35
Weakly positive (+)	3	5	17	8
Moderately positive (++)	19	3	36	2
Strongly positive (+++)	33	0	13	0
Positivity rate (%)	68.75	17.78	82.50	22.22
χ^2	32.600		53.127	
p Value	<0.001		<0.001	

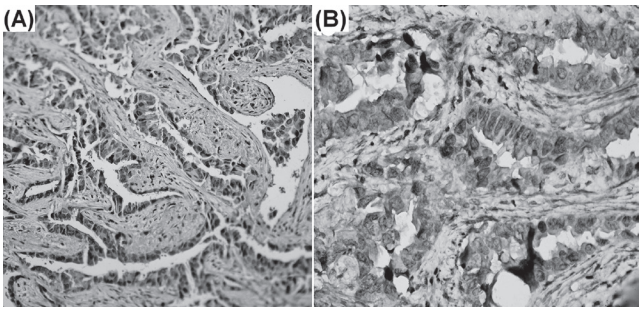

FIGURE 1 Expression of insulin-like growth factor 1 (IGF-1) and IGF-1 receptor (IGF-1R) in a surgical specimen of non-small-cell lung cancer. Positive cells appear yellowish brown or have yellowish brown granules. (A) IGF-1 is distributed mainly in the cytoplasm and on the cell membrane, and the membrane is strongly positive for IGF-1 (200× original magnification). (B) IGF-1R expression is seen in cytoplasm and the cell membrane, and the cell membrane is strongly positive for IGF-1R (400× original magnification).

Serum IGF-1 Changes and Clinicopathologic Parameters in NSCLC

In Table I, no differences in the extent of the change in serum IGF-1 are evident according to clinicopathologic parameters such as tumour grade, sex, age, smoking index, and pathologic type. However, serum IGF-1 declined significantly in patients with a tumour size of 3 cm or larger [T2 ($p = 0.0051$)], with lymph node metastasis ($p = 0.0131$), with advanced disease [stages III–IV ($p = 0.0092$)], and with low tumour differentiation ($p < 0.001$).

TABLE III Correlation of the expression of insulin-like growth factor 1 (IGF-1) and its receptor (IGF-1R) with clinicopathologic characteristics of non-small-cell lung cancer

Characteristic		IGF-1		*p* Value[a]	IGF-1R		*p* Value[a]
		Negative	Positive		Negative	Positive	
Sex	Men	11	32	0.486	8	35	0.308
	Women	8	29		12	25	
Age	<55 Years	12	14	0.247	10	16	0.222
	≥55 Years	20	34		21	33	
Smoking index	<400	10	13	0.605	8	15	0.315
	≥400	25	32		26	31	
Histologic type	Squamous cell carcinoma	26	20	0.520	27	19	0.289
	Adenocarcinoma	11	23		10	24	
Lymph node metastasis	N0	16	9	**0.012**	8	15	**0.017**
	N1–3	15	42		16	41	
TNM stage	IA–IIB	10	24	**0.034**	8	26	**0.029**
	IIIA–IV	17	29		12	34	
Differentiation	Moderate to well	20	13	**0.011**	11	22	0.652
	Poor	20	27		19	28	
Tumour diameter	<3 cm	12	14	**0.021**	15	11	0.142
	≥3 cm	14	40		21	33	

[a] Significant values appear in boldface type.

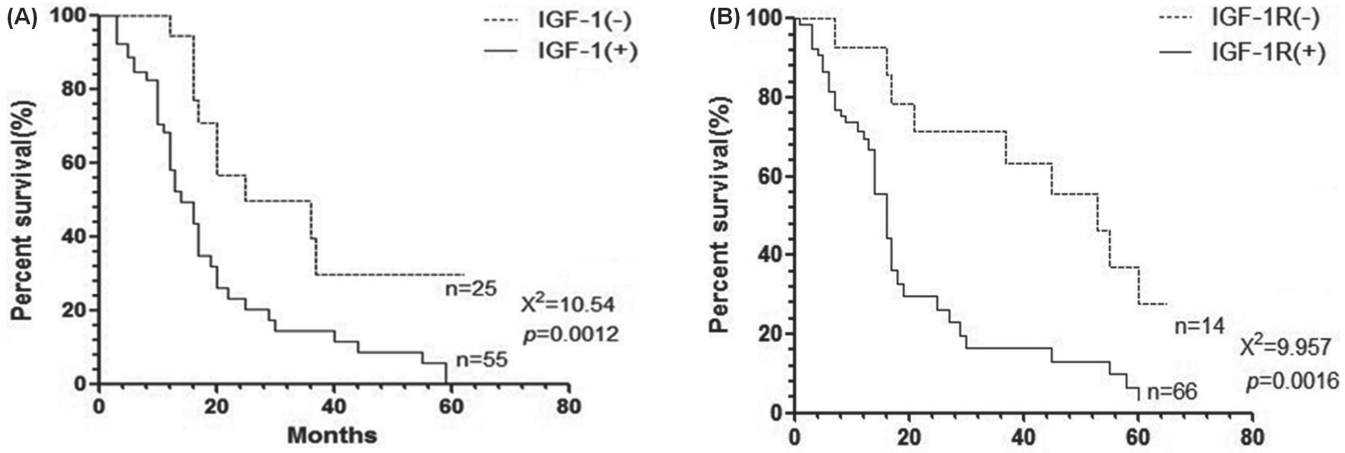

FIGURE 2 Association of the expression, in tissue and serum from patients with non-small-cell lung cancer, of insulin-like growth factor 1 (IGF-1) and its receptor (IGF-1R) with survival. Kaplan–Meier survival curves for patients (A) based on IGF-1 positivity (+) or negativity (–), and (B) based on IGF-1R positivity (+) or negativity (–).

Preoperative Serum IGF-1 and Intensity of IGF-1 Expression in NSCLC Specimens

Serum IGF-1 concentrations were significantly different ($p < 0.05$) and positively correlated (eta = 0.254) at various levels of IGF-1 expression intensity in tissue specimens from NSCLC patients.

DISCUSSION

The polypeptide IGF-1 (76 amino acids) is a mitogenic and antiapoptotic factor that regulates cell growth. Insulin-like growth factor 1 is produced not only in the liver, but also in numerous non-hepatic tissues. Earlier studies showed that locally produced IGF-1 mediates growth in a autocrine/paracrine manner. Ohlsson *et al.*[17] suggested a revision in the classical somatomedin hypothesis and proposed the "dual somatomedin hypothesis" in which autocrine/paracrine IGF-1 is the main determinant of postnatal body growth, and liver-derived endocrine-acting IGF-1 supplies negative feedback to GH secretion and possibly exerts other effects on carbohydrate and lipid metabolism.

Paracrine/autocrine IGF-1 promotes the growth of many cancers, such as acute myeloid leukemia[18], chronic myelogenous leukemia[19], neuroendocrine tumour[20], breast

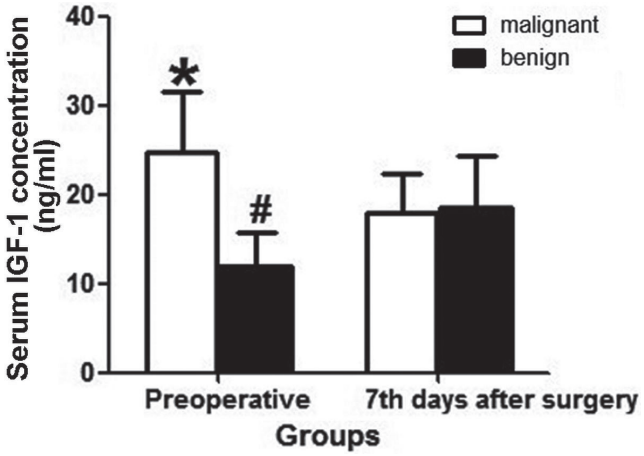

FIGURE 3 Mean serum insulin-like growth factor 1 (IGF-1) before surgery and at day 7 after surgery in patients with benign pulmonary lesions (BPLs) or with non-small-cell lung cancer (NSCLC). Compared with preoperative circulating serum IGF-1, postoperative circulating serum IGF-1 was significantly lower in patients with primary NSCLC (21.59 ± 9.04 ng/mL vs. 16.80 ± 5.66 ng/mL, * $p = 0.0030$), but significantly higher in patients with BPLs (12.37 ± 4.51 ng/mL vs. 18.60 ± 6.53 ng/mL, # $p = 0.0346$).

cancer[21], and cervical cancer[22]. Pulmonary neuroendocrine tumours such as carcinoids can produce ectopic GH in sufficient quantities to manifest as acromegaly, potentially contributing to local and distant IGF activation loops[23]. An earlier study by our group found that circulating serum IGF-1 was significantly higher in patients with larger NSCLC tumours, demonstrating that IGF-1 can exerts its effects by the paracrine/autocrine route[8]. In accord with our earlier studies, immunologic and molecular analyses have shown that IGF-1 is produced in human lung parenchyma and lung cancer cell lines[9–12].

In the present study, we observed serum IGF-1 concentrations that were significantly higher in NSCLC patients than in control patients with BPLs. However, serum IGF-1 declined by about 20.80% on the 7th day after surgery in the NSCLC patients, and it rose after the removal of BPLs. During the 7 days after surgery, serum IGF-1 differed significantly between the groups with benign and malignant disease. The increase in serum IGF-1 after the removal of BPLs suggests that IGF-1 might be involved in the process of tissue healing. McAnulty *et al.*[24] found that unilateral pneumonectomy in rats caused compensatory growth of the remaining lung, which was greatly increased between days 2 and 6 post-pneumonectomy, but which returned to normal by day 14. Serum IGF-1 increased by about 100% 2 days after pneumonectomy, which demonstrated that the IGF-1 messenger RNA synthesized post-injury correlated with the autocrine/paracrine action of IGF-1. Earlier studies by different groups showed that other components of the IGF-1 axis are induced shortly after an injury, thus resulting in an increased ability of the tissue to respond to IGF-1[25–30]. Our results align with those studies in suggesting that IGF-1 plays a key role in the protective response to trauma.

The decline in serum IGF-1 after surgery in NSCLC patients was similar to the variation seen in gastric cancer patients after surgery[31,32]. The reason for the decline in serum IGF-1 after surgery in patients with NSCLC remains uncertain. It could potentially be the result of a metabolic response to the stress caused by surgery. However, circulating IGF-1 rose after surgery in patients with BPLs, which could not be explained by a metabolic response to the surgery. The decline in circulating IGF-1 after surgery in patients with NSCLC therefore appears to relate directly to tumour removal. The possibility that the tumour itself might contribute to IGF-1 production is supported by the observation that the decline in IGF-1 was greater after the removal of larger tumours (3 cm or more in diameter) than after the removal of smaller lesions (less than 3 cm in diameter) and that serum IGF-1 was positively correlated with the intensity of IGF-1 expression in lung cancer tissue. The significance of the latter finding remains uncertain. It might be the result of an influence by the lung cancer on production of IGF-1 in lung tissues, or it might indicate a direct release of IGF-1 by NSCLC tissue as previously shown during *in vitro* studies.

Pulmonary macrophages, lymphocytes, mononuclear phagocytes, epithelial cells of the respiratory tract, and vascular endothelial cells can secrete IGF-1[9–12]. Tumour tissue or stroma (or both) might specifically contribute to the circulating pool of IGF-1, or NSCLC might influence systemic production of IGF-1 from other tissues, such as liver. The present study showed that circulating serum IGF-1 declined after surgery in patients with advanced-stage disease (III or IV) or with lymph node metastasis. In NSCLC of advanced stage or with lymph node metastasis, secretion of IGF-1 might be increased, a hypothesis that requires further investigation.

Other studies indicate that autocrine/paracrine IGF-1 might be more important than endocrine IGF-1 in the development of NSCLC. Hohla *et al.*[33] investigated the effect of antagonists of growth hormone–releasing hormone (MZ-J-7-138 and JV-1-92) on H460 human NSCLC orthotopically xenografted into nude mice. Those antagonists inhibited the orthotopic growth of H460 NSCLC by 52%–65% and reduced the tumour volume by 30%–36%. However, they did not affect circulating IGF-1. Those findings suggest that the antiproliferative effects of growth hormone–releasing hormone antagonists in H460 NSCLC are associated with autocrine/paracrine IGF-1, but not with endocrine IGF-1. Autocrine/paracrine IGF-1 plays an important role in the development of NSCLC and can be regulated by GH[34,35]. That role might be the reason that antagonists of growth hormone–releasing hormone inhibit the growth of NSCLC.

In the present study, we correlated the immunohistochemical expression of IGF-1 and IGF-1R with clinicopathologic parameters and overall survival in 80 patients with NSCLC and 45 with BPLs. The expression of both IGF-1 and IGF-1R was significantly higher in NSCLC than in BPL specimens. Moreover, the intensity of the IGF-1 and IGF-1R expression was significantly associated with stage III and IV disease and with lymph node metastasis. That finding suggests that the IGF-1 signalling system might be correlated with prognosis in NSCLC. Univariate analysis revealed that IGF-1 or IGF-1R positivity was correlated with significantly shorter patient survival, suggesting that the IGF-1 signalling system might be correlated with NSCLC

tumour aggressiveness, as has previously been reported[36]. Predicting prognosis for patients with operable NSCLC is important in choosing adjuvant therapy, especially for patients with advanced-stage tumours, because the local recurrence rate in NSCLC is high, even in patients receiving curative R0 operations.

One limitation of our study is that the analysis was based only on tissue and serum samples. Neither cell lines nor animal models were included to prove the function and prognostic significance of IGF-1 and IGF-1R. Further studies to provide more mechanistic data about possible interactions between IGF-1 in tissue and IGF-1 in serum are warranted. Nevertheless, our data suggest that evaluation of IGF-1 and IGF-1R expression could be a useful prognostic factor in NSCLC.

CONCLUSIONS

In NSCLC, IGF-1 signalling might be associated with tumour aggressiveness. Upregulation and expression of IGF-1 and IGF-1R in NSCLC were correlated with tumour progression and patient prognosis. To some extent, radical resection of NSCLC appears to directly influence the serum concentration of IGF-1, possibly by removing direct tumour production. Autocrine/paracrine IGF-1 might play an important role in the development of lung cancer, but further investigations, such as in primary cell cultures and animal experiments, are required.

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CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology*'s policy on disclosing conflicts of interest, and we declare that we have none.

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