Surgical Results in T2N0M0 Nonsmall Cell Lung Cancer Patients With Large Tumors 5 cm or Greater in Diameter: What Regulates Outcome?

Surgical Results in T2N0M0 Non-small Cell Lung Cancer Patients With Large Tumors 5 cm or Greater in Diameter: What regulates outcome?

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Running head: Large lung cancer

Key words: Lung cancer, Surgery, Recurrence

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Background: We assessed the surgical results along with the clinical and biological features of non-small cell lung cancer (NSCLC) patients with localized large tumors.

Methods: The study population consisted of 86 NSCLC patients who underwent complete resection of tumors 5 cm or larger in diameter in stage IB (T2N0M0). We immunohistochemically assessed the expression of angiostatin and endostatin.

Results: The median tumor size was 6.0 cm (range, 5–14 cm). The operative procedures used were lobectomy in 71 cases, bilobectomy in 8 cases, and pneumonectomy in 11 cases. Fifty patients (58.1%) relapsed during the mean follow-up period of 33.6 ± 4.5 months. The median disease-free interval (DFI) was 9 months. Of 44 recurrent patients whose DFI could be identified, 25 patients (56.8%) relapsed within 12 months after the operation. The overall 5- and 10-year survival rates were 42.0% and 24.2%, respectively. Multivariate analysis showed that the degree of pleural involvement and angiostatin expression within the tumor were independent prognostic indicators. The endostatin expression within tumors also had a weaker relationship with outcome.

Conclusions: Long-term surgical results were poor and early relapse was common in this cohort. In addition to pleural involvement, the tumor-induced expression of angiostatin and endostatin merit further investigation to gain possible insights into selection of patients who will benefit from surgery as the first line treatment.

Introduction

Surgery remains the mainstay treatment for localized non-small cell lung cancer (NSCLC) patients irrespective of the tumor size. As tumor size is crucial for the oncological outcome in NSCLC patients, the effects of surgical treatment are still uncertain in patients with large tumors. Although lung cancer is considered a surgically treatable disease in the relatively early stages, recent retrospective clinical assessment showed that a diameter of more than 5 cm is the threshold predicting poor prognosis in lung carcinoma, suggesting that the current staging system should be revised [1-4]. On the other hand, some investigators suggested that localized large tumors also include those with less metastatic potential[5]. However, the biological features specific to large tumors have not been clarified.

There have been a number of studies using the experimental metastasis paradigm in the areas of both basic and clinical research. Based on such studies, it has begun to become clear that the removal of the primary tumor occasionally results in stimulation of cancer cell proliferation in metastatic foci [6-9]. Interestingly, the inhibition of proliferation of these metastatic foci can be explained partly by the production of anti-angiogenic factors, such as angiostatin and endostatin [10,11]. These factors are both endogenous angiogenesis inhibitors, which are related to the inhibition of

metastatic growth by the primary neoplasms. From this specific oncological viewpoint, the appropriateness of removal of the primary tumor as the first line of treatment remains an open question, especially in patients with large primary tumors. On a hypothesis that large tumors have a high risk of systemic micrometastasis and produce inhibitory anti-angiogenic factors, we performed the present retrospective review to assess surgical results by highlighting angiostatin and endostatin expression in NSCLC patients with large tumors in the relatively early stage of disease.

Patients and Methods

Patients

From January 1981 to December 2004, a total of 1,436 NSCLC patients underwent pulmonary resection at Kanazawa University Hospital. Of these patients, 283 (19.7%) had bulky primary tumors 5 cm or larger in diameter, and complete resection of the primary tumor could be achieved in 259 patients. Among these 259 patients, 86 (72 men and 14 women) patients in pathological stage IB (T2N0M0) according to the TNM classification [12] who had undergone complete resection with systematic lymphadenectomy were included in this retrospective review. Lymph node biopsy through mediastinoscopy or thoracoscopy was performed selectively in 19 patients with

clinical N2 diagnosis based on the results of CT and ^{201}Tl or FDG-PET scan. None of the patients underwent any preoperative induction treatment. The degree of pleural involvement was classified pathologically as follows: p0, no invasion; p1, tumor invasion reached the visceral pleura but not beyond; p2, tumor invasion penetrated the visceral pleura but not the parietal pleura. In this study, N1 nodal station number was designated according to Naruke's map [13]. Written informed consent was obtained from all patients.

Immunohistochemical assessment of angiostatin, endostatin, and VEGF-A

Resected tumor specimens could be collected from 83 patients. After reviewing the hematoxylin and eosin-stained slides of the tumor specimens, we selected blocks of the invasive edge in the tumor area. Paraffin-embedded tumor tissues were cut into consecutive sections 4 μm thick, deparaffinized, and subjected to immunohistochemical staining using the labeled streptavidin-biotin method, as described previously [14]. The primary antibodies used in the present study were a rabbit polyclonal antibody to angiostatin (Oncogene Research Products, Cambridge, MA) diluted 150-fold [15], a rabbit polyclonal antibody to endostatin (Lab Vision Corp., Fremont, CA), and a rabbit polyclonal antibody to VEGF (Santa Cruz Biotechnology Inc., Santa Cruz, CA) diluted

100-fold. Negative controls were stained using all reagents except the primary antibodies. As positive controls for angiostatin and endostatin, we used formalin-fixed, paraffin-embedded human melanoma and tonsil tissues, respectively, according to the manufacturers' recommendations.

The immunoreactivities were graded as (-), (+), and (++) according to the intensity of the tumor cells: (-) represents positive staining of 0%–10% of the total area, (+) represents 10%–50% positive staining, and (++) represents the strongest staining of more than 50% at ×200 magnification. For assessment of angiostatin and endostatin expression, tumors with (-) staining were classified as negative. For assessment of VEGF-A expression, tumors with the strongest staining were defined as overexpressing VEGF-A [14]. The positivity of these markers was evaluated by two independent viewers without knowledge of the clinical factors.

Statistical analysis

Survival time was measured from the date of surgery. The disease-free interval (DFI) was calculated for patients who showed relapse and was defined as the interval between time of lung surgery and clinical or radiographic demonstration of the first recurrence. Survival curves were plotted by the Kaplan-Meier method and univariate comparisons

were performed by the log-rank test. Zero time was the date of surgical treatment. The effects of age, gender, pathological type (squamous *vs*. other), location of primary tumor (right *vs*. left and upper lobe *vs*. lower lobe), nodal status (N0 *vs*. N1), pleural involvement (p0/1 *vs*. p2), adjuvant systemic chemotherapy, VEGF-A, angiostatin, and endostatin on overall survival and DFI were assessed. Age and tumor size were classified as being in the high or low group relative to the median value. For multivariate analysis, Cox's proportional hazards regression was used and factors with $p<0.10$ were included in the final model. Associations between variables were analyzed with the χ^2 test. The Mann-Whitney U test for differences in mean values was used for comparison of nominal data. Mean values are shown ± the standard errors.

Results

The basic clinical and pathological features of the patients are shown in Table 1. The patients ranged in age from 45 to 84 years (median age, 69.0). The median tumor size was 6.0 cm (range, 5–14 cm). The pathological types were as follows: 50 squamous cell carcinomas, 31 adenocarcinomas, 3 adenosquamous cell carcinomas, and 2 large cell carcinomas. The operative procedures used were lobectomy in 71 cases, pneumonectomy in 7 cases, and bilobectomy in 8 cases. Postoperative adjuvant

chemotherapy was performed in 24 patients. In-hospital mortality for all 86 patients was 0.01% (1/86). Information about overall survival and DFI could be obtained for all patients and for 44 patients, respectively. In the mean follow-up period of 33.6 ± 4.5 months (range, 1–146 months), 50 patients (58.1%) relapsed. Of the 44 recurrent patients whose DFI could be identified, 25 (56.8%) relapsed within 12 months after surgical removal of the primary tumors. The median DFI was 9 months (range, 2–105 months). Among the 22 patients whose recurrent pattern could be identified clearly, distant and local recurrence developed in 19 and 3 cases, respectively. In the total cohort, the overall 5- and 10-year survival rates were 42.0% and 24.2%, respectively. The disease-free survival rate at 5 years was only 10.3%. With respect to the pleural involvement, the patients with p2 showed significantly poorer survival and shorter period of DFI as compared to those with p0−1 (Fig. 1).

VEGF-A, angiostatin, and endostatin antigens were identified mainly in the cytoplasm of tumor cells (Fig. 2). These antigens were also identified in vascular endothelial cells, and angiostatin antigen was also found in some smooth muscle cells and lymphatic cells.

The percentage of patients with VEGF-A overexpression was 75.9% (63 of 83 patients). Four cases were classified as (-) and 16 cases were classified as (+). On the

other hand, the percentages of patients positive for angiostatin and endostatin expression were 13.3% (11 of 83 patients) and 54.2% (45 of 83 patients), respectively.

In univariate analysis, pleural involvement (p2), negativity for angiostatin, and negativity for endostatin had significant negative impact on overall survival (Table 2). While the overall 5- and 10-year survival rates of angiostatin-positive or endostatin-positive patients were 47.0% and 29.1%, respectively, those of angiostatin-negative and endostatin-negative patients were 21.6% and 0%, respectively ($p=0.0049$)(Fig. 3). Although the number of patients was limited ($n=7$), the 10-year survival rate of angiostatin-positive and endostatin-positive patients was 83.3%. Multivariate analysis showed that pleural involvement and angiostatin were independent prognostic indicators on overall survival (Table 3).

Comments

Currently, surgical resection represents the standard first line of treatment for localized NSCLC in the early stages irrespective of tumor size. However, earlier studies indicated that the surgical outcome of NSCLC with large tumors more than 5 cm in diameter is poor. Carbone and associates [2] reported that 5-year survival rate of 111 NSCLC patients with T2 tumors more than 5 cm in diameter was 35.1%. In their series, the

significant survival difference based on this threshold remained in N0 and N2-3 status. In the assessment of 60 pN0-NSCLC patients with a tumor size greater than 5 cm, Takeda *et al.* [4] reported that 5-year survival rate after surgery was 46.6%. Cangir *et al.* [3] also found that the 5-year survival rate of patients with tumors larger than 5 cm in diameter was significantly poorer than that of patients with tumors measuring 3.1–5 cm in diameter when patients at various stages were included. In our series highlighting localized pT2N0M0 patients with a tumor size of 5 cm or larger in diameter, we similarly found poor outcomes with a 5-year survival rate of 42.0%. Consistent with previous data, the surgical results of patients with large tumors 5 cm or greater in diameter were poor after the initial operation even in those at the earliest stage.

Although it is still not clear which population among these localized NSCLC with large tumors would benefit from surgery, the findings of the present study have some implications for the selection of patients that may benefit from surgical resection. Considering the possibility that the lack of tumor-induced growth inhibitory factors may accelerate residual micrometastatic foci after removal of the primary site, we performed immunohistochemical assessment of the expression of two noble markers, angiostatin and endostatin. These two biological markers appeared to be associated with surgical outcomes in the present study. Angiostatin is a proteolytic fragment of plasminogen,

which was found originally in a primary Lewis lung carcinoma [10]. There has been only one previous report of immunohistochemical determination of angiostatin expression in lung cancer, in which the results obtained in 143 NSCLC patients in stage I-IIIA indicated that angiostatin expression within the tumor is a favorable prognostic factor [15]. Our observations regarding the relationship between angiostatin expression and patients outcome were consistent with the findings of this previous study. Further, while the percentages of patients with tumors positive for angiostatin were 24% (34/143) in the total patient population in various stages and 24% (11/45) in patients in stage I-II in this previous study, the percentage of patients positive for angiostatin expression was only 13.3% (11/83) in our series using the same antibody under the same experimental conditions. Therefore, the expression of angiostatin within the tumor may be suppressed in large lung cancer lesions. On the other hand, endostatin was isolated originally from murine hemangioendothelioma [11] and identified as the C-terminal fragment of its precursor, collagen XVIII [16]. With regard to endostatin expression in lung cancer, the presence of its precursor, collagen XVIII, in lung cancer cells was confirmed previously [17]. Notably, some investigators from the same institute reported previously that its expression in lung cancer tissue was associated with tumor progression and poor outcomes in assessment of patients at various stages [17,18].

The endostatin expression in tumor tissue was also reported to be associated with tumor grade and poor outcome in other neoplasms [19]. Interestingly, in hepatocellular carcinoma, collagen XVIII expression decreases with both increasing tumor size and tumor progression [20]. Considering these conflicting phenomena, the relationship between endostatin expression within the tumor and patient outcome may be altered according to the tumor type and their phase or stage of progression. The presence or absence of micrometastases will also inevitably affect this relationship. In the present study, we found that both of these anti-angiogenic factors were actually expressed in some tumors. In our series of large tumors 5 cm or more in diameter, although the percentage of tumors positive for endostatin expression was relatively high as compared to that of tumors positive for angiostatin expression, both endostatin and angiostatin expression at the primary site were associated with preferable outcome on overall survival. Therefore, it is unlikely that loss of tumor-induced angiostatin/endostatin expression at the primary site after removal of the tumor affects early relapse. Our findings support the idea that the presence of these anti-angiogenic factors within the tumor is associated with preferable outcome after removal of the primary tumor as the first line of treatment.

We also found that the degree of pleural invasion was associated with surgical results,

consistent with previous findings reported by Carbone and colleagues [2]. The appropriateness of initial operation in patients with pleural invasion must be determined carefully in patients with the disease.

In conclusion, long-term surgical results were not satisfactory and the frequency of early relapse was high in this cohort. Pleural involvement was determined as a significant independent prognostic indicator of overall survival. Further, angiostatin/endostatin expression within tumors should be investigated further to gain possible insights into the selection of patients who will benefit from surgery as the first line of treatment.

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Figure Legends

Figure 1. Kaplan-Meier overall survival plots for 120 T2N0-1M0 non-small cell lung cancer patients with large tumors 5 cm or greater in diameter stratified by pleural involvement. The degree of pleural involvement was classified pathologically as: p0, no invasion; p1, tumor invasion reached the visceral pleura but not beyond; p2, tumor invasion penetrate the visceral pleura but not the parietal pleura. The patients with p2 showed significantly poorer survival as compared to those with p0−1 (p=0.008). The survival curves are combined with lines representing the 90% confidence interval.

Figure 2. Examples of immunohistochemical staining for (A) angiostatin and (B) endostatin. Cytoplasmic staining was positive in tumor cells. (Original magnification, ×400.)

Figure 3. Kaplan-Meier overall survival plots of T2N0M0 non-small cell lung cancer patients with large tumors 5 cm or greater in diameter stratified by the expression of angiostatin and endostatin. The difference was statistically significant (p=0.0049). The survival curves are combined with lines representing the 90% confidence interval.

Table 1. Patient Demographics

Characteristics	Values
No. of patients	**86**
Gender	
Male	**72**
Female	**14**
Median age (years)	**69**
Median tumor size (cm)	**6.0**
Location-1	
Right	**51**
Left	**35**
Location-2	
Upper lobe	**39**
Middle lobe	**1**
Lower lobe	**46**
Histology	
Adenocarcinoma	**31**
Squamous cell carcinoma	**50**
Others	**5**
Pleural involvement	
Negative (p0)	**52**
Reached visceral pleura but not beyond (p1)	**22**
Penetrate visceral pleura but not the parietal pleura (p2)	**7**
Unknown	**5**
Adjuvant chemotherapy	**24**
Operative procedures	
Lobectomy	**71**
Pneumonectomy	**7**
Bilobectomy	**8**

Table 2. Univariate Analysis of Prognostic Factors

Factors	Hazard ratio (95% CI)	*p* Value
Age	**0.824 (0.624-1.077)**	**0.158**
Gender	**1.372 (0.954-2.137)**	**0.091**
Tumor size	**0.859 (0.659-1.129)**	**0.271**
Histology (Adenocarcinoma vs others)	**1.066 (0.818-1.401)**	**0.636**
Pleural involvement	**3.496 (1.420-7.411)**	**0.008**
Adjuvant therapy	**1.071 (0.822-1.407)**	**0.609**
Location(1)(rt vs. lt)	**1.228 (0.936-1.634)**	**0.137**
Location(2)(upper lobe vs. lower lobe)	**1.280(0.971-1.682)**	**0.078**
VEGF-A	**1.310 (0.699-2.678)**	**0.412**
Angiostatin	**7.550 (2.689-31.583)**	**<0.00001**
Endostatin	**1.691 (1.000-2.798)**	**0.049**

Table 3. Multivariate Analysis of Prognostic Factors

Factors	Favorable	Unfavorable	Hazard ratio (95% CI)	*p* Value
Pleural involvement	**p0-1**	**p2**	**2.727 (1.096-5.887)**	**0.032**
Angiostatin	**Positive**	**Negative**	**7.222 (2.172-44.794)**	**0.0003**
Endostatin	**Positive**	**Negative**	**1.701 (0.976-2.997)**	**0.060**