Changes of Circulating Transforming Growth Factor-β1 Level During Radiation Therapy Are Correlated with the Prognosis of Locally Advanced Non-small Cell Lung Cancer

Lujun Zhao, MD, PhD,† Wei Ji, MD, PhD,*‡ Li Zhang, MD, PhD,*§ Guangfei Ou, MD, PhD,* Qinfu Feng, MD,* Zongmei Zhou, MD,* Mingfang Lei, MSc,* Weizhi Yang, MD,* and Luhua Wang, MD**

Introduction: We hypothesized that plasma transforming growth factor-β1 (TGF-β1) level and its dynamic change are correlated with the prognosis of locally advanced non-small cell lung cancer (NSCLC) treated with radiation therapy (RT).

Methods: Patients with stage IIIA or IIIB NSCLC treated with RT with or without chemotherapy were eligible for this study. Platelet poor plasma was collected from each patient within 1 week before RT (pre-RT) and at the 4th week during RT (during-RT). TGF-β1 level was measured with enzyme-linked immunosorbent assay. The primary end point was overall survival (OS) and the secondary end point was progression-free survival (PFS). Kaplan-Meier and Cox regression were used for risk factor evaluation.

Results: A total of 65 patients were eligible for the study. The median OS and PFS were 17.7 and 13.7 months, respectively. In univariate analysis, performance status, weight loss, radiation dose, and TGF-β1 ratio (during-RT/pre-RT TGF-β1 level) were all significantly correlated with OS. In the multivariate analysis, performance status, radiation dose, and TGF-β1 ratio were still significantly correlated with OS. The median OS was 30.7 months for patients with TGF-β1 ratio ≤1 versus 13.3 months for those with TGF-β1 ratio more than 1 ($p = 0.0029$); and the median PFS was 16.8 months versus 7.2 months, respectively ($p = 0.010$).

Conclusions: In locally advanced NSCLC, the decrease of TGF-β1 level during RT is correlated with favorable prognosis.

*Department of Radiation Oncology, Cancer Hospital (Institute), Peking Union Medical College, Chinese Academy of Medical Science, Beijing; †Department of Radiation Oncology, Key Laboratory of Cancer Prevention and Therapy, Tianjin Medical University Cancer Institute and Hospital, Tianjin; ‡Department of Radiation Oncology, Fudan University, Zhongshan Hospital, Shanghai; and §Cancer Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China.

Disclosure: The authors declare no conflicts of interest.

Address for correspondence: Luhua Wang, MD, Department of Radiation Oncology, Cancer Hospital and Institute, Peking Union Medical College, Chinese Academy of Medical Science, P.O. Box 2258, Beijing 100021, China. E-mail: wlhwq@yahoo.com

Zhao, L. and Ji, W. contributed equally to this study.

ISSN: 1556-0864/10/0504-0521

Key Words: Non-small cell lung cancer, Radiation therapy, Transforming growth factor-β1, Prognosis.

(*J Thorac Oncol.* 2010;5: 521–525)

There are many obstacles to the improvement of prognosis in locally advanced non-small cell lung cancer (NSCLC). Among those factors, two factors were studied most extensively, one is the limitation to the escalation of radiation dose and the concurrent administration of chemotherapy because of radiation-induced lung toxicities (RILT),[1–5] and the other is the immune escape of cancer cells partly due to various cytokines secreted by cancer cells and its surrounding tissues.[6] Transforming growth factor-β1 (TGF-β1) plays an important role both in RILT[7–9] and in the immune escape of cancer cells.[10–12]

It has been reported that TGF-β1 level in lung cancer tissues[13–16] or in the plasma of patients with lung cancer was correlated with the prognosis of the disease.[17,18] However, to the best of our knowledge, there is no study published so far reporting the relationship between the plasma TGF-β1 level or its dynamic changes during the fractionated radiation therapy (RT) and the overall survival (OS) in locally advanced NSCLC. The purpose of this study was to evaluate whether the plasma TGF-β1 level before RT and its change during the radiation correlate with the prognosis in locally advanced NSCLC.

PATIENTS AND METHODS

Patient Selection

This was a retrospective study, which was approved by our institutional review board. All data were from a prospective study that was performed to evaluate the correlations between circulating cytokine levels (including TGF-β1) and the risk of RILT in patients with unresectable or medically inoperable NSCLC. The inclusion criteria of the prospective trial have been described in detail previously.[19] Briefly, patients must have histopathologically confirmed stage I–III NSCLC, with a Karnofsky performance status more than 60, with an expected survival duration more than 6 months, and with forced expiratory volume in the first 1 second more than

50% of the predictive value. Only patients with stage III NSCLC with TGF-β1 measurement available both before RT and at the 4th week during RT were eligible for this retrospective study. The disease stage was reassessed for all patients based on 2002 American Joint Committee on Cancer tumor, node, metastasis classification system.

Treatment Regimen

RT was given by a consistent three-dimensional conformal technique. Treatment-planning computed tomography (CT) scans were performed with patients breathing comfortably while lying supine in the treatment position with proper immobilization. The gross tumor volume (GTV) included the primary tumor, any hilar or mediastinal lymph nodes with a short-axis diameter of at least 1 cm on CT or fluorodeoxyglucose avidity on fluorodeoxyglucose positron emission tomography, and any abnormal findings detected on bronchoscopy or mediastinoscopy. The clinical target volume (CTV) was uniformly created by expanding the GTV by a 0.5 to 1.0 cm margin. Hilar, mediastinal, and supraclavicular nodal regions that appeared to be uninvolved by CT or positron emission tomography were not purposely included in the CTV. The planning target volume was created by expanding the CTV by a minimum of 0.5 cm for setup error. A lung dose-volume histogram was generated with inclusion of both right and left lungs with subtraction of GTV. Tissue inhomogeneity correction was applied for all plans.

Chemotherapy was given before, during, and/or after RT. When chemotherapy was given sequentially with RT, platinum-based doublets (carboplatin/cisplatin combined with vinorelbine, paclitaxel, or gemcitabine) were the commonly used regimens, for 3 to 4 cycles before or after RT. When chemotherapy was given concurrently, the combination of carboplatin and paclitaxel were commonly used, with the carboplatin dose of area under the curve = 2 and the paclitaxel dose of 45 mg/m^2, once a week, for 4 to 6 weeks.

Sample Collection and TGF-β1 Measurement

Blood samples were collected with K2EDTA as the anticoagulant within 1 week before RT (pre-RT) and at the 4th week during RT (during-RT) from all patients. Blood samples from 26 healthy volunteers were also collected as normal controls. The samples were kept on ice for no longer than 1 hour before centrifuging at 1000 *g* for 15 minutes. The upper one-third of the supernatants were collected and stored at −70°C. Such processing generates platelet-rich plasma, thus the samples were recentrifuged at 10,000 *g* for 30 minutes before measurement of TGF-β1. Plasma TGF-β1 levels were measured with enzyme-linked immunosorbent assay using human TGF-β1 Quantikine Kit (R&D Systems Inc., Minneapolis, MN).

Study End Points and Statistical Analysis

Patients were seen weekly during RT and then, after the first follow-up at 1 month after RT, every 3 months for 2 years and every 6 months until the patient was dead. The primary end point was OS, which was defined as the duration between the start of RT and the date of death. The second end point was progression-free survival (PFS), which was defined as the duration between the start of RT and the date of disease progression. Data were considered as right censored for PFS if patients died without evidence of disease progression. TGF-β1 ratio, which was defined as the ratio of during-RT TGF-β1 level compared with its pre-RT level, was used to present the dynamic change of circulating TGF-β1 level. As used previously in our study,[9] changes of plasma TGF-β1 level during fractionated RT were divided into two groups: TGF-β1 ratio more than 1 and TGF-β1 ratio $\leq$1, for the calculation of OS and PFS.

Cox proportional hazard model was used to estimate the impacts of TGF-β1 level and clinical factors on OS and PFS. The Kaplan-Meier method and log-rank test were used to compare the OS and PFS in different groups. All the data were presented as mean ± SD unless otherwise specified. All *p* values were two-sided.

RESULTS

Clinical Characteristics

A total of 139 patients were included in the initial prospective study. Seventy-four patients were excluded from this study for the following reasons: 27 patients were identified with stage I–II disease, 8 patients were identified with stage IV disease, and 39 patients had only pre-RT TGF-β1 measured. The 65 remaining patients were included in the final analysis. The clinical characteristics of the eligible patients and the treatments given to those patients are listed in Table 1.

TGF-β1 Level Measurement

The mean TGF-β1 level was 4.8 ± 5.6 ng/ml in pre-RT patients, which was significantly higher than in normal controls (0.5 ± 0.2 ng/ml, $p < 0.001$). No significant correlation was found between pre-RT TGF-β1 level and any of the following factors: smoking status ($p = 0.366$), histopathology

TABLE 1. Clinical Characteristics and Treatment Regimens

Parameters	Value
Age, yr, median (range)	59 (36–80)
Gender (male/female)	58/7
Stage (IIIA/IIIB)	16/49
Pathology (SCC/AC/other)	42/19/4
KPS (≥80/<80)	58/7
Weight loss ≥5% (yes/no)	21/44
Ever smoking (yes/no)	49/16
Chemotherapy (yes/no)	55/10
Concurrent chemoradiotherapy (yes/no)	41/24
Radiation dose, Gy, median (range)	60 (45–70)
V20 (%)	25.5% ± 5.9%
Mean lung dose (Gy)	15.3 ± 4.2
Pre-RT TGF-β1 level (ng/ml)	4.8 ± 5.6
During-RT TGF-β1 level (ng/ml)	3.7 ± 3.4
TGF-β1 ratio (>1/<1)	27/38

KPS, Kamofsky performance status; V20, the percentage of the whole lung which received a dose more than 20 Gy; RT, Radiation therapy; TGF-β1, transforming growth factor β1; SCC, squamous cell carcinoma; AC, adenocarcinoma; TGF-β1 ratio, the ratio of during-RT TGF-β1 level compared with its pre-RT level.

($p = 0.295$), stage of disease (stage IIIA versus stage IIIB, $p = 0.288$), and GTV ($p = 0.124$). At the 4th week during RT, the mean level of plasma TGF-β1 of the entire group was not significantly different from its pre-RT level (3.7 ± 3.4 ng/ml versus 4.8 ± 5.6 ng/ml, respectively, paired *t* test, $p = 0.116$). Thirty-eight patients had decreased TGF-β1 level and the other 27 patients had increased TGF-β1 level.

Factors Associated with OS and PFS

The median OS and PFS for the entire group of patients were 17.7 months (95% confidential interval [CI]: 10.8–24.5 months) and 13.7 months (95% CI: 8.0–19.4 months), respectively (Fig. 1). In univariate analysis, performance status, weight loss, radiation dose, and TGF-β1 ratio were all significantly correlated with OS, and only weight loss, during-RT TGF-β1 level, and TGF-β1 ratio were significantly correlated with PFS; radiation dose was marginally correlated with PFS (Table 2). When performance status, weight loss, radiation dose, and TGF-β1 ratio were included together into the multivariate Cox regression analysis, it was found that only performance status, radiation dose, and TGF-β1 ratio were significantly correlated with OS, and only weight loss and TGF-β1 ratio were significantly correlated with PFS (Table 3).

The median OS was 30.7 months (95% CI: 15.9–45.5 months) for patients with TGF-β1 ratio ≤1 ($n = 38$) versus 13.3 months (95% CI: 10.9–15.7 months) for those with TGF-β1 ratio more than 1 ($n = 27$), and the 3-year OS was 46.7% versus 19.8%, respectively (Fig. 2, $p = 0.003$). The corresponding median PFS were 16.8 months (95% CI: 12.5–21.2 months) and 7.2 months (95% CI: 6.2–8.2 months), and the 3-year PFS were 30.5% and 0.0%, respectively (Fig. 3, $p = 0.010$).

In patients with low pre-RT TGF-β1 level, the change of TGF-β1 level is more "upside potential" than "downside potential," and vice versa. To clarify this issue, all the patients were divided into 2 groups for further analysis: those with pre-RT TGF-β1 level ≤3 ng/ml, the median level of the pre-RT TGF-β1, and those with TGF-β1 level more than 3 ng/ml. In patients with low pre-RT TGF-β1 level, the median OS was 30.7 months (95% CI: unavailable) for those with TGF-β1 ratio ≤1 ($n = 16$) versus 13.3 months (95% CI: 10.7–15.9 months) for those with TGF-β1 ratio more than 1 ($n = 17$), and the 3-year OS was 45.1% versus 11.8%, respectively ($p < 0.001$). The median PFS was 17.9 months (95% CI: 7.7–28.1 months) versus 6.7 months (95% CI: 5.0–8.3 months), and the 3-year PFS was 31.6% versus 0.0%, respectively ($p = 0.003$). In patients with high pre-RT TGF-β1 level, the median OS was 22.2 months (95% CI: 8.4–35.9 months) for those with TGF-β1 ratio ≤1 ($n = 22$) versus 11.7 months (95% CI: 7.0–16.4 months) for those with TGF-β1 ratio more than 1 ($n = 10$), and the 3-year OS was 36.4% versus 12.5%, respectively ($p = 0.064$). The median PFS was 13.7 months (95% CI: 2.7–24.7 months) versus 7.3 months (95% CI: 1.9–12.6 months), and the 3-year PFS was 19.3% versus 0.0%, respectively ($p = 0.048$).

As reported previously, elevation of TGF-β1 during-RT is significantly correlated with RILT.[7,8] To clarify the confounding effect of RILT, another analysis was made with patients who experienced grade 3 and above RILT excluded. In this selected group of patients, the median OS was 36.6

TABLE 2. Factors Correlated with OS and PFS in Univariate Cox Regression Analysis

Factors	Odds Ratio for OS	*p*	Odds Ratio for PFS	*p*
Female compared with male	0.712	0.520	0.710	0.472
Age	1.016	0.224	1.005	0.728
KPS	0.930	0.019	0.965	0.212
Weight loss ≥5%	2.073	0.023	2.245	0.009
Ever smoking	1.561	0.241	0.994	0.987
Histopathology	—	0.497	—	0.733
Stage IIIB compared with stage IIIA	1.112	0.771	1.394	0.338
Radiation dose	0.911	0.001	0.949	0.064
Administration of chemotherapy	0.791	0.573	1.535	0.333
Pre-RT TGF-β1 level	1.021	0.520	1.041	0.152
During-RT TGF-β1 level	1.032	0.469	1.091	0.020
TGF-β1 ratio	1.422	0.002	1.380	0.004

KPS, Kamofsky performance status; RT, radiation therapy; TGF-β1, transforming growth factor β1; OR, odds ratio; OS, overall survival; PFS, progression-free survival; TGF-β1 ratio, the ratio of during-RT TGF-β1 level compared with its pre-RT level.

TABLE 3. Factors Correlated with OS and PFS in Multivariate Cox Regression Analysis

Factors	Odds Ratio for OS	*p*	Odds Ratio for PFS	*p*
KPS	0.933	0.042	0.978	0.489
Weight loss ≥5%	1.630	0.126	2.153	0.015
Radiation dose	0.918	0.002	0.954	0.112
TGF-β1 ratio	1.279	0.042	1.374	0.006

KPS, Kamofsky performance status; TGF-β1, transforming growth factor β1; OR, odds ratio; OS, overall survival; PFS, progression-free survival; TGF-β1 ratio, the ratio of during-RT TGF-β1 level compared with its pre-RT level.

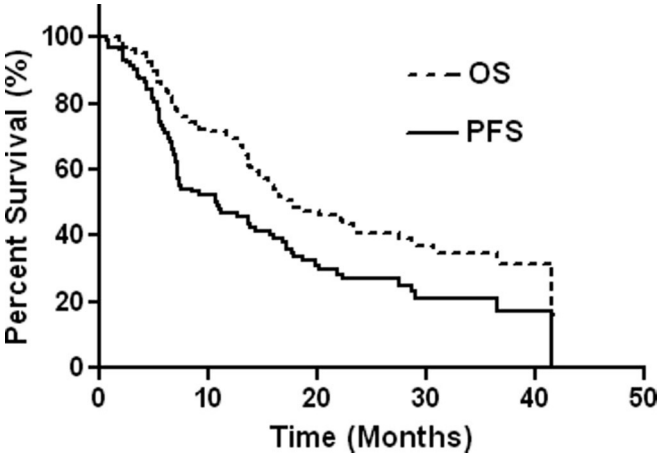

FIGURE 1. The overall survival (OS) and progression-free survival (PFS) for the whole group of patients.

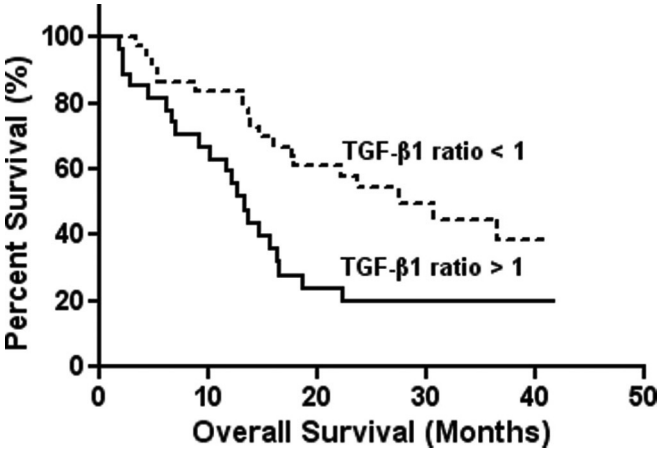

FIGURE 2. Difference in overall survival between patients with transforming growth factor-β1 (TGF-β1) ratio (during-RT/pre-RT TGF-β1 level) $\leq$1 (n = 38) and those with TGF-β1 ratio $>$1 (n = 27) (p = 0.003).

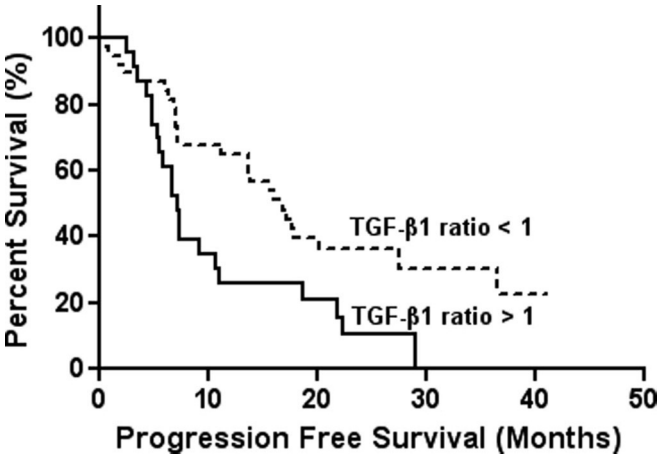

FIGURE 3. Difference in progression-free survival between patients with transforming growth factor-β1 (TGF-β1) ratio (during-RT/pre-RT TGF-β1 level) $\leq$1 (n = 38) and those with TGF-β1 ratio $>$1 (n = 27) (p = 0.010).

months (95% CI: 21.1–52.0 months) and the 3-year OS was 43.5% in patients with TGF-β1 ratio $\leq$1, and they were 13.6 months (95% CI: 10.7–16.5 months) and 24.5% in those with TGF-β1 ratio more than 1, respectively (p = 0.010). The corresponding PFS were 17.2 months (95% CI: 14.7–19.6 months) and 24.9% versus 7.2 months (95% CI: 6.2–8.2 months) and 0.0%, respectively, (p = 0.008).

DISCUSSION

As reported in the literatures,[20,21] this retrospective study found that performance status, weight loss, and radiation dose are all important prognostic factors for OS in patients with locally advanced NSCLC. Most importantly, this study suggested that patients with decreased circulating TGF-β1 level at the 4th week during RT compared with its baseline level may have a favorable prognosis, with longer OS and PFS.

However, in this study, disease stage and the administration of chemotherapy failed to show significant influence on OS and PFS. This may be at least partly due to that only patients with stage III disease were included, which weakened the prognosis value of disease stage, and that only 10 patients received RT alone, which made it impossible to get a reasonable comparison with combined treatment modality.

TGF-β1 plays an important role in the development and progression of NSCLC. Almost all human tumors overproduce TGF-β1, whose autocrine and paracrine actions promote tumor cell invasiveness and metastasis.[22] TGF-β1 also suppresses proliferation and differentiation of lymphocytes including cytolytic T cells, natural killer cells, and macrophages, thus preventing immune surveillance of the developing tumor.[22] Studies on specimens from patients with NSCLC showed that the overall prognosis is significantly poorer for patients showing positive TGF-β1 expression in tumors,[13,14] and this may be due to the immune suppression effect of TGF-β1 through $CD4^+$ $CD25^+$ regulatory T cells.[16]

Circulating TGF-β1 level was also reported to be correlated with the prognosis of NSCLC. In 1999, Kong et al.[18] reported that the pre-RT plasma TGF-β1 level was significantly higher in patients with lung cancer than in normal controls, and it was significantly higher in patients with disease at the last follow-up compared with that in the no evidence of disease group. In this study, we also found that patients with NSCLC had significantly higher TGF-β1 level than normal controls. Most importantly, for the first time, we found that patients with decreased circulating level of TGF-β1 at the 4th week during RT compared with its baseline level may have a prolonged overall survival. Further analysis demonstrated that no matter in patients with higher pre-RT TGF-β1 level or in patients with lower pre-RT level, the decrease of TGF-β1 level during RT all correlated with an improved prognosis. Our results also suggested that lower absolute level of circulating TGF-β1 during RT may predict an improved tumor control. Decreased tumor burden may contribute to the decreased level of TGF-β1 and this may result in an improved antitumor immunity. Much work needs to be done to further clarify the underlying mechanisms.

It was reported that increased circulating TGF-β1 level correlated with increased risk of RILT.[7,23] This may contribute to the increased risk of death. However, in this study, the change of circulating TGF-β1 level was independently correlated not only with the OS but also with the PFS. Furthermore, even when patients with grade 3 and above RILT were excluded, those with decreased level of TGF-β1 still had a favorable prognosis. These results suggested that TGF-β1 level plays an important role in the progression of NSCLC and the decrease of circulating TGF-β1 level during RT may predict a favorable prognosis. Much research needs to be done to further clarify the underlying mechanism.

It should be noted that this is a retrospective study and suffers the pitfalls of all such kind of studies. The chemotherapy given to these patients were inconsistent, and the radiation dose varied from 45 to 70 Gy. All these confounding factors might interfere with the conclusions of this study and should be taken into consideration.

In summary, the results of this retrospective study suggest that TGF-β1 level is significantly higher in locally advanced NSCLC than in healthy controls and the decrease of circulating TGF-β1 level at the 4th week during RT may predict a prolonged OS and PFS. The findings need to be validated and the underlying mechanism and the potential of TGF-β1 signal pathway as a novel target for the treatment of locally advanced NSCLC are worthy of further study.

ACKNOWLEDGMENTS

Supported in part by grant #2002-101 from the Beijing Medicine Development Foundation (to L.W.).

REFERENCES

1. Zhao L, West BT, Hayman JA, et al. High radiation dose may reduce the negative effect of large gross tumor volume in patients with medically inoperable early-stage non-small cell lung cancer. *Int J Radiat Oncol Biol Phys* 2007;68:103–110.
2. Hayman JA, Martel MK, Ten Haken RK, et al. Dose escalation in non-small-cell lung cancer using three-dimensional conformal radiation therapy: update of a phase I trial. *J Clin Oncol* 2001;19:127–136.
3. Yorke ED, Jackson A, Rosenzweig KE, et al. Correlation of dosimetric factors and radiation pneumonitis for non-small-cell lung cancer patients in a recently completed dose escalation study. *Int J Radiat Oncol Biol Phys* 2005;63:672–682.
4. Grills IS, Yan D, Martinez AA, et al. Potential for reduced toxicity and dose escalation in the treatment of inoperable non-small-cell lung cancer: a comparison of intensity-modulated radiation therapy (IMRT), 3D conformal radiation, and elective nodal irradiation. *Int J Radiat Oncol Biol Phys* 2003;57:875–890.
5. Kong FM, Ten Haken RK, Schipper MJ, et al. High-dose radiation improved local tumor control and overall survival in patients with inoperable/unresectable non-small-cell lung cancer: long-term results of a radiation dose escalation study. *Int J Radiat Oncol Biol Phys* 2005; 63:324–333.
6. Sheu BC, Chang WC, Cheng CY, et al. Cytokine regulation networks in the cancer microenvironment. *Front Biosci* 2008;13:6255–6268.
7. Zhao L, Sheldon K, Chen M, et al. The predictive role of plasma TGF-beta1 during radiation therapy for radiation-induced lung toxicity deserves further study in patients with non-small cell lung cancer. *Lung Cancer* 2008;59:232–239.
8. Anscher MS, Kong FM, Jirtle RL. The relevance of transforming growth factor beta 1 in pulmonary injury after radiation therapy. *Lung Cancer* 1998;19:109–120.
9. Zhao L, Wang L, Ji W, et al. Elevation of plasma TGF-beta1 during radiation therapy predicts radiation-induced lung toxicity in patients with non-small-cell lung cancer: a combined analysis from Beijing and Michigan. *Int J Radiat Oncol Biol Phys* 2009;74:1385–1390.
10. Takayama S, Hatori M, Kurihara Y, et al. Inhibition of TGF-beta1 suppresses motility and invasiveness of oral squamous cell carcinoma cell lines via modulation of integrins and down-regulation of matrix-metalloproteinases. *Oncol Rep* 2009;21:205–210.
11. Sun L. Tumor-suppressive and promoting function of transforming growth factor beta. *Front Biosci* 2004;9:1925–1935.
12. Vicent S, Luis-Ravelo D, Anton I, et al. A novel lung cancer signature mediates metastatic bone colonization by a dual mechanism. *Cancer Res* 2008;68:2275–2285.
13. Takanami I, Tanaka F, Hashizume T, et al. Roles of the transforming growth factor beta 1 and its type I and II receptors in the development of a pulmonary adenocarcinoma: results of an immunohistochemical study. *J Surg Oncol* 1997;64:262–267.
14. Saji H, Nakamura H, Awut I, et al. Significance of expression of TGF-beta in pulmonary metastasis in non-small cell lung cancer tissues. *Ann Thorac Cardiovasc Surg* 2003;9:295–300.
15. Hasegawa Y, Takanashi S, Kanehira Y, et al. Transforming growth factor-beta1 level correlates with angiogenesis, tumor progression, and prognosis in patients with nonsmall cell lung carcinoma. *Cancer* 2001; 91:964–971.
16. Su YJ, Ren K, Li H, et al. [Clinical significance of $CD4^+$ $CD25^+$ regulatory T-cells detection in tumor-draining lymph nodes of nonsmall cell lung cancer patients]. *Zhonghua Zhong Liu Za Zhi* 2007;29:922–926.
17. Vujaskovic Z, Groen HJ. TGF-beta, radiation-induced pulmonary injury and lung cancer. *Int J Radiat Biol* 2000;76:511–516.
18. Kong F, Jirtle RL, Huang DH, et al. Plasma transforming growth factor-beta1 level before radiotherapy correlates with long term outcome of patients with lung carcinoma. *Cancer* 1999;86:1712–1719.
19. Zhao L, Wang L, Ji W, et al. Association between plasma angiotensin-converting enzyme level and radiation pneumonitis. *Cytokine* 2007;37: 71–75.
20. Wang L, Correa CR, Hayman JA, et al. Time to treatment in patients with stage III non-small cell lung cancer. *Int J Radiat Oncol Biol Phys* 2009;74:790–795.
21. Wang L, Correa CR, Zhao L, et al. The effect of radiation dose and chemotherapy on overall survival in 237 patients with stage III non-small-cell lung cancer. *Int J Radiat Oncol Biol Phys* 2009;73:1383–1390.
22. Pardali K, Moustakas A. Actions of TGF-beta as tumor suppressor and pro-metastatic factor in human cancer. *Biochim Biophys Acta* 2007; 1775:21–62.
23. Anscher MS, Kong FM, Andrews K, et al. Plasma transforming growth factor beta1 as a predictor of radiation pneumonitis. *Int J Radiat Oncol Biol Phys* 1998;41:1029–1035.

