ANTICANCER RESEARCH *37*: 5565-5571 (2017)
doi:10.21873/anticanres.11989

Association Between Endothelial Progenitor Cells and Treatment Response in Non-Squamous Non-small Cell Lung Cancer Treated with Bevacizumab

KAZUHISA SUDO[1], KAZUHIRO SATO[1], SHO SAKAMOTO[1], YUKIYASU HASEGAWA[1], MARIKO ASANO[1], YUJI OKUDA[1], MASAHIDE TAKEDA[1], MASAAKI SANO[1], HIROYUKI WATANABE[1], TAKANOBU SHIOYA[2] and HIROSHI ITO[1]

[1]*Department of Cardiovascular and Respiratory Medicine, Akita University Graduate School of Medicine, Akita, Japan;*
[2]*Department of Physical Therapy, Akita University Graduate School of Health Sciences, Akita, Japan*

Abstract. *Background/Aim: To investigate the association between the number of circulating endothelial progenitor cells (EPCs) in non-squamous non-small cell lung cancer (NSCLC) and disease outcome, in combination chemotherapy with and without bevacizumab. Materials and Methods: We retrospectively identified 25 non-squamous NSCLC cases, and divided them into high-EPC and low-EPC groups. Within each group, we compared disease outcomes, with or without the administration of bevacizumab. Results: In the high-EPC group, chemotherapy with bevacizumab produced a significantly higher tumor reduction rate and objective response rate, with significantly longer progression-free survival, compared to chemotherapy without bevacizumab ($p<0.001$, $p=0.010$, and $p<0.001$, respectively). However, in the low-EPC group, there were no significant differences in disease outcomes in groups with versus those without bevacizumab. Conclusion: The number of EPCs may be a useful biomarker to guide decision-making in the use of bevacizumab in non-squamous NSCLC.*

Non-small-cell lung cancer (NSCLC) is the most common type of lung cancer, accounting for approximately 80% of the total cases (1). Recently, it has become possible to tailor chemotherapy treatment for lung cancer according to its histological type or the presence of genetic variation (2-4). For non-squamous NSCLC, the use of bevacizumab is the standard approach in combination with chemotherapy (5, 6).

Bevacizumab is a humanized monoclonal antibody that specifically targets vascular endothelial growth factor (VEGF). One study reported bevacizumab down-regulated the expression of B-cell lymphoma 2 protein, VEGF and E-cadherin and inhibited vasculogenesis in MCF-7 breast cancer cell implantation onto chick embryo chorioallantoic membrane (7). In non-squamous NSCLC patients, response rates and progression-free survival were improved by treatment with conventional chemotherapy in combination with bevacizumab (5, 6). It is known that bevacizumab prevents tumor neovascularization and improves the arrival rate of chemotherapy for an additional anti-tumor effect. However, bevacizumab sometimes causes severe adverse events, including hypertension, proteinuria, and gastrointestinal hemorrhage (5). In addition, in terms of health economics, the use of bevacizumab increases the cost of treatment (8). Thus, the situations in which the effects of chemotherapy could be enhanced by the use of bevacizumab remain unknown.

Bevacizumab targets tumor angiogenesis, which is involved in tumor growth and metastasis (9). Neoplastic cells, stimulated by cytokines from pre-existing cells, secrete various cytokines including VEGF, platelet-derived growth factor (PDGF), interleukin (IL)-8, and IL-10. These cytokines promote tumor neovascularization (6, 10, 11). Endothelial progenitor cells (EPCs) are cells showing the presence of typical immunological cell surface markers, CD34, CD133, and VEGF receptor-2 (VEGFR-2), and originate from bone marrow (12, 13). Cytokines released from tumors induce EPCs that mobilize in the blood, where they differentiate into tumor vessels and cause tumor neovascularization (9). These findings suggest that EPCs

Correspondence to: Associate Prof. Kazuhiro Sato, Akita University Graduate School of Medicine, Hondo 1-1-1, Akita, Akita 0108543, Japan. Tel: +81 188846110, Fax: +81 188362612, e-mail: kazuhiro@doc.med.akita-u.ac.jp

Key Words: Non-squamous non-small-cell lung cancer, endothelial progenitor cell, tumor angiogenesis, bevacizumab, chemotherapy.

could be a potential biomarker for evaluation of the extent of tumor angiogenesis (3). Results from a previous study demonstrated that EPC counts in peripheral blood were higher in NSCLC patients than in healthy individuals, and treatment outcomes were improved in NSCLC patients with low EPC counts relative to those with high EPC counts (4). It is possible that high EPC counts cause increased tumor neovascularization and diminish the effects of chemotherapy. However, a previous report found no significant difference in outcome between 'high EPC' and 'low EPC' cancer patient groups, when both were treated with chemotherapy in combination with bevacizumab (14). Thus, in cancer patients with greater numbers of EPCs, bevacizumab may enhance chemotherapy treatment efficacy more than in those with fewer.

Therefore, we aimed to investigate whether treatment with chemotherapy in combination with bevacizumab increased efficacy more in patients who had non-squamous NSCLC and high EPC counts than in those with low numbers of EPCs. Then, we hypothesized that the number of EPCs might become a useful biomarker for deciding whether or not bevacizumab should be administered, in terms of adverse events and health economics.

Materials and Methods

Patient selection. Patients who had been diagnosed with advanced non-squamous NSCLC (inoperable stage IIIA, stage IIIB, or stage IV), but who had not yet received any treatment, were selected. We selected patients continually after diagnosis. Patients with brain metastases, bronchial luminal lesions, or infiltration of the large vessels were treated with carboplatin and paclitaxel. Other patients were treated with carboplatin, paclitaxel, and bevacizumab. Patients were diagnosed in Akita University Hospital from January 2012 to November 2015, and were free of additional malignant tumors, severe diabetes mellitus, wounds, or inflammatory diseases that might influence the number of EPCs. Before patient enrollment, the study was approved by our institutional ethics committee, and all patients provided informed consent before participating. Patients were staged according to the seventh edition of the tumor, node, metastasis (TNM) classification (15).

Study design. The aim of this study was to determine whether any association exists between EPC counts in non-squamous NSCLC patients and their response to combined chemotherapy and bevacizumab treatment. EPCs were defined as CD34- and VEGFR-2-positive cells. We divided patients into two groups according to their EPC level ('high EPC' and 'low EPC' groups) to evaluate whether there was a treatment benefit of bevacizumab according to the number of EPCs. The cutoff for a high *versus* low level of EPCs was 1,000 EPCs/ml of peripheral blood, in accordance with previous studies (4, 16). Both groups (with *vs*. without bevacizumab) received chemotherapy and the tumor reduction rate, progression-free survival, objective response rate, and disease control rate were compared between the patients who were treated with chemotherapy only and those who had the combination regimen. Treatment efficacy was assessed using computed tomography (CT) after two courses of chemotherapy, according to the Response Evaluation Criteria in Solid Tumors (RECIST; ver. 1.1) (17). In addition, we evaluated the correlation between the number of EPCs and the curative effects, and serum levels, of VEGF-A, IL-8, IL-10, and PDGF-BB.

Flow cytometry. Flow cytometry was used to measure the number of EPCs in peripheral blood before chemotherapy. Peripheral blood was collected into tubes containing EDTA using 21-gauge needles. To quantify the EPC content, fluorescence-activated cell sorting (FACS) analysis was performed, following the procedure from a previous study (4). Mononuclear cells were isolated from peripheral blood using Ficoll-Hypaque density gradient centrifugation. The expression of cell surface antigens was determined by two-color immunofluorescence staining, as described in a previous study (18). After centrifugation, the mononuclear cell layer was carefully transferred into a new tube, mixed with red cell lysis buffer for >10 min at 4˚C, and washed twice in phosphate-buffered saline containing 0.3% bovine serum albumin (BSA). A 100 μl volume of mononuclear cells from peripheral blood was incubated at 4˚C for 30 min while being protected from light, along with 20 μl of fluorescein isothiocyanate (FITC)-conjugated anti-human CD34 (BD Biosciences, San Jose, CA, USA) and 20 μl of R-Phycoerythrin (PE)-conjugated anti-human VEGFR-2 (R&D Systems, Minneapolis, MN, USA). Appropriate fluorochrome-conjugated isotype-matched immunoglobulin (Ig) G1 and IgG2a antibodies were used as negative controls for each patient. Cells were washed three times to remove unbound antibodies and finally re-suspended in 900 μl of FACS solution. After appropriate gating, cells that were identified as CD34- and VEGFR-2-positive were determined to be circulating EPCs in peripheral blood, and expressed as the number of cells per ml of blood using a Cytomics FC500 flow cytometer and CXP software (both Beckman Coulter, Miami, FL, USA).

Enzyme-linked immunosorbent assay (ELISA). The concentrations of VEGF-A, IL-8, IL-10, and PDGF-BB (all from Cloud-Clone Corp., Houston, TX, USA) were assessed using ELISA in serum samples obtained from all patients *via* 21-gauge needles prior to chemotherapy. ELISA was performed according to the manufacturer's instructions.

Statistical analysis. Categorical data were compared between groups using Fisher's exact probability test. Continuous data were compared using Student's *t*-test if the sample distribution was normal, or with the Mann-Whitney *U*-test if the sample distribution was asymmetric. Progression-free survival was defined as the time from commencement of treatment to disease progression or death from any cause; the curve was estimated using the Kaplan-Meier method and evaluated with a log-rank test. p-Values<0.05 were considered to be significant. All statistical analyses were carried out using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) (19), a graphical user interface for R software (ver. 2.13.0; R Development Core Team, Vienna, Austria). EZR is a modified version of R commander software (ver. 1.6.3) that includes statistical functions used frequently in biostatistics.

Results

Patient characteristics. From January 2012 to November 2015, 25 patients with untreated non-squamous NSCLC were

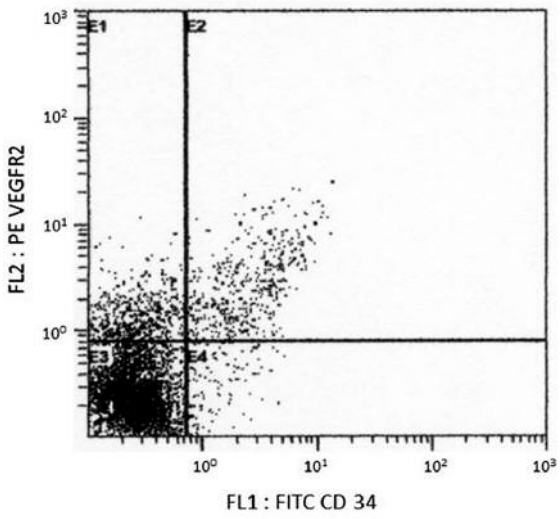

Figure 1. *Representative flow cytometric analysis of the number of CD34/vascular endothelial growth factor receptor-2 (VEGFR-2) double-positive cells. E1: CD34-/VEGFR-2+, E2: CD34+/VEGFR-2+, E3: CD34-/VEGFR-2–, E4: CD34+/VEGFR-2–.*

Table I. *Characteristics of the 'high EPC' and 'low EPC' patients.*

	EPC high (N=12)	EPC low (N=13)	*p*-Value
Age (years)			
Median	63 (56-65)	71 (65-74)	p=0.051
Gender (male)	10 (83%)	10 (77%)	p=0.59
Smoking History			p=0.32
Never-smoker	1 (8.3%)	4 (31%)	
Smoker	11 (92%)	9 (69%)	
Pathologic stage			p=0.22
III	0 (0%)	3 (23%)	
IV	12 (100%)	10 (77%)	
Therapy			p=0.43
Carboplatin+Paclitaxel	4 (33%)	7 (54%)	
Carboplatin+Paclitaxel+ Bevacizumab	8 (67%)	6 (46%)	

Data are n (%) or median (IQR).

enrolled in this study. The patients were 20 men and 5 women with a median age of 65 years (interquartile range (IQR)=62-71 years). Five patients had hypertension, three had dyslipidemia and one had bronchial asthma. Of the total of 25 patients, 20 were former or current smokers and 5 had never smoked. All patients were diagnosed with adenocarcinoma. One patient was Stage IIIa, two were Stage IIIB and twenty-two were Stage IV. One group of 14 patients received chemotherapy, consisting of carboplatin (area under the curve [AUC]=5 mg/l/h on day 1), paclitaxel (200 mg/m^2 on day 1), and bevacizumab (15 mg/kg on day 1). The remaining 11 patients received chemotherapy consisting of carboplatin (AUC=5 mg/l/h on day 1) and paclitaxel (200 mg/m^2 on day 1). Chemotherapy was administered over a 3-week period.

EPC, VEGF-A, IL-8, IL-10, and PDGF-BB levels and their correlations with EPC count. The number of cells that were both CD34- and VEGFR-2-positive (*i.e.*, EPCs) was determined by FACS analysis of peripheral blood samples (Figure 1). Among our 25 patients, the mean circulating EPC count was 1,138±680/ml (mean±standard deviation [SD]). Serum levels of VEGF-A, IL-8, IL-10, and PDGF-BB were measured using ELISA prior to administration of chemotherapy. The VEGF-A level was 1,207.0±570.8 pg/ml, the IL-8 level was 343.1±1,280.6 pg/ml, and the IL-10 level was 58.4±105.1 pg/ml. The PDGF-BB level was 6,008.8±3,865.3 pg/ml. No correlation was apparent between the number of EPCs and levels of VEGF-A, IL-8, IL-10, or PDGF-BB.

Next, patients were divided into two groups according to EPC level ('high EPC' and 'low EPC' groups). The cut-off for a high *versus* low level of EPCs was defined as 1,000 EPCs/ml of peripheral blood. Patient characteristics in the two groups are shown in Table I. The high EPC group comprised 12 patients and the low EPC group had 13 patients. There were no significant differences in any of the measured characteristics between the high and low EPC groups.

Efficacy of chemotherapy. We compared the tumor reduction rate, progression-free survival, objective response rate, and disease control rate between the high and low EPC groups, and according to whether patients had received either chemotherapy alone or the combination of chemotherapy and bevacizumab. In the high-EPC group, the tumor reduction rate of patients treated with bevacizumab was significantly higher than that of those who had received chemotherapy alone ($p<0.001$; Figure 2a, Table II). However, in the low-EPC group, there was no significant difference in the tumor reduction rate according to whether bevacizumab was part of the treatment ($p=0.82$; Figure 2b, Table II). In the high EPC group, progression-free survival of patients who had received bevacizumab was significantly longer compared with that of those who did not ($p<0.001$; Figure 3a). However, in the low-EPC group, there was no significant difference in progression-free survival according to whether the patients had received bevacizumab ($p=0.67$; Figure 3b). In the high-EPC group, the objective response rate of patients who received bevacizumab was significantly higher than that of those who received chemotherapy alone ($p=0.010$; Table II). However, in the low-EPC group, there

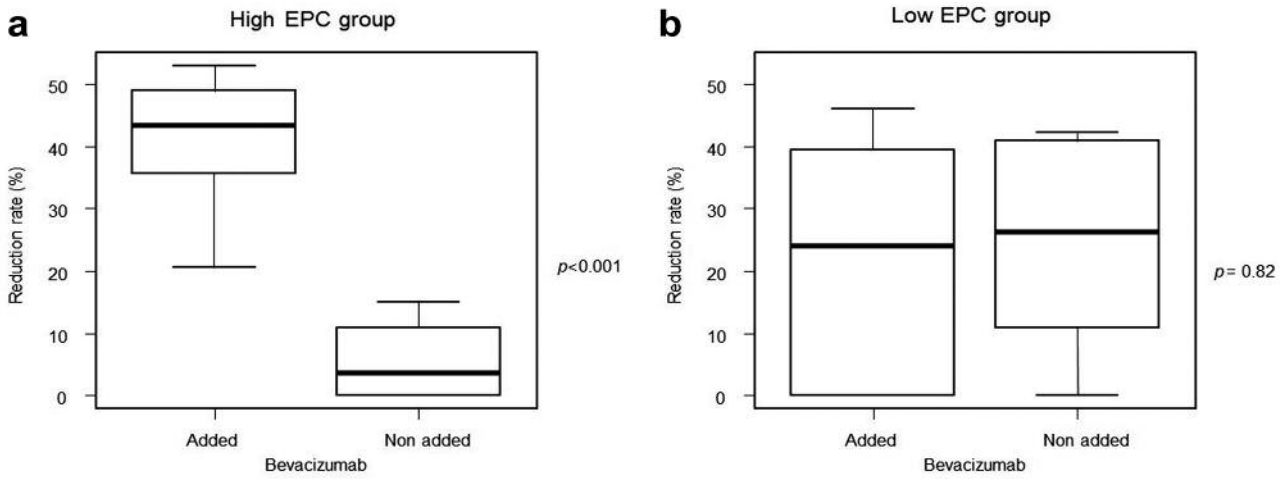

Figure 2. *Comparison of reduction rate according to the presence or absence of bevacizumab in the high endothelial progenitor cell (EPC) group (a). Comparison of reduction rate according to the presence or absence of bevacizumab in the low EPC group (b).*

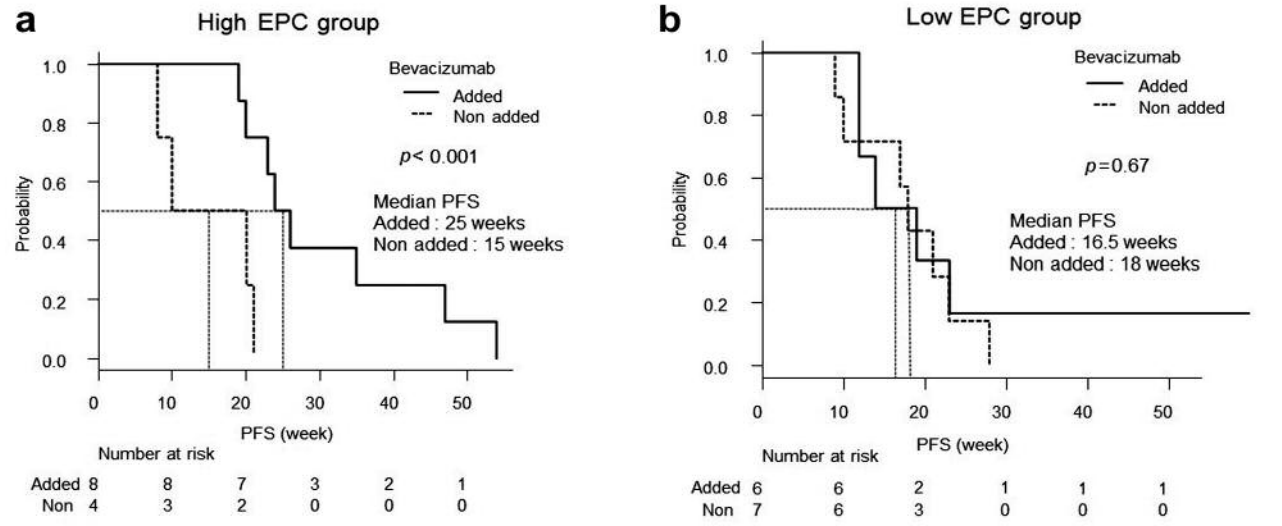

Figure 3. *Comparison of progression-free survival according to the presence or absence of bevacizumab in the high EPC group (a). Comparison of progression-free survival according to the presence or absence of bevacizumab in the low EPC group (b).*

was no significant difference in the objective response rate according to whether the patients had received bevacizumab (p=0.59; Table II). In the high- and low-EPC group, there was no significant difference in the disease control rate according to the presence or absence of bevacizumab (p=1; Table II). There was no correlation between improved outcomes and levels of VEGF-A, IL-8, IL-10, or PDGF-BB in either the high- or low-EPC group.

Discussion

This study investigated the relationship between the number of EPCs in non-squamous NSCLC patients and their disease outcomes following treatment with chemotherapy in combination with bevacizumab. It was demonstrated that the tumor reduction rate and objective response rate were significantly higher in patients with high EPC levels who

Table II. *Comparison of the reduction rate, objective response rate, and disease control rate according to the presence or absence of bevacizumab in the high- and low-EPC groups.*

	EPC high Bev (+)	EPC high Bev (–)	*p*-Value	EPC low Bev (+)	EPC low Bev (–)	*p*-Value
Reduction rate (%)						
mean (±SD)	41.3 (±10.6)	5.4 (±6.9)	<0.001	22.4 (±19.6)	24.9 (±17.2)	0.82
Objective response rate (%)	87.5	0	0.010	33.3	57.1	0.59
Disease control rate (%)	100	100	1	100	100	1

received chemotherapy in combination with bevacizumab compared with those who received chemotherapy alone. In addition, progression-free survival was significantly prolonged in the 'high EPC' patients who had received the combination treatment. Conversely, there was no significant difference in the tumor reduction rate, progression-free survival, objective response rate, or disease control rate among patients with low EPC levels according to the presence or absence of bevacizumab. There were no correlations between the level of EPCs and levels of VEGF-A, IL-8, IL-10, and PDGF-BB, nor between the levels of VEGF-A, IL-8, IL-10, and PDGF-BB and disease outcome. These results support our hypothesis that the efficacy of chemotherapy may be improved in 'high EPC' non-squamous NSCLC patients by administering bevacizumab.

Prior studies have shown that angiogenesis plays an important role in tumor progression, and that EPCs are mobilized from bone marrow into sites of tumor neovascularization (20). Recently, several studies have shown that tumor angiogenesis does not arise solely from endothelial cell proliferation and the sprouting of new capillaries; EPCs also participate in tumor angiogenesis and the development of tumor tissue vasculature (18, 21). Therefore, EPCs could potentially serve as surrogate markers of tumor angiogenesis status (3). Previous studies have reported that the number of peripheral blood EPCs in lung cancer patients is significantly higher than in healthy controls (4, 16, 22). These results support the idea that tumor angiogenesis is accelerated, and that greater numbers of EPCs are mobilized from bone marrow (and thus are found in peripheral blood), in lung cancer patients relative to healthy individuals.

There have been several reports investigating the relationship between EPC levels in peripheral blood and cancer patients' clinical presentations. One study reported that overall survival in NSCLC patients with high pre-treatment EPC numbers in peripheral blood was significantly shorter compared with that of patients with low pre-treatment EPC levels. (16). Morita *et al*. reported that, in NSCLC patients with low pre-treatment EPC levels in peripheral blood, progression-free survival was prolonged, and tumor reduction rates were higher, than in patients with high pre-treatment EPC numbers (4). It is possible that high levels of EPCs reflect less normalized tumor vessels and a chemotherapy response that is worse than in patients with low EPC levels. However, another study reported that the number of EPCs decreased rapidly in rectal cancer patients receiving bevacizumab (23). In addition, in rectal cancer, the number of EPCs was not predictive of clinical outcome when patients were treated with chemotherapy in combination with bevacizumab (14). Following these studies, we assessed the hypothesis that, in patients with high EPC levels, the use of bevacizumab would normalize the tumor vasculature when administered along with chemotherapy to non-squamous NSCLC patients (24). We also developed the hypothesis that the gap in clinical outcomes, between high and low EPC non-squamous NSCLC patients treated with chemotherapy in combination with bevacizumab, could be narrowed. Therefore, in our study, we divided these patients into 'high EPC' and 'low EPC' groups. We found that the reduction rate and objective response rate were significantly higher, and progression-free survival was significantly prolonged, among the 'high EPC' patients receiving chemotherapy plus bevacizumab compared with those receiving chemotherapy alone. However, in the low EPC group, there was no significant difference in clinical outcome according to whether or not bevacizumab was administered. Regarding the disease control rate in the high EPC group, there was no significant difference with *versus* without bevacizumab administration. One reason for this result might be that treatment efficacy was assessed after only two courses of chemotherapy.

Bevacizumab sometimes causes severe adverse events, including hypertension, proteinuria, and gastrointestinal hemorrhage (5). In addition, bevacizumab therapy is expensive (8). We investigated whether we could predict the patients who would respond well or poorly to bevacizumab; the results of our study indicate that the efficacy of chemotherapy may be improved in high EPC non-squamous NSCLC patients by administering bevacizumab. Thus, the EPC count in peripheral blood may become a useful biomarker, indicating whether treatment of non-squamous NSCLC with chemotherapy should or should not include bevacizumab.

It has been reported that several cytokines, such as VEGF, PDGF, IL-8, and IL-10, play important roles in the mobilization of EPCs and tumor angiogenesis. These cytokines have potential as biomarkers of tumor angiogenesis status, so we examined the correlation between the levels of VEGF-A, PDGF-BB, IL-8, and IL-10 and the number of EPCs, as well as the effects of treatment. One study reported that the level of serum VEGF-A in cancer patients was significantly higher than that in healthy controls (25). Also, high levels of PDGF are known to be related to poor disease-free and overall survival in NSCLC (26). PDGF-BB in particular acts on the PDGF β receptor, which stimulates pericyte development and promotes angiogenesis (27). IL-8, which was originally described as a proinflammatory chemotactic factor for neutrophils, is known to promote tumor angiogenesis (28). Another report found that high IL-8 levels in the serum of advanced NSCLC patients were correlated with short overall survival (29). IL-10 expression was also correlated with clinical outcome in NSCLC patients (11). Recently, several studies have shown that the genomic variants of IL-10 may serve as biomarkers for various types of cancer, such as leukemia (30), renal cell carcinoma (31) and lung cancer (32). In NSCLC, IL-10 polymorphisms may become biomarkers that predict prognosis. However, in our study, there was no correlation between EPC level, or clinical outcome, and levels of VEGF-A, IL-8, IL-10, or PDGF-BB. We surmise that EPCs are mobilized by multiple factors, including various cytokines, and the level of EPCs is reflected in the tumor angiogenesis status.

We acknowledge several limitations associated with our study. First, the sample size was small at 25. It will be necessary for us to recruit more patients in future studies. Second, there may have been a selection bias with respect to whether patients received chemotherapy with or without bevacizumab. In some patients not treated with bevacizumab, their tumors had invaded major blood vessels or had produced a brain metastasis, which may already confer a poor prognosis. However, as the patients in our study were chosen in the same condition between the high EPC group and low EPC group, we do not consider it likely that this bias significantly influenced our results. Third, we conducted only univariate analyses. In the future, we will recruit more patients to this study and conduct multivariate analyses.

In conclusion, in our study, the clinically beneficial effects of chemotherapy in combination with bevacizumab were significantly greater, compared to those of chemotherapy alone, among 'high EPC' non-squamous NSCLC patients. However, there was no significant difference in the clinical outcomes of 'low EPC' non-squamous NSCLC patients according to whether bevacizumab was administered. The EPC count may become a useful biomarker of whether bevacizumab would be advantageous for certain non-squamous NSCLC patients.

Conflicts of Interest

The Authors declare that they have no conflicts of interest.

References

1 Gridelli C, Rossi A and Maione P: Treatment of non-small-cell lung cancer: state of the art and development of new biologic agents. Oncogene *22*: 6629-6638, 2003.

2 Herbst RS, Onn A and Sandler A: Angiogenesis and lung cancer: prognostic and therapeutic implications. J Clin Oncol *23*: 3243-3256, 2005.

3 Maeda R, Ishii G, Ito M, Hishida T, Yoshida J, Nishimura M, Haga H, Nagai K and Ochiai A: Number of circulating endothelial progenitor cells and intratumoral microvessel density in non-small cell lung cancer patients: differences in angiogenic status between adenocarcinoma histologic subtypes. J Thorac Oncol *7*: 503-511, 2012.

4 Morita R, Sato K, Nakano M, Miura H, Odaka H, Nobori K, Kosaka T, Sano M, Watanabe H, Shioya T and Ito H: Endothelial progenitor cells are associated with response to chemotherapy in human non-small-cell lung cancer. J Cancer Res Clin Oncol *137*: 1849-1857, 2011.

5 Reck M, von Pawel J, Zatloukal P, Ramlau R, Gorbounova V, Hirsh V, Leighl N, Mezger J, Archer V, Moore N and Manegold C: Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer: AVAil. J Clin Oncol *27*: 1227-1234, 2009.

6 Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilenbaum R and Johnson DH: Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med *355*: 2542-2550, 2006.

7 Comsa S, Popescu R, Avram S, Ceausu RA, Cimpean AM, and Raica M: Bevacizumab Modulation of the Interaction Between the MCF-7 Cell Line and the Chick Embryo Chorioallantoic Membrane. In Vivo *31*: 199-203, 2017.

8 Gonzalez Garcia J, Gutierrez Nicolas F, Nazco Casariego GJ, Valcarcel Nazco C, Batista Lopez JN and Oramas Rodriguez J: Cost-effectiveness of pemetrexed in combination with cisplatin as first line treatment for patients with advanced non-squamous non-small-cell lung cancer in Spain. Farm Hosp *41*: 3-13, 2017.

9 Folkman J, Watson K, Ingber D and Hanahan D: Induction of angiogenesis during the transition from hyperplasia to neoplasia. Nature *339*: 58-61, 1989.

10 Angelo LS and Kurzrock R: Vascular endothelial growth factor and its relationship to inflammatory mediators. Clin Cancer Res *13*: 2825-2830, 2007.

11 Hatanaka H, Abe Y, Naruke M, Tokunaga T, Oshika Y, Kawakami T, Osada H, Nagata J, Kamochi J, Tsuchida T, Kijima H, Yamazaki H, Inoue H, Ueyama Y and Nakamura M: Significant correlation between interleukin 10 expression and vascularization through angiopoietin/TIE2 networks in non-small cell lung cancer. Clin Cancer Res *7*: 1287-1292, 2001.

12 Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G and Isner JM: Isolation of putative progenitor endothelial cells for angiogenesis. Science *275*: 964-967, 1997.

13 Hristov M, Erl W and Weber PC: Endothelial progenitor cells: isolation and characterization. Trends Cardiovasc Med *13*: 201-206, 2003.

14 Ronzoni M, Manzoni M, Mariucci S, Loupakis F, Brugnatelli S, Bencardino K, Rovati B, Tinelli C, Falcone A, Villa E and Danova M: Circulating endothelial cells and endothelial progenitors as predictive markers of clinical response to bevacizumab-based first-line treatment in advanced colorectal cancer patients. Ann Oncol *21*: 2382-2389, 2010.
15 Goldstraw P, Crowley J, Chansky K, Giroux DJ, Groome PA, Rami-Porta R, Postmus PE, Rusch V and Sobin L: The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. J Thorac Oncol *2*: 706-714, 2007.
16 Dome B, Timar J, Dobos J, Meszaros L, Raso E, Paku S, Kenessey I, Ostoros G, Magyar M, Ladanyi A, Bogos K and Tovari J: Identification and clinical significance of circulating endothelial progenitor cells in human non-small cell lung cancer. Cancer Res *66*: 7341-7347, 2006.
17 Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D and Verweij J: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer *45*: 228-247, 2009.
18 Dome B, Hendrix MJ, Paku S, Tovari J and Timar J: Alternative vascularization mechanisms in cancer: Pathology and therapeutic implications. Am J Pathol *170*: 1-15, 2007.
19 Kanda Y: Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant *48*: 452-458, 2013.
20 Siegel RL, Miller KD and Jemal A: Cancer statistics, 2016. CA Cancer J Clin *66*: 7-30, 2016.
21 Hilbe W, Dirnhofer S, Oberwasserlechner F, Schmid T, Gunsilius E, Hilbe G, Woll E and Kahler CM: CD133 positive endothelial progenitor cells contribute to the tumour vasculature in non-small cell lung cancer. J Clin Pathol *57*: 965-969, 2004.
22 Nowak K, Rafat N, Belle S, Weiss C, Hanusch C, Hohenberger P and Beck G: Circulating endothelial progenitor cells are increased in human lung cancer and correlate with stage of disease. Eur J Cardiothorac Surg *37*: 758-763, 2010.
23 Willett CG, Boucher Y, Duda DG, di Tomaso E, Munn LL, Tong RT, Kozin SV, Petit L, Jain RK, Chung DC, Sahani DV, Kalva SP, Cohen KS, Scadden DT, Fischman AJ, Clark JW, Ryan DP, Zhu AX, Blaszkowsky LS, Shellito PC, Mino-Kenudson M and Lauwers GY: Surrogate markers for antiangiogenic therapy and dose-limiting toxicities for bevacizumab with radiation and chemotherapy: continued experience of a phase I trial in rectal cancer patients. J Clin Oncol *23*: 8136-8139, 2005.
24 Yu M, Men H-T, Niu Z-M, Zhu Y-X, Tan B-X, Li L-H and Jiang J: Meta-Analysis of Circulating Endothelial Cells and Circulating Endothelial Progenitor Cells as Prognostic Factors in Lung Cancer. Asian Pac J Cancer Prev *16*: 6123-6128, 2015.
25 George ML, Tutton MG, Janssen F, Arnaout A, Abulafi AM, Eccles SA and Swift RI: VEGF-A, VEGF-C, and VEGF-D in colorectal cancer progression. Neoplasia *3*: 420-427, 2001.
26 Horinouchi H: Anti-vascular endothelial growth factor therapies at the crossroads: linifanib for non-small cell lung cancer. Transl Lung Cancer Res *5*: 78-81, 2016.
27 Hellberg C, Ostman A and Heldin CH: PDGF and vessel maturation. Recent Results Cancer Res *180*: 103-114, 2010.
28 Sanmamed MF, Carranza-Rua O, Alfaro C, Onate C, Martin-Algarra S, Perez G, Landazuri SF, Gonzalez A, Gross S, Rodriguez I, Munoz-Calleja C, Rodriguez-Ruiz M, Sangro B, Lopez-Picazo JM, Rizzo M, Mazzolini G, Pascual JI, Andueza MP, Perez-Gracia JL and Melero I: Serum interleukin-8 reflects tumor burden and treatment response across malignancies of multiple tissue origins. Clin Cancer Res *20*: 5697-5707, 2014.
29 Orditura M, De Vita F, Catalano G, Infusino S, Lieto E, Martinelli E, Morgillo F, Castellano P, Pignatelli C and Galizia G: Elevated serum levels of interleukin-8 in advanced non-small cell lung cancer patients: relationship with prognosis. J Interferon Cytokine Res *22*: 1129-1135, 2002.
30 Lo WJ, Chang WS, Hsu HF, Ji HX, Hsiao CL, Tsai CW, Yeh SP, Chen CM, and Bau DT: Significant Association of Interleukin-10 Polymorphisms with Childhood Leukemia Susceptibility in Taiwan. In Vivo *30*: 265-269, 2016.
31 Chang WS, Liao CH, Tsai CW, Hu PS, Wu HC, Hsu SW, Ji HX, Hsiao CL and Bau DT: The Role of IL-10 Promoter Polymorphisms in Renal Cell Carcinoma. Anticancer Res *36*: 2205-2209, 2016.
32 Hsia TC, Chang WS, Liang SJ, Chen WC, Tu CY, Chen HJ, Yang MD, Tsai CW, Hsu CM, Tsai CH, and Bau DT: Interleukin-10 (IL-10) promoter genotypes are associated with lung cancer risk in Taiwan males and smokers. Anticancer Res *34*: 7039-7044, 2014.

Received August 8, 2017
Revised August 23, 2017
Accepted August 24, 2017