Tumor Angiogenesis in 75 Cases of Pleomorphic Carcinoma of the Lung

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Abstract. *Background: Pleomorphic carcinoma (PC) of the lung is a rare tumor that usually has an aggressive clinical course and a poor prognosis. In this study, 75 cases of PC were reviewed to identify its clinical features, and we examined the expression of angiogenic factors. Patients and Methods: We immunohistochemically examined the expression of angiogenic factors in tissue specimens of PC. Results: 66 males and 9 females were examined. The median survival time was 16.5 months. The stage and symptomatical diagnosis were significantly associated with the survival. In the immunohistochemical analyses, vascular endothelial growth factor (VEGF) was expressed in many cases of PC. A high score for angiogenesis was significantly related to a poorer prognosis. Conclusion: We conclude that PC should be considered an aggressive disease, and that the stage and symptomatical diagnosis are strong prognostic factors. Furthermore, tumor angiogenesis provides significant prognostic information about the clinical outcome in PC.*

Pleomorphic carcinoma (PC) of the lung is a comparatively rare tumor, which constitutes approximately 0.1-0.4% of all lung cancer cases. PC is usually of high grade, aggressive and associated with a poor prognosis (1-3). Histologically, PC exhibits characteristic tumor components, *i.e.* malignant epithelial and homologous sarcomatoid spindle/giant cells. The WHO classification defines pleomorphic carcinoma as a "poorly differentiated non-small cell carcinoma, namely a squamous cell carcinoma, adenocarcinoma or large cell carcinoma, containing spindle cells and giant cells, in which the pleomorphic component comprise at least 10% of the neoplasm" (4). This subtype consists of large cell lung carcinomas that exhibit a spindle cell component, or both a large cell and spindle cell carcinoma, of 10% or more. In the clinic, PC tends to be negative for tumor markers and difficult to diagnose cytologically or histologically using bronchoscopic or computed tomography-guided biopsy. Therefore, the clinical features and behavior of PC remain unclear.

It is well-established that tumor growth beyond the size of 1-2 mm is angiogenesis-dependent (5). It is also known that many malignancies, including lung cancer, overexpress angiogenic factors (6-12). One of the major regulators of the neovasculization process is vascular endothelial growth factor (VEGF), which was originally discovered as a vascular permeability factor (13). Several studies demonstrated that an increase in microvessel density (MVD) was found to be closely associated with the expression of VEGF, and that MVD and VEGF expression had prognostic value for predicting the metastasis of various types of malignant solid tumors (6-10).

While there have been a few case reports of an association between a bleeding tendency in tumor tissues and abnormalities

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of angiogenesis, there have been few studies that have made a comprehensive evaluation of angiogenesis in PC. In addition, distant metastasis is frequently observed in cases of PC, and such metastases have tended to expand into regions such as the small intestine, peritoneum, skin, and lymph nodes (14, 15). Moreover, inflammation and a hypoxic state are also widely recognized to play an important role in tumor angiogenesis through the up-regulation of cyclooxygenase (COX)-2 and hypoxia-inducible factor (HIF)-1α, respectively (16-18). COX-2 and HIF-1α are important pro-angiogenic factors that activate the transcription of *VEGF*. The aim of the present study was to investigate the clinical features and mechanisms of angiogenesis in PC, which could lead to the development of new treatments and a more precise diagnosis for patients with non-small cell lung cancer (NSCLC). We retrospectively analyzed the background and the disease prognosis in 75 cases of PC, and performed an analysis of the expression of tumor angiogenic factors, such as VEGF, COX-2, CD31 and HIF-1α.

Patients and Methods

Patients and samples. We used formalin-fixed, paraffin-embedded blocks from 75 patients with PC who had undergone surgical resection between August 2001 and May 2010, which were retrieved from the Hiroshima City Hospital, the Higashi Hiroshima Medical Center, Showa University Fujigaoka Hospital, the Yoshijima Hospital, Nishigunma National Hospital, the Japanese Red Cross Nagaoka Hospital, the Shimane Prefectural Central Hospital, the Kure Medical Center and the Shimane University Hospital. All of the PC cases were diagnosed strictly according to the WHO classification by an experienced pathologist. All available clinical information was obtained from the clinical records and reports of the referring physicians, and e-mails or letters were sent to contributors for additional information. These records were reviewed for patient age, gender, smoking status, presenting symptoms, location of the primary tumor, follow-up tumor markers, the stage at the time of diagnosis, and the duration of follow-up. Surgical or pathological staging was performed according to the TNM classification of the International Union Against Cancer criteria version 6.0 or 7.0 (19). This study was approved by the Institutional Review Boards of each institute.

Immunohistochemistry. Formalin-fixed, paraffin-embedded tumor samples were cut into 4 μm-thick sections. Tissue samples were routinely deparaffinized in xylene and rehydrated through a series of graded alcohols. Antigen retrieval was carried out using an appropriate heat-induced procedure, and samples were immunostained using a BioGenex AutoStainer i6000® automated staining system, with antibodies against VEGF (A-20; dilution 1:200; Santa Cruz Biotech, CA, USA), COX-2 (CX-294; pre-diluted; Dako, CA, USA), CD31 (JC70A; pre-diluted; Dako) and HIF-1α (H1α67; dilution 1:50; Santa Cruz Biotech.). After buffer washes, samples were visualized using a Dako Envision System (Dako). Finally, the sections were lightly counterstained with Mayer's haematoxylin.

Evaluation of immunostaining. All of the immunostained sections were evaluated by four of the Authors (Y.T., T.O., A.S. and T.I.). The staining of VEGF and HIF-1α was assessed semi-quantitatively according to three indices: (i) the percentage of area stained (<10%, 25%, 50%, 75% and 100%), (ii) the intensity of staining (none, 0; weak, +1; moderate, +2; and strong, +3), and (iii) the final score [product of the area and intensity, called the histological (H) score]. In the evaluation of the staining for COX-2, the reactions in vascular endothelial cells, which were present in all specimens, were used as internal "built-in" controls, and cases with tumor cells showing significantly more intense staining than the internal control cells were recorded as being positive. The intensity of staining was graded as follows: weak, +1; moderate, +2; and strong, +3 [called the intensive (I) score].

Vascularity was measured by the average of the MVD. The MVD was measured by assessing CD31 immunostaining according to the international consensus report (20). The tumor areas with the three highest densities of distinctly highlighted hot spots were selected by light microscopy under low power magnification. The count of CD31-positive vessels was determined in three separate fields in each of these areas, and a ×200 field (0.785 mm^2 per field) was used for all evaluations. The counting score was calculated as the sum of the vessel counts of nine of these fields. The MVD was expressed as the number of microvessels/field.

According to the H score, the I score or the MVD score, the angiogenic activity of the histological specimens was graded as follows: low (0 point), intermediate (1 point) and high (2 points). On the basis of the sum of the four scores, the angiogenic score was classified as belonging to the high-score group (5 or more points) or the low-score group (4 or fewer points).

Statistical analysis. Student's *t*-test or Mann-Whitney *U*-test were applied to assess the immunoreactivity and patients' characteristics. The

Table I. *Patients' characteristics (N=75).*

Characteristic		Number/Value (%)
Age, median, years		69
(range)		39-88
Gender	Male	66 (88.0)
	Female	9 (12.0)
Surgical or pathological stage	IA	8 (10.7)
	IB	12 (16.0)
	IIB	16 (21.3)
	IIIA	15 (20.0)
	IIIB	12 (16.0)
	IV	12 (16.0)
Smoking status	Yes	66 (88.0)
	No	9 (12.0)
(Average B.I.)		808
Site of the primary tumor	RUL	33 (45.2)
	RML	4 (5.5)
	RLL	7 (9.6)
	LUL	18 (24.6)
	LLL	11 (15.1)
Positive tumor markers	Yes	38 (50.7)
(CEA, CYFRA, SLX, SCC)	No	37 (49.3)
Symptoms at diagnosis	Yes	47 (62.7)
	No	28 (37.3)

B.I., Brinkman index; RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper robe; LLL, left lower lobe; CEA, carcinoembryonic antigen; CYFRA, cytokeratin 19 fragment; SLX, sialyl Lewis-X antigen; SCC, squamous cell carcinoma antigen.

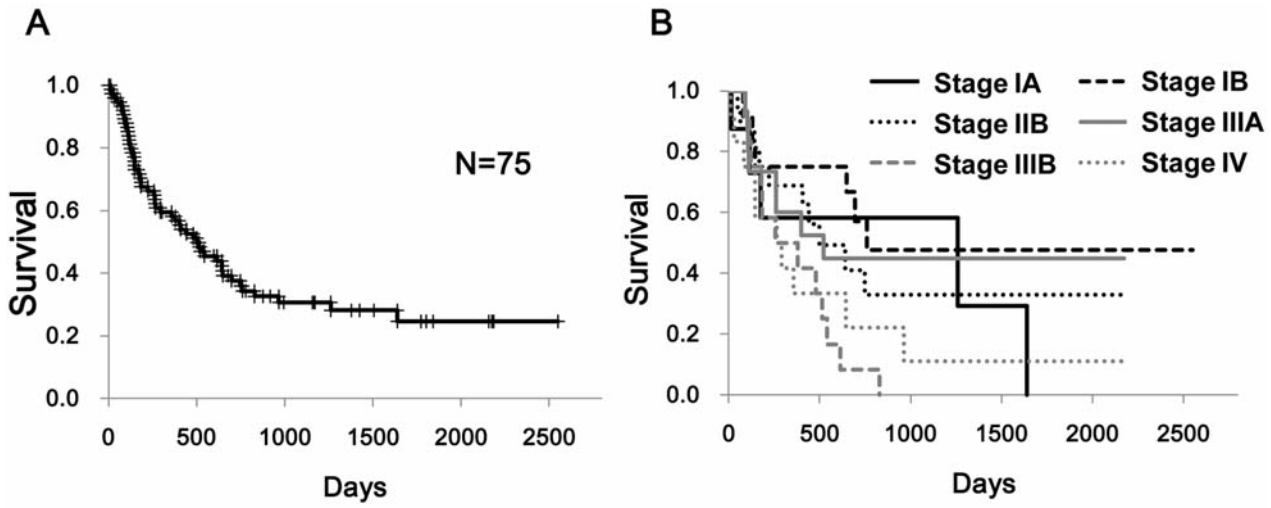

Figure 1. *The overall survival of patients with pleomorphic carcinoma. Survival curves according to all patients (A), and surgical or pathological stage (B). The patient numbers were 8/12/16/15/12/12 for stage IA/IB/IIB/IIIA/IIIB/IV, respectively.*

median survival time (MST) was calculated using the Kaplan-Meier method, and significance was determined using the log-rank test. For the multivariate analyses, the Cox proportional hazards model was used. We included possible factors affecting the overall survival, including the stage, symptoms, tumor markers, expression of VEGF, COX-2 and HIF-1α and the MVD. All of the calculations were performed using the SPSS software version 19 (IBM, Tokyo, Japan).

Results

Clinical findings. This study included 75 patients (66 males and 9 females), ranging in age from 39 to 88 years (median age; 69 years), 66 (88.0%) of them were current or former smokers, and 30 of them were heavy smokers (Brinkman index over 600) (Table I). No history of symptoms was available for 28 patients, but the other 47 patients had experienced one or more of the following symptoms: cough (27.7%), hemoptysis (25.5%), chest or upper back pain (14.9%) and dyspnea (6.4%). The right upper lobe (45.2%) was the most common location of the primary tumor. Unresectable disease of stage IIIB or IV was present in 24 cases (32.0%). The average follow-up time was 21 months, and the MST was 16.5 months (Figure 1A). An MST of 42.0 months was observed in stage IA parients, whereas the MST values were 25.3, 16.7, 17.3, 12.6 and 9.7 months, respectively, in those with stage IB, IIB, IIIA, IIIB and IV disease (Figure 1B).

Immunohistochemical findings. An immunohistochemical analysis was performed on tumor samples from 75 cases of PC. Figure 2 shows an example of the staining for tumor angiogenic factors in a representative sample. A cytoplasmic staining pattern was found for VEGF and COX-2. On the other hand, HIF-1α exhibited a nuclear staining pattern. The staining of VEGF, COX-2 and HIF-1α was assessed semiquantitatively according to the H or I score. The distribution of the intensity of each factor is shown in Figure 3. The VEGF expression was positive in many cases of PC, so the H scores were high (Figure 3A). Vascular areas were identified by immunohistochemical reaction to CD31 (Figure 3D). The mean of all MVDs was 22.8 microvessels/field. We also analyzed the angiogenic score to identify whether the level of angiogenesis in PC may affect the clinical course. According to the angiogenic score, 16 patients were classified in the high-score group, and the other 59 were classified in the low-score group. The MST was significantly different between the groups, with a value of 6.0 months for the high-score group and 21.4 months for the low-score group (p=0.008) (Figure 4). The surgical and pathological stage (stage IA-IIIA *vs*. IIIB, IV) and the presence of symptoms at the time of diagnosis (yes *vs*. no) also significantly differed according to the angiogenic score (p=0.005 and p=0.02) (Figure 5). No significant differences were detected in the tumor markers between the two groups.

When a Cox proportional hazards model was constructed, which included the stage, symptoms, tumor markers, expression levels of VEGF, COX-2 and HIF-1α, and the MVD, only the stage and symptoms predicted the overall survival (Table II, p=0.006 and 0.026, respectively).

Discussion

We retrospectively examined and analyzed the clinical background of a large number of PC cases. This is the first report that has focused specifically on the angiogenesis of PC and its relationship to the accompanied clinical features.

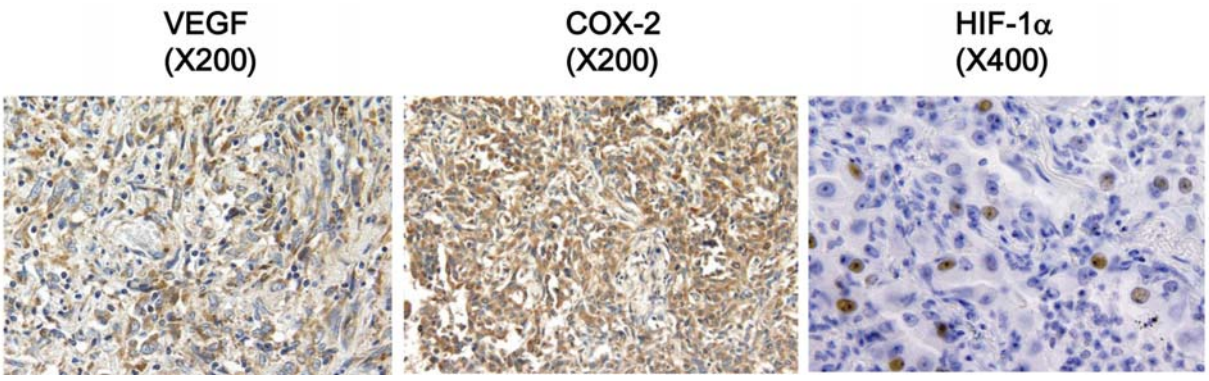

Figure 2. *Immunohistochemical staining for angiogenic factors in pleomorphic carcinoma. PC, Pleomorphic carcinoma; VEGF, vascular endothelial growth factor; COX-2, cyclooxygenase-2; HIF-1α, hypoxia-inducible factor-1α.*

The results of the present study indicate that the expression of angiogenic factors is related to the clinical course and prognosis in PC. In the present study, PC was found to be an aggressive tumor, and was strongly related to smoking. The majority of the tumors arose in the right upper lobe. The surgical and pathological stage and the presence of symptoms at the time of diagnosis were significantly associated with survival. Because VEGF, COX-2 and HIF-1α are overexpressed in PC, the results of this study suggest that prolonged inflammation and the hypoxic state of tumor cells strongly affect angiogenesis, at least partially through the up-regulation of VEGF in the PC. We therefore determined that the angiogenic score, obtained *via* an immunohistochemical examination-based classifier that can partition PC patients into subgroups, is associated with significantly different prognoses.

In the clinical setting, PC predominantly occurs in male heavy smokers with an average age at diagnosis of 60 years, and the disease has a very poor prognosis (3, 21). Several unique clinical features of PC have been demonstrated in past studies, such as the fact that the majority of PC patients have at least one symptom at the time of the diagnosis, that there are often large peripheral tumors, with chest wall invasion and distant metastasis to unusual organs (3, 14-15). Since the WHO pathological definition became widely recognized, PC has been diagnosed more frequently. However, although the number of case reports about the clinical background of PC has recently increased, there are few reports which have accumulated cases of PC to better understand its clinical features. Fishback *et al.* examined the clinical background of 78 cases of PC in 1994, and pointed out that the prognostic factors were the tumor size, metastasis to lymph nodes and the clinical stage (1). Rossi *et al.* (21) and Mochizuki *et al.* (3) analyzed the clinicopathological features in over 70 cases of PC, and both studies showed that PC was a high-grade, aggressive disease, which was associated with a poor prognosis.

Table II. *The results of the multivariate analysis of the prognostic factors for pleomorphic carcinoma of the lung.*

Variable	Hazard ratio	95% CI	*p*-Value
Surgical or pathological stage	2.708	1.325-5.533	0.006
Symptoms at diagnosis (+)	0.454	0.227-0.909	0.026
Positive tumor marker(s) (+)	1.664	0.903-3.067	0.103
High VEGF score	0.789	0.416-1.495	0.467
High COX-2 score	1.216	0.593-2.495	0.594
High HIF-1α score	1.292	0.724-2.305	0.387
High MVD score	0.712	0.385-1.316	0.279

CI, Confidence interval; VEGF, vascular endothelial growth factor; COX, cyclooxygenase; HIF-1α, hypoxia inducible factor-1α; MVD, microvessel density.

Our observations are compatible with these studies, and indicate that patients with PC often present with symptoms such as hemoptysis, chest pain and bloody pleural effusion at the time of diagnosis. The results of the present study also showed that PC has a poor prognosis, especially when symptoms are present at diagnosis. In addition, in our study, the majority of primary tumors arose from the right upper lobe. Moreover, in addition to the presence of symptoms at diagnosis, the surgical and pathological stage were also significantly associated with patient survival.

Angiogenesis is the formation of new blood vessels from pre-existing vessels, and it is essential for tumor growth, development and metastasis (5, 12). It is frequently assessed in tumors by evaluating the MVD, using an antibody to CD31. Previous reports have suggested that VEGF is overexpressed in 30-80% of NSCLCs (22). Several studies have found a correlation between the MVD and the expression of angiogenic factors, especially VEGF, and a poor prognosis and increased incidence of metastasis in patients with NSCLC (23-24). The MVD is thought to closely reflect the extent of intratumoral angiogenesis, and to be a good

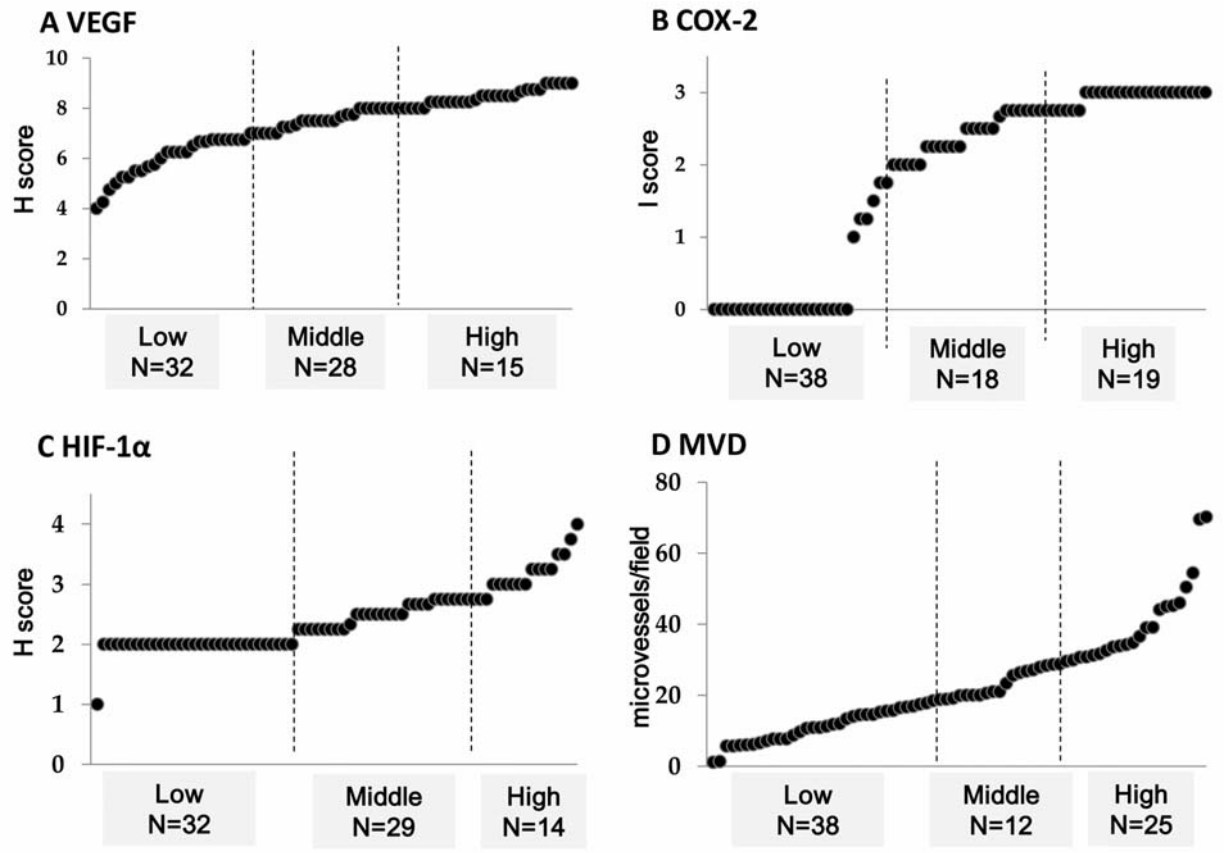

Figure 3. *The distribution of the intensity of angiogenic factors in immunohistochemical staining. VEGF, Vascular endothelial growth factor; COX-2, cyclooxygenase-2; HIF-1α, hypoxia inducible factor-1α; MVD, microvessel density.*

prognostic factor in cases of NSCLC. The present study showed that PC is associated with a very high MVD, and that the score for VEGF expression is high. Although the expression of angiogenic factors, as determined by an immunohistochemical analysis, was not a prognostic factor, patients with a high angiogenic score had a poorer prognosis than those with low scores. We also found that early-stage disease and the presence of symptoms at diagnosis were related to a higher expression of angiogenic markers. These results suggest that a poor prognosis and the frequency of distant metastasis in PC are influenced by angiogenesis.

The present study indicates that COX-2 and HIF-1α are affected and involved in angiogenesis, at least in part, in association with the up-regulation of VEGF in PC. COX-2 is selectively overexpressed in inflammatory and tumor tissues (13). The prognostic significance of elevated COX-2 expression in lung cancer was evaluated in several previous studies (25, 26). In addition, COX-2-mediated production of prostaglandin E2 and interleukin-1α appears to play an important role in tumor angiogenesis through the induction of VEGF (13). Some reports have shown the possibility that VEGF expression is regulated by the COX-2 pathway (27, 28).

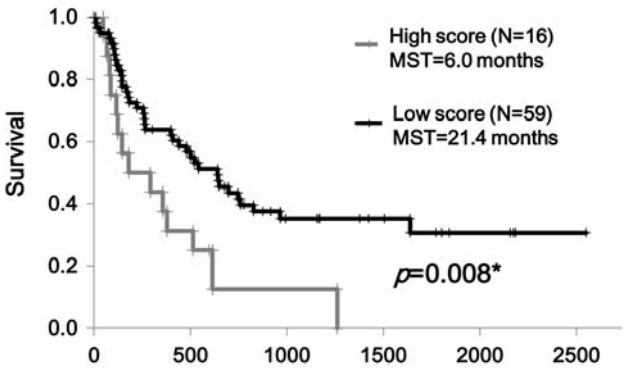

Figure 4. *The overall survival of patients with pleomorphic carcinoma based on the angiogenic score. MST, Median survival time. *Log-rank test.*

On the other hand, intratumoral hypoxia can lead to HIF-1α overexpression. This phenomenon is associated with a poor prognosis, resistance to chemotherapy and radiotherapy, and an increased potential for invasion, metastasis and patient mortality (17, 29, 30). Many researchers have reviewed the importance of intratumoral hypoxia in the regulation of tumor

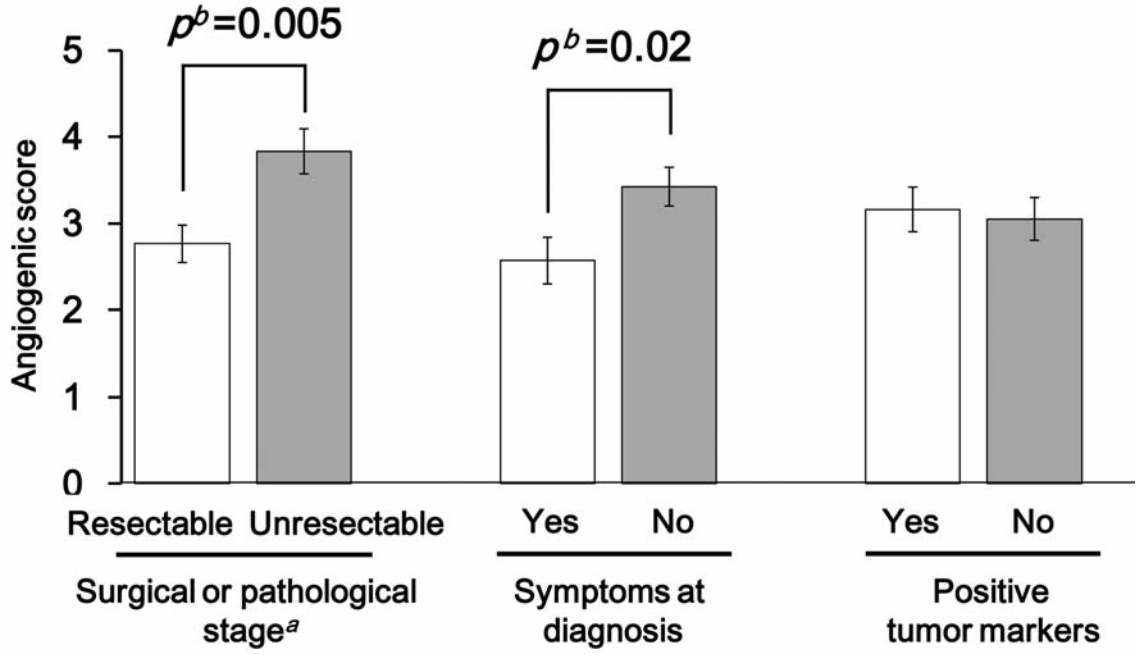

Figure 5. *Correlation of the angiogenic score and of the clinical features in pleomorphic carcinoma.* [a]*Early stage was stage I-IIIA, and advanced stage was stage IIIB and IV.* [b]*All p-values are from the Mann Whitney U-test.*

angiogenesis, which is associated with the production of VEGF (17, 18). The inhibition of HIF-1α activity had marked effects on tumor growth in preclinical studies (31, 32). In the clinic, efforts are currently underway to test the efficacy of HIF-1α inhibitors as anticancer therapeutics (33-35). Fishback *et al.* and Rossi *et al.* pointed out that an inflammatory background was present in some cases of PC (1, 21). We support the view that the tumor microenvironment, such as the presence of prolonged hypoxia and inflammation, leads to an increase in the MVD, resulting from the up-regulation of angiogenesis in PC. It might be considered that inhibiting or preventing angiogenesis could be used as a new treatment strategy for PC, and agents such as bevacizumab (an anti-human VEGF-A monoclonal antibody), COX-2 inhibitors (*e.g.* celecoxib) and HIF-1α inhibitors, should be considered.

Conclusion

In conclusion, the results of the current study showed that the stage and presence of symptoms at the time of diagnosis are prognostic factors in PC. Furthermore, tumor angiogenesis provides significant prognostic information about the clinical outcome. These findings suggest that prolonged inflammation and the hypoxic state of tumor cells affect angiogenesis of the tumor, and angiogenesis is related to the progression of PC. Since PCs are rare tumors, the optimal treatment and precise diagnosis remain unclear, with the only data currently available having been obtained from retrospective studies. We believe that our findings will improve the understanding over the clinical characteristics and the behavior of PC of the lung.

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References

1 Fishback NF, Travis WD, Moran CA, Guinee DG Jr., McCarthy WF and Koss MN: Pleomorphic (spindle/giant cell) carcinoma of the lung. A clinicopathologic correlation of 78 cases. Cancer *73*: 2936-2945, 1994.

2 Travis WD, Tlavis LB and Devesa SS: Lung cancer. Cancer *75*: 191-202, 1995.

3 Mochizuki T, Ishii G, Nagai K, Yoshida J, Mizuno T, Yokose T, Suzuki K and Ochiai A: Pleomorphic carcinoma of the lung. Clinicopathologic characteristics of 70 cases. Am J Surg Pathol *32*: 1727-1735, 2008.

4 Brambilla E, Travis WD, Colby TV, Corrin B and Shimosato Y: The new World Health Organization classification of lung tumors. Eur Respir J *18*: 1059-1068, 2001.

5 Folkman J: What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst *82*: 4-6, 1990.
6 Toi M, Kondo S, Suzuki H, Yamamoto Y, Inada K, Imazawa T, Taniguchi T and Tominaga T: Quantitative analysis of vascular endothelial growth factor in primary breast cancer. Cancer *77*: 1101-1106, 1996.
7 Paley PJ, Staskus KA, Gebhard K, Mohanraj D, Twiggs LB, Carson LF and Ramakrishnan S: Vascular endothelial growth factor expression in early stage ovarian carcinoma. Cancer *80*: 98-106, 1997.
8 Takahashi Y, Tucker SL, Kitadai Y, Koura AN, Bucana CD, Cleary KR and Ellis LM: Vessel counts and expression of vascular endothelial growth factor as prognostic factors in node-negative colon cancer. Arch Surg *132*: 541-546, 1997.
9 Eisma RJ, Spiro JD and Kreutzer DL: Vascular endothelial growth factor expression in head and neck squamous cell carcinoma. Am J Surg *174*: 513-517, 1997.
10 Samoto K, Ikezaki K, Ono M, Shono T, Kohno K, Kuwano M and Fukui M: Expression of vascular endothelial growth factor and its possible relation with neo-vascularization in human brain tumors. Cancer Res *55*: 1189-1193, 1995.
11 Yamazaki K, Abe S, Takekawa H, Sukoh N, Watanabe N, Ogura S, Nakajima H, Isobe H, Inoue K and Kawakami Y: Tumor angiogenesis in human lung adenocarcinoma. Cancer *74*: 2245-2250, 1994.
12 Yano T, Tanikawa S, Fujie T, Masutani M and Horie T: Vascular endothelial growth factor expression and neovascularisation in non-small cell lung cancer. Eur J Cancer *36*: 601-609, 2000.
13 Senger DR, Perruzzi CA and Feder J: A highly conserved vascular permeability factor secreted by a variety of human and rodent tumor cell lines. Cancer Res *46*: 5629-5632, 1986.
14 Sagisaka S, Uehara M, Harada M, Sato J, Uchiyama H, Imokawa S, Yasuda K, Tanioka F, Suda T and Chida K: Clinicopathological investigation of 5 autopsy cases of pulmonary pleomorphic carcinoma. Jpn J Lung Cancer *50*: 803-808, 2010.
15 Ito K, Oizumi S, Fukumoto S, Harada M, Ishida T, Fujita Y, Harada T, Kojima T, Yokouchi H and Nishimura M, Hokkaido Lung Cancer Clinical Study Group: Clinical characteristic of pleomorphic carcinoma of the lung. Lung Cancer *68*: 204-210, 2010.
16 Ramalingam S and Belani CP: Cycoloxygenase-2 inhibitors in lung cancer. Clin Lung Cancer *5*: 245-253, 2004.
17 Harris AL: Hypoxia – A key regulatory factor in tumour growth. Nat Rev Cancer *1*: 38-47, 2002.
18 Hockel M and Vaupel P: Tumor hypoxia : definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst *93*: 266-276, 2001.
19 UICC: TNM classification of malignant tumours. 6th and 7th eds. Geneva: UICC, 2002 and 2009.
20 Vermeulen PB, Gasparini G, Fox SB, Toi M, Martin L, McCulloch P, Pezzella F, Viale G, Weidner N, Harris AL and Dirix LY: Quantification of angiogenesis in solid human tumours: an international consensus on the methodology and criteria of evaluation. Eur J Cancer *32A*: 2474-2484, 1996.
21 Rossi G, Cavazza A, Sturm N, Migaldi M, Facciolongo N, Longo L, Mariorana A and Brambilla E: Pulmonary carcinomas with pleomorphic, sarcomatoid, or sarcomatous elements. A clinicopathologic and immunohistochemical study of 75 cases. Am J Surg Pathol *27*: 311-324, 2003.
22 Isobe T, Herbst RS and Onn A: Current management of advanced non-small cell lung cancer: Targeted therapy. Semin Oncol *32*: 315-328, 2005.
23 Meert AP, Paesmans M, Martin B, Delmotte P, Berghmans T, Verdebout JM, Lafitte JJ, Mascaux C and Sculier JP: The role of microvessel density on the survival of patients with lung cancer: a systematic review of the literature with meta-analysis. Br J Cancer *87*: 694-701, 2002.
24 Macchiarini P, Fontanini G, Hardin MJ, Squartini F and Angeletti CA: Relation of neovascularization to metastasis of non-small cell lung cancer. Lancet *340*: 145-146, 1992.
25 Achiwa H, Yatabe Y, Hida Y, Kuroishi T, Kozaki K, Nakamura S, Ogawa M, Sugiura T, Mitsudomi T and Takahashi T: Prognostic significance of elevated cyclooxygenase 2 expression in primary, resected lung adenocarcinoma. Clin Cancer Res *5*: 1001-1005, 1999.
26 Khuri FR, Wu H, Lee JJ, Kemp BL, Lotan R, Lippman SM, Feng L, Hong WK and Xu XC: Cycoloxygenase-2 overexpression is a marker of poor prognosis in stage I non-small cell lung cancer. Clin Cancer Res *7*: 861-867, 2001.
27 Zhu YM, Azahri NS, Yu DC and Woll PJ: Effects of COX-2 inhibition on expression of vascular endothelial growth factor and interleukin-8 in lung cancer cells. BMC Cancer *31*: 218-228, 2008.
28 Kim HS, Youm HR, Lee JS, Min KW, Chung JH and Park CS: Correlation between cyclooxygenase-2 and tumor angiogenesis in non-small cell lung cancer. Lung Cancer *42*: 163-170, 2003.
29 Giatromanolaki A, Koukourakis MI, Sivridis E, Turley H, Talks K, Pezzella F, Gatter KC and Harris AL: Relation of hypoxia inducible factor 1α and 2α in operable non-small cell lung cancer to angiogenic/molecular profile of tumors and survival. Br J Cancer *85*: 881-890, 2001.
30 Yohena T, Yoshino I, Takenaka T, Kameyama T, Ohba T, Kuniyoshi Y and Maehara Y: Upregulation of hypoxia-inducible factor 1alpha mRNA and its clinical significance in non-small cell lung cancer. J Thorac Oncol *4*: 284-290, 2009.
31 Semenza GL: Targeting HIF-1 for cancer therapy. Nat Rev Cancer *3*: 721-732, 2003.
32 Jacoby JJ, Erez B, Korshunova MV, Williams RR, Furutani K, Takahashi O, Kirkpatrick L, Lippman SM, Powis G, O'Reilly MS and Herbst RS: Treatment with HIF-1α antagonist PX-478 inhibits progression and spread of orthotopic human small cell lung cancer and lung adenocarcinoma in mice. J Thorac Oncol *5*: 940-949, 2010.
33 Ramalingam SS, Egorin MJ, Ramanathan RK, Remick SC, Sikorski RP, Lagattuta TF, Chatta GS, Friedlamd DM, Stoller RG, Potter DM, Ivy SP and Belani CP: A phase I study of 17-allylamino-17-demethoxygeldanamycin combined with paclitaxel in patients with advanced solid malignancies. Clin Cancer Res *14*: 3456-3461, 2008.
34 Provencio M, Sanchez A, Garrido P, and Valcarcel F: New molecular-targeted therapies integrated with radiation therapy in lung cancer. Clin Lung Cancer *1*: 91-97, 2010.
35 Goetz MP, Toft D, Reid J, Ames M, Stensgard B, Safgren S, Adjei AA, Sloan J, Atherton P, Vasile V, Salazaar S, Adjei A, Croghan G and Erlichman C: Phase I trial of 17-allylamino-17-demethoxygeldanamycin in patients with advanced cancer. J Clin Oncol *23*: 1078-1087, 2005.

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