Prognostic value of insulin-like growth factor 2 mRNA-binding protein 3 and vascular endothelial growth factor-A in patients with primary non-small-cell lung cancer

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Abstract. Insulin-like growth factor 2 mRNA-binding protein 3 (IMP3) and vascular endothelial growth factor-A (VEGF-A) may play important roles in the process of tumor progression and tumor angiogenesis. The aim of the present study was to examine the co-expression of IMP3 and VEGF-A in primary human non-small cell lung cancer (NSCLC), to investigate the association between these two expression levels and determine the clinicopathological implications, including changes to microvessel density (MVD), and to assess the prognostic value of co-expression. Using immunohistochemical staining, the expression of IMP3, VEGF-A and CD34 expression was detected in 128 primary NSCLC tissue samples. According to the expression of IMP3 and VEGF-A, the cases were divided into four groups. Next, the clinicopathological features, MVD and survival time were investigated across the different groups. The immunohistochemical analyses demonstrated that there was a significant correlation between IMP3 and VEGF-A expression in NSCLC (r=0.181; P=0.041). Co-expression of IMP3 and VEGF-A was significantly associated with larger primary tumor size (P=0.016), poorer differentiation (P=0.014), more advanced Tumor-Node-Metastasis stage (P=0.012), increased MVD (P=0.004) and positive lymph node metastasis (P=0.002). Survival analysis demonstrated that cases with IMP3 and VEGF-A double-positive staining were significantly associated with lower survival rates compared with cases with double-negative staining (P=0.039). In the early NSCLC (I-IIa) subgroup, the mean survival time of the double-positive staining group was significantly shorter compared with that of the double-negative staining group (P=0.015). Co-expression of IMP3 and VEGF-A was associated with angiogenesis and a poorer prognosis in NSCLC, and may therefore play a critical role in NSCLC progression.

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Introduction

Lung cancer is the most common malignant tumor and the leading cause of cancer-associated mortality worldwide (1). The majority of all histological types of lung cancer (~80%) are classified as non-small cell lung cancer (NSCLC) (2). Despite the introduction of targeted therapies and recently immune checkpoint inhibitors that have changed the prognosis of NSCLC, the 5-year survival rate was only 18.4% (3). One of the most important factors directly affecting the prognosis and therapeutic strategy in NSCLC is tumor angiogenesis. Tumor angiogenesis is regulated by complex interactions of multiple pro-angiogenic and anti-angiogenic factors (4). Therefore, it is necessary to understand the mechanism underlying tumor angiogenesis, in order to improve NSCLC prognosis.

Angiogenesis is estimated by determining the mean microvessel density (MVD) (5). To the best of our knowledge, vascular endothelial growth factor-A (VEGF-A) is the most efficient and specific pro-angiogenic factor known thus far; it plays a critical role in tumor growth, metastasis and angiogenesis (6). VEGF-A binding to its receptors triggers multiple signaling cascades, resulting in endothelial cell proliferation, migration and differentiation (7). In fact, the FDA has approved certain anti-angiogenesis antibodies, such as Bevacizumab and Ramucirumab, for the treatment of solid tumors (8).

Insulin-like growth factor 2 mRNA-binding protein 3 (IMP3/IGF2BP3) acts as an oncofetal protein. IMP3 is known to be involved in the regulation of cell proliferation and migration during embryogenesis (9). In adult tissues, IMP3 expression is low or undetectable; however, it has been found to be overexpressed in malignant tumors (10). The overexpression of IMP3 promotes cell proliferation, tumor migration and

invasion (11). It has also been reported that the expression of IMP3 is associated with tumor angiogenesis (12,13).

Based on the above data, IMP3 and VEGF-A may play important roles in tumor progression and angiogenesis. Although IMP3 and VEGF-A have been widely investigated in a variety of cancer types, to the best of our knowledge, whether the expression of IMP3 in tumor cells is associated with VEGF-A expression in NSCLC, and whether their co-expression has clinicopathological significance, particularly for angiogenesis and survival time, has never been evaluated. Therefore, the aim of the present study was to focus on these aspects.

Materials and methods

Patient characteristics. Tumor specimens were obtained from 128 patients with primary NSCLC who underwent surgery at the Yantai Yuhuangding Hospital (Yantai, China) between February 2014 and October 2015. The present study included 92 male and 36 female patients, with a median age of 60 years (range, 39-78 years) at the time of diagnosis. NSCLC was diagnosed by histology or cytology in all 128 cases, 58 of which were squamous cell carcinomas (SQC) and 70 were adenocarcinomas (ADC). The cell differentiation degree was evaluated, and 92 cases were well and moderately differentiated, while 36 cases were poorly differentiated. According to the Union for International Cancer Control 8th edition staging system for NSCLC (14,15), the Tumor-Node-Metastasis (TNM) classification system was used, and 45 patients with early NSCLC (I-IIa) and 83 with advanced NSCLC (IIb-III) were found. The clinical features of all patients are summarized in Table I. Eastern Cooperative Oncology Group (ECOG) Performance Status of less than or equal to 1 and patients with adequate organ function were included. Patients with prior history of cancer, other than basal cell carcinoma after appropriate treatment, or prior systemic chemotherapy treatment or radiation treatment were excluded. All patients were followed up for at least 3 years, with a median follow-up period of 41 months (range, 3-55 months), after surgery. The overall survival (OS) time was calculated as the period from the date of surgery to death or the last follow-up. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board (Medical Ethics Committee of Yantai Yuhuangding Hospital), and written informed consent was obtained from all patients.

Main reagents. The main reagents were as follows: Monoclonal mouse anti-human CD34 antibody (1:100; cat. no. TA320263; OriGene Technologies, Inc.); anti-IGF2BP3 rabbit polyclonal antibody (1:200; cat. no. TA322483; OriGene Technologies, Inc.); anti-VEGF-A rabbit monoclonal antibody (1:100; cat. no. ab52917; Abcam); immunohistochemical SP (Streptavidin-Peroxidase) DAKO Envision detection kit (cat. no. K4065; Dako; Agilent Technologies, Inc.) and 3,3'-diaminobenzidine (DAB) color reagent (cat. no. MAX-001 MAX007TM; Fuzhou Maixin Biotech Co., Ltd.).

Immunohistochemistry. Immunohistochemical staining was performed using the DAKO Envision detection kit (cat. no. K4065; Dako; Agilent Technologies, Inc.), according to the manufacturer's protocols. Briefly, all specimens were fixed with 10% formalin for 6-24 h at room temperature, paraffin embedded, cut into 4-μm sections, and dried. To remove paraffin wax, sections were placed in two containers of xylene for 5 min each. Fresh xylene should be used as incomplete deparaffinization can also lead to inconsistent staining. To start rehydration, place sections in three containers of 100% ethanol, 95% ethanol, 85% ethanol for 5 min each. To complete the rehydration process, sections were washed two times in dH_2O for 5 min each. Antigen retrieval was performed by incubating the tissue sections in 0.01 M citric acid buffer (pH 6.0) at 100˚C for 15 min. After cooling for 30 min at room temperature, the slides were incubated with 3% H_2O_2 in methanol for 15 min at room temperature to eliminate endogenous peroxidase activity. The slides were then blocked with 10% normal goat serum in use (cat. no. K4065; Dako; Agilent Technologies, Inc.) for 10 min at room temperature, and incubated with an appropriately diluted primary monoclonal mouse anti-human CD34 antibody (dilution, 1:100; cat. no. TA320263; OriGene Technologies, Inc.), anti-VEGF-A rabbit monoclonal antibody (dilution, 1:200; cat. no. TA322483; OriGene Technologies, Inc.) or anti-IGF2BP3 rabbit polyclonal antibody (dilution, 1:200; cat. no. TA322483; OriGene Technologies, Inc.) at 4˚C overnight. After incubation with biotinylated secondary antibodies (ready-to use, no dilution; cat. no. K4065; Dako; Agilent Technologies, Inc.) for 30 min at room temperature, the slides were subsequently incubated with streptavidin-peroxidase complex for 30 min at room temperature. Each of the previous steps was followed by PBS washes for 5 times. The slides were stained with 3,3'-diaminobenzidine as a chromogen, and then counterstained with Mayer's hematoxylin at 25˚C for 1 min. Negative controls were prepared using normal mouse and rabbit immunoglobulin G instead of the primary antibody. The immunostained sections were viewed using light-microscopy at a magnification of x200 (Nikon eclipse 80i). Semi-quantitative analysis of sections was used with image pro plus software 6.0 (Media Cybernetics, Inc.).

Immunohistochemical evaluation of VEGF-A and IMP3. Two independent pathologists, who were blinded to the clinical data, examined all specimens simultaneously using a double-headed microscope. The percentage of stained cells was recorded by counting at least 5 random fields at a magnification of x200. Discordant results were reviewed by a senior pathologist, and a consensus was reached. Only a membranous and/or cytoplasmic expression pattern was considered to indicate positive staining. As cancer cells exhibited heterogeneous staining, the dominant staining pattern was used for scoring. A combined scoring system was used according to a previously published methodology (16) that considers the intensity of staining, as well as the percentage of stained cells. The intensity of staining was graded from 0 to 3 as follows: 0, negative; 1, weak; 2, moderate; and 3, strong. The percentage of stained cells was defined as follows: 0, negative; 1, <10% positive cells; 2, 10-50% positive cells; 3, 51-80% positive cells; and 4, >80% positive cells. When the staining intensities varied significantly in the same field of view, the mean of the least intense and most intense staining was recorded. These two variables (staining intensity and percentage) were then

Table I. Correlations of IMP3 and VEGF-A co-expression with clinicopathological factors in primary human NSCLC.

Variables	I^-V^-	I^+V^+	P-value[a]	I^+V^-	P-value[b]	I^-V^+	P-value[c]
Sex			0.062		0.698		0.970
Male	17	42		18		15	
Female	2	20		7		7	
Age, years			0.222		0.127		0.513
>60	11	26		15		11	
≤60	8	36		10		11	
Histology			0.170		0.112		0.136
SQC	7	34		9		8	
ADC	12	28		16		14	
Tumor size, cm			0.016		0.207		0.048
>5	3	29		8		5	
≤5	16	33		17		17	
Differentiation			0.014		0.096		0.333
WD, MD	18	41		21		12	
PD	1	21		4		10	
TNM stage			0.012		0.019		0.098
I-IIa	10	14		12		9	
IIb-III	9	48		13		13	
Nodal status			0.002		0.165		0.105
Positive	3	35		10		8	
Negative	16	27		15		14	

[a]P-value between I^+V^+ and I^-V^-; [b]P-value between I^+V^- and I^-V^-; [c]P-value between I^-V^+ and I^-V^-; NSCLC, non-small cell lung cancer; IMP3, insulin-like growth factor 2 mRNA-binding protein 3; VEGF-A, vascular endothelial growth factor-A; NSCLC, non-small cell lung cancer; I^+V^+, IMP3- and VEGF-A-positive expression; I^+V^-, IMP3-positive and VEGF-A-negative expression; I^-V^+, VEGF-A-positive and IMP3-negative expression; I^-V^-, IMP3- and VEGF-A-negative expression; SQC, squamous cell carcinoma; ADC, adenocarcinoma; WD, well-differentiated; MD, moderately differentiated; PD, poorly differentiated; TNM, Tumor-Node-Metastasis; I-IIa, early NSCLC; IIb-III, advanced NSCLC.

multiplied to obtain a final score for each sample (range, 0-12). For the statistical analyses, cases with scores ranging from 0 to 5 were defined as negative, and all others were considered to be positive.

Immunohistochemical evaluation of MVD. MVD was detected via CD34 immunohistochemical staining of tumor vessels and was measured according to a modification of the Weidner's method (17). Any single endothelial cell cluster identified by positive CD34 staining, with or without lumen, was counted as a single microvessel. The five most hypervascular areas (hot spots) were selected under light-microscopy at low magnification (x40). MVD was then determined by counting the number of immune-stained microvessels per field (x200) in the five hot spots for each case. The mean number of microvessels across the five fields/hotspots was recorded as the MVD for each sample.

Statistical analysis. SPSS version 18.0 (SPSS, Inc.) was used for data analyses. A χ^2 test was used to assess the association between IMP3 and VEGF-A. Spearman's correlation coefficient was used to analyze the correlation between IMP3 and VEGF-A expression. An analysis of variance test was used for comparisons of MVD between multiple groups along with the least significant difference post hoc test. OS curves were generated using the Kaplan-Meier method and compared with a log-rank test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

IMP3 and VEGF-A co-expression in primary human NSCLC and its association with clinical characteristics. The expression of IMP3 was observed in the nuclei and cytoplasm of cancer cells (Fig. 1A and B), while VEGF-A expression (Fig. 1C and D) was mainly observed in the cytoplasm. Negative or weak staining for IMP3 and VEGF-A was observed in stromal cells and peritumoral normal lung tissue. High expression of IMP3 and VEGF-A was observed in 67.97 (87/128) and 65.63% (84/128) of the 128 NSCLC cases, respectively. The present study demonstrated that IMP3 and VEGF-A were co-expressed in NSCLC tumor cells, and a positive correlation existed between IMP3 and VEGF-A ($r=0.181$; $P=0.041$; Fig. 2).

According to the expression of IMP3 and VEGF-A, all patients with primary NSCLC were classified into 4 groups (Table I). A total of 48.44% patients (62/128) had positive expression of IMP3 and VEGF-A (I^+V^+), 19.53% patients (25/128) had positive expression of IMP3 but negative expression of VEGF-A (I^+V^-),

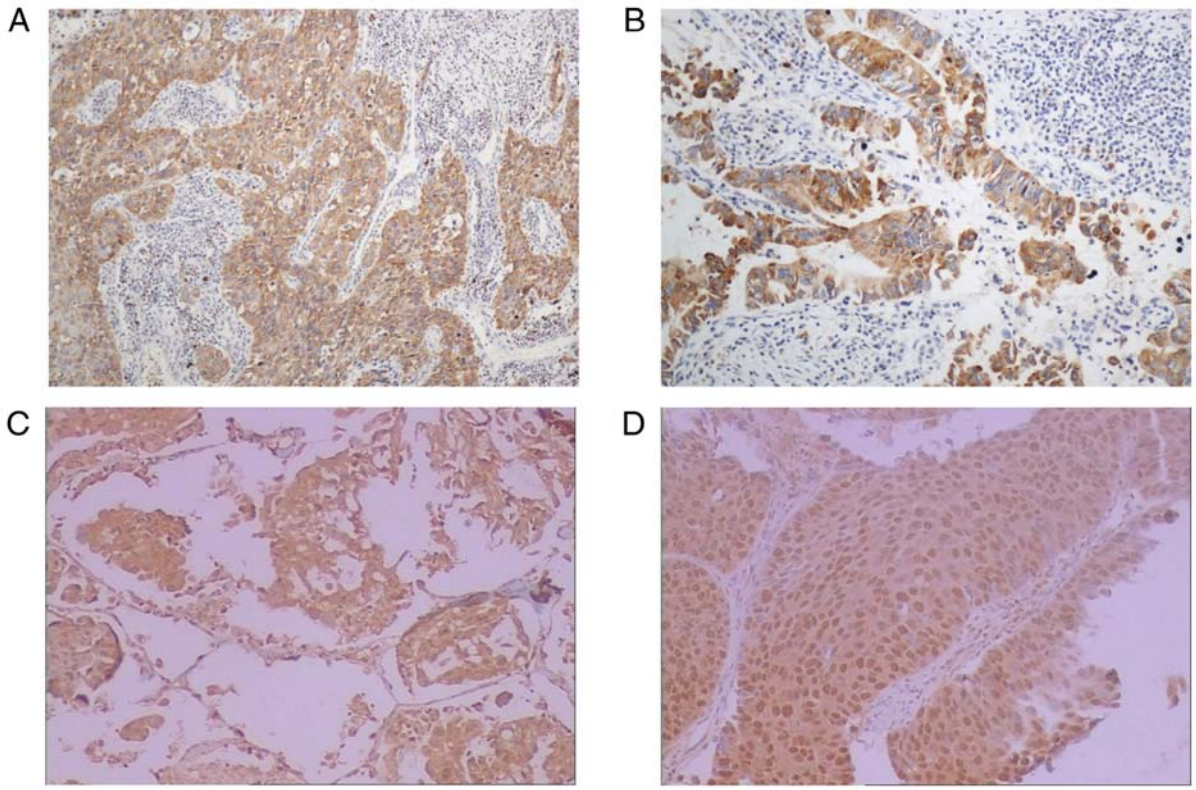

Figure 1. Immunohistochemical staining of IMP3 and VEGF-A in primary non-small cell lung cancer tissues. IMP3 expression in (A) adenocarcinoma and (B) squamous cell carcinoma. VEGF-A expression in (C) adenocarcinoma and (D) squamous cell carcinoma. Magnification, x200. IMP3, insulin-like growth factor 2 mRNA-binding protein 3; VEGF-A, vascular endothelial growth factor-A.

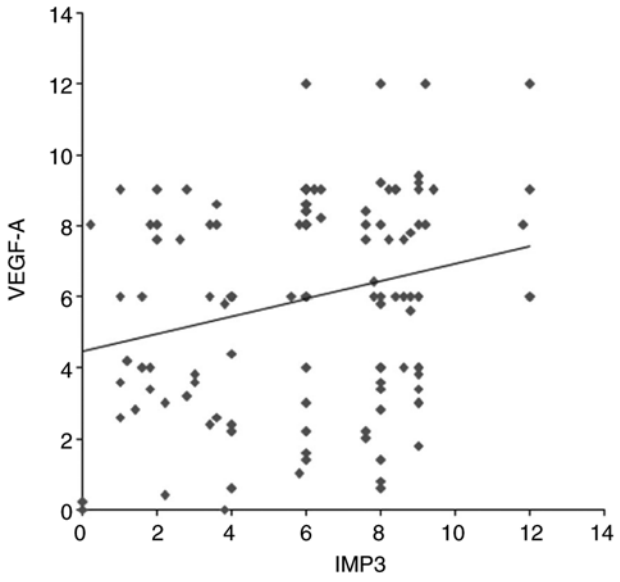

Figure 2. Significant correlation between IMP3 and VEGF-A expression in non-small-cell lung cancer tumor cells (r=0.181; P=0.041). IMP3, insulin-like growth factor 2 mRNA-binding protein 3; VEGF-A, vascular endothelial growth factor-A.

17.19% patients (22/128) had positive expression of VEGF-A but negative expression of IMP3 (I^-V^+) and 14.84% patients (19/128) had negative expression of both IMP3 and VEGF-A (I^-V^-). The association between the clinical characteristics and the expression of IPM3/VEGF-A across the four groups is shown in Table I. Compared with the I^-V^- group, the I^+V^+ group was significantly associated with larger primary tumor size (P=0.016), poorer differentiation (P=0.014), more advanced TNM stages (P=0.012) and positive lymph node metastasis (P=0.002). In addition, the I^+V^- group was associated with more advanced TNM stage (P=0.019) compared with the I^-V^- group, and the I^-V^+ group was characterized by larger primary tumor size (P=0.048).

Association between MVD and co-expression of both IPM3 and VEGF-A in primary human NSCLC. CD34 expression was strictly detected in the cytoplasm of vascular endothelial cells (Fig. 3A and B). The amount of intratumoral MVD, observed by CD34 staining, was significantly higher in the IMP3-positive group compared with that in the IMP3-negative group (48.69±24.25 vs. 36.71±24.36; P=0.01), similar to that observed in VEGF-A positive and -negative groups (48.30±25.15 vs. 38.27±23.08; P=0.029). The association between IMP3/VEGF-A co-expression and the amount of MVD was also evaluated. The mean MVD level was 51.15±23.99 in the I^+V^+ group, 42.60±24.28 in the I^+V^- group, 40.27±27.14 in the I^-V^+ group and 32.58±20.63 in the I^-V^- group. Compared with the I^-V^- group, the I^+V^+ group exhibited a significantly increased MVD level (P=0.004; Fig. 4). No other significant differences were identified in the remaining pairwise comparisons.

Association between IMP3/VEGF co-expression and OS. The mean survival time of the entire NSCLC group was 25.1 months. The mean survival time of the I^+ group was shorter compared with that of the I^- group (24.5 vs. 27.4 months, respectively;

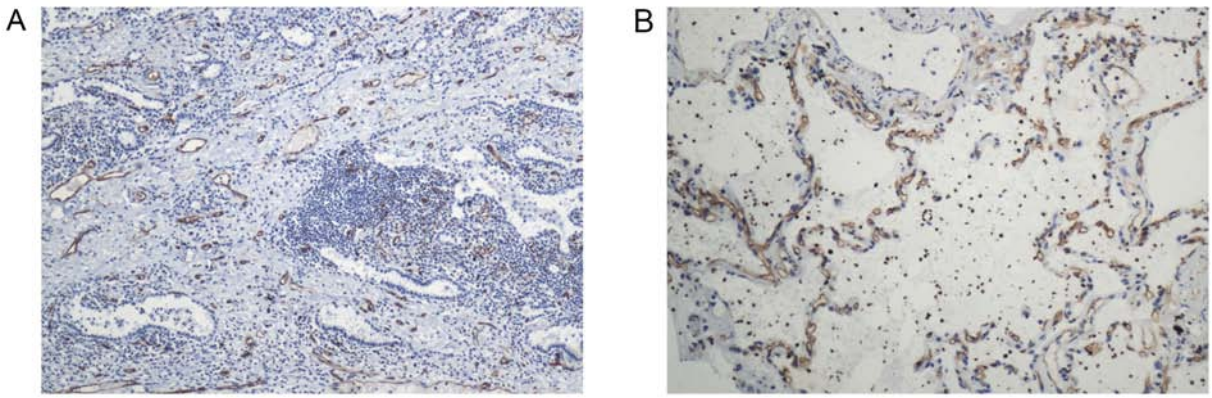

Figure 3. Immunohistochemical staining of CD34 in primary non-small cell lung cancer tissues. CD34 expression in (A) adenocarcinoma and (B) squamous cell carcinoma. Magnification, x200.

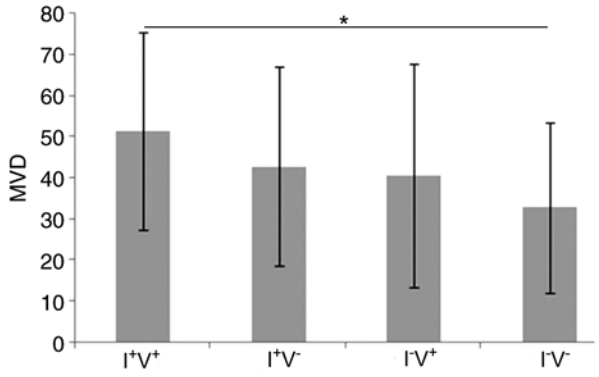

Figure 4. Comparison of intratumoral MVD among patients with I^+V^+, I^+V^-, I^-V^+ and I^-V^-. The error bars represent the standard deviation. *P<0.01. MVD, micro vessel density; IMP3, insulin-like growth factor 2 mRNA-binding protein 3; VEGF-A, vascular endothelial growth factor-A; I^+V^+, IMP3- and VEGF-A-positive expression; I^+V^-, IMP3-positive and VEGF-A-negative expression; I^-V^+, VEGF-A-positive and IMP3-negative expression; I^-V^-, IMP3- and VEGF-A-negative expression.

P=0.06; Fig. 5A), and the mean survival time in the V^+ group was shorter compared with that in the V^- group (23.9 vs. 27.9 months, respectively; P=0.095; Fig. 5B); however, these differences were not statistically significant. The mean survival time of the I^+V^+ group was 23.5 months, which was significantly shorter compared with that of the I^-V^- group (31.4 months; P=0.039); no other significant differences were observed between other pairwise comparisons (Fig. 5C). Survival was evaluated separately in early (I-IIa) and advanced (IIb-III) NSCLC subgroups. In the early (I-IIa) NSCLC subgroup, the mean survival time in the I^+V^+ group was significantly shorter compared with that of I^-V^- group (25.8 vs. 33.4 months, respectively; P=0.015; Fig. 5D). In the advanced (IIb-III) NSCLC subgroup, the mean survival time of the I^+V^+ group was shorter compared with that of I^-V^- group (22.8 vs. 27.4 months, Fig. 5E); however the differences were not statistically significant (P=0.338).

Discussion

Patients with NSCLC usually have a variable prognosis, which depends on the biological characteristics of the tumor. Angiogenesis is one of the important prerequisites for tumor growth and metastasis. The development of anti-angiogenic compounds for the treatment of tumors increases the importance of angiogenesis evaluation in cancer (4). MVD, a measure used to evaluate angiogenesis, has been validated as an independent prognostic factor for patients with cancer (18,19). VEGF-A has also been identified as an effective pro-angiogenic factor that has been found to be upregulated in various types of human tumors, including breast, colorectal and gastric cancer, and NSCLC, and it is positively correlated with MVD (20-23). In the present study, the levels of intratumoral MVD were found to be significantly higher in the VEGF-A-positive group compared with those in the VEGF-A-negative group, as reported by previous studies (24-26). However, the results regarding the association between VEGF-A expression and survival time in patients with cancer were conflicting. VEGF-A has been proven to be associated with survival time in certain studies (27,28), whereas other studies (19,29,30) have not found such an association. The results of the present study showed that high expression of VEGF-A occurred in the majority of the patients; however, no correlation between VEGF-A and survival time was observed.

Various angiogenic factors and their receptors are simultaneously expressed, and their overlapping expression may contribute to aggressive tumor growth. In addition to VEGF-A, other factors may play an important role in tumor angiogenesis. The association between IMP3 and tumor development has attracted research attention, with several studies linking IMP3 and cancer (16). IMP3 may play an essential and multifaceted role in tumorigenesis, and has been implicated in the migration and invasion of tumor cells by affecting the epithelial-to-mesenchymal transition of cancer cells (31,32). In addition, IMP3 has been demonstrated to promote the adhesion and proliferation of tumor cells by increasing the translation of IGF-2 mRNA (33,34). Further studies have revealed that IMP3 is upregulated in a variety of cancer types, including renal cell carcinoma, breast cancer and ovarian carcinoma (35-38). The overexpression of IMP3 in lung cancer has also been documented (39,40). Patients with IMP3 overexpression more often develop distant metastases compared with patients with negative IMP3 expression in lung adenocarcinoma (41). An association between IMP3 and angiogenesis has also been reported. In bone giant cell tumors, MVD was observed to be significantly higher in the IMP3-positive group

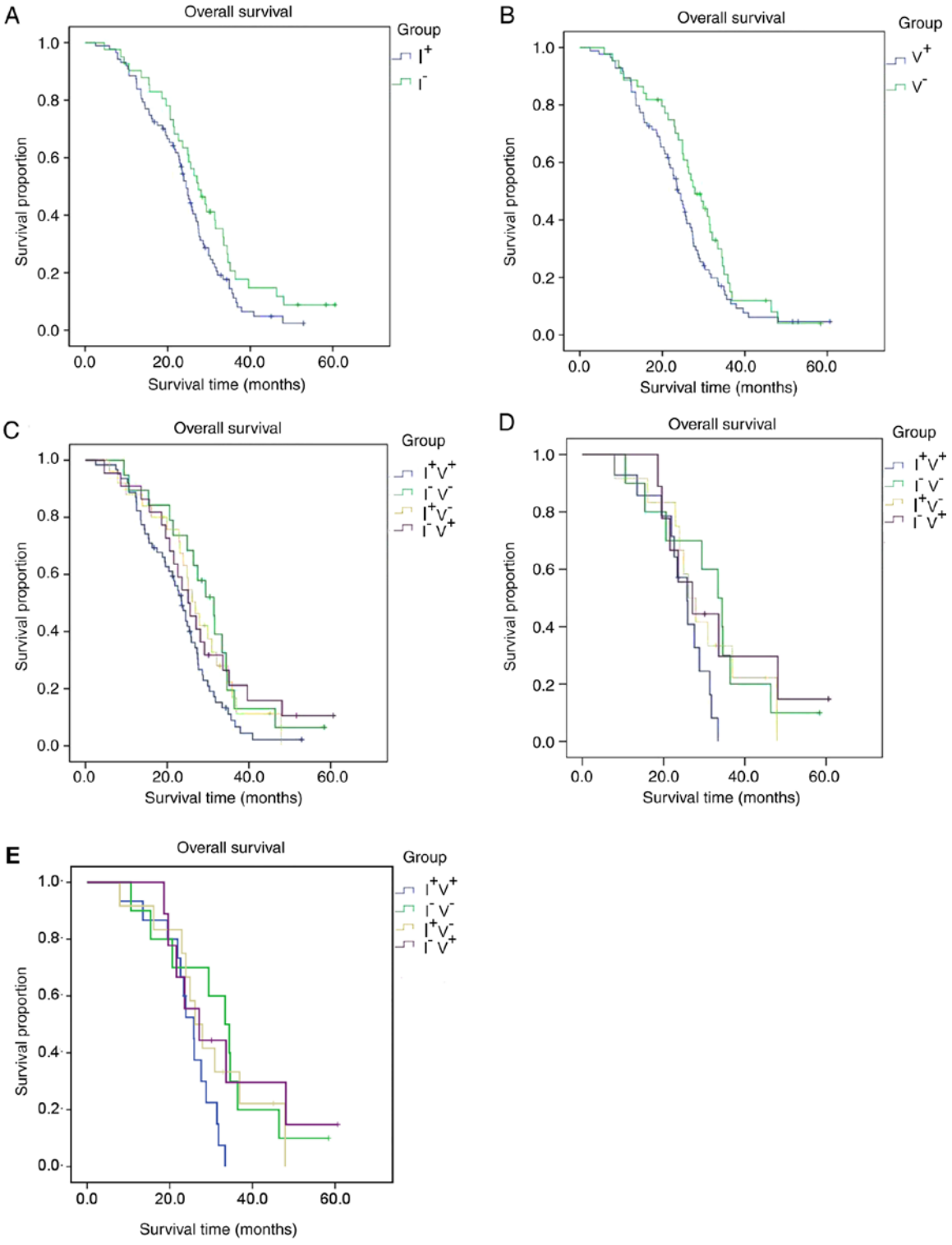

Figure 5. Associations between the expression of IMP3 and VEGF-A and overall survival in patients with NSCLC. (A) Differences in the overall survival rates of I^+ and I^- patients. (B) Differences in the overall survival rates of V^+ and V^- patients. (C) Differences in the overall survival rates among patients in the I^+V^+, I^+V^-, I^-V^+ and I^-V^- subgroups. (D) Differences in the overall survival rates in the early (I-IIa) NSCLC subgroup among patients who are I^+V^+, I^+V^-, I^-V^+ and I^-V^-. (E) Differences in the overall survival rates in the the advanced (IIb-III) NSCLC subgroup among patients who are I^+V^+, I^+V^-, I^-V^+ and I^-V^-. IMP3, insulin-like growth factor 2 mRNA binding protein 3; VEGF-A, vascular endothelial growth factor-A; NSCLC, non-small cell lung cancer; I^+V^+, IMP3- and VEGF-A-positive expression; I^+V^-, IMP3-positive and VEGF-A-negative expression; I^-V^+, VEGF-A-positive and IMP3-negative expression; I^-V^-, IMP3- and VEGF-A-negative expression.

compared with that in the IMP3-negative group, and therefore, IMP3 may play a role in regulating angiogenesis (12). Similar results were indicated in the present study, as IMP3 expression was observed in lung cancer cell nuclei and cytoplasm, with high expression of IMP3 occurring in the majority patients with NSCLC. MVD was also significantly higher in the IMP3-positive group compared with that in the IMP3-negative group. All these results indicate that IMP3 may play a role in

tumor angiogenesis; however, its exact function in angiogenesis remains to be fully elucidated.

Similar to VEGF-A, the results on the correlation between IMP3 expression and survival are also conflicting. Some studies identified IMP3 as an independent risk factor for poor prognosis in lung cancer and renal cell carcinoma patients (42-44). However, in the present study, the difference in survival time between the IMP3-positive and negative groups was not statistically significant, as reported by another previous study (41).

Although both IMP3 and VEGF-A have been observed to be upregulated in tumor cells across a number of types of cancer, the results on the association between the expression of IMP3 or VEGF and survival are conflicting. In order to identify a useful prognostic biomarker for determining tumor malignancy, their co-expression pattern was evaluated in the present study. IMP3 and VEGF-A were observed to be co-expressed in NSCLC tumor cells, and a positive correlation was indicated. Compared with that in the I^-V^- group, the MVD in the I^+V^+ group was higher. These results suggested that IMP3 and VEGF-A may act synergistically to promote angiogenesis in NSCLC. This result is consistent with previously published reports. Gharib *et al* (45) observed a significant correlation between VEGF and IGFBP3 mRNA in lung adenocarcinomas; however, no such correlation was detected in normal lung samples, and the associations between the co-expression of the two factors and clinical characteristics or survival time were not evaluated. The results of the present study demonstrated that the co-expression of IMP3 and VEGF-A was significantly associated with larger primary tumor size, poorer differentiation, advanced TNM stage and positive lymph node metastasis. In addition, co-expression of both factors was correlated with poorer survival, as the mean survival time of the I^+V^+ group was significantly shorter compared with that of the I^-V^- group. The results of the survival analysis in the early (I-IIa) NSCLC subgroup indicated that the co-expression of IMP3 and VEGF-3 may be a good prognostic marker for patients with early NSCLC. This correlation in patients with advanced NSCLC will be further investigated in the future by increasing the sample size.

Different agents including antibodies and small molecules have been extensively investigated to block VEGF and its pro-angiogenic functions. Bevacizumab and ramucirumab have been approved by the Food and Drug Administration agency. Moreover, some VEGFR tyrosine kinase inhibitors such as sunitinib, sorafenib and pazopanib are undergoing clinical trials. Combination therapies are also being pursued for better tumor control (46). The present study demonstrated that IMP3 and VEGF-A may act cooperatively to promote tumor angiogenesis, resulting in a poorer prognosis. Therefore, targeting both IMP3 and VEGF-A may be a promising strategy for anticancer therapy.

However, the results of the present study have certain limitations. A small sample size led to a low correlation coefficient for the co-expression of IMP3 and VEGF-A. The exact association between IMP3 and VEGF-A remains unknown and controversial. It has been previously demonstrated that IMP3 may induce VEGF expression in a human keratinocyte cell line (47). In a number of other studies, IMP3 was shown to suppress VEGF expression in NSCLC and other cell types (48-51). For these reasons, further studies will be conducted on their correlation through expanding the sample size, and their correlation through the level of DNA will be further analyzed.

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Availability of data and materials

The datasets used and/or analyzed for the present study are available from the corresponding author on reasonable request.

Authors' contributions

JL and YL designed the study, analyzed the data and drafted the manuscript. WG assisted with the design of the study and collected clinical data. XK and CW performed the immunohistochemistry and collected clinical data. SW and AL conceived and designed the study, analyzed the data and edited the manuscript.

Ethics approval and consent to participate

All tissue samples from patients were collected and protocols were performed according to the procedures approved by the Institutional Review Board of the Independent Medical Ethics Committee of Yantai Yuhuangding Hospital [approval no. (2018) 115]. Written informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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