

Circulating Vascular Endothelial Growth Factors and their Soluble Receptors in Pre-invasive, Invasive and Recurrent Cervical Cancer

SHERKO KUEMMEL¹, ANKE THOMAS², SOLVEIG LANDT¹, ANDREA FUGER², PETER SCHMID³, MONIKA KRINER⁴, JENS-UWE BLOHMER⁵, JALID SEHOULI⁶, GERHARD SCHALLER⁷, WERNER LICHTENEGGER⁶, ANGELA KÖNINGER¹ and ILKA FUCHS⁶

¹Department of Gynecology and Obstetrics, University of Duisburg-Essen, Essen;

²Department of Gynecology and Obstetrics, University Medicine Berlin, Campus Charité Mitte, Berlin, Germany;

³Charing Cross and Hammersmith Hospital, Imperial College London, London, United Kingdom;

⁴Department of Medical Statistics and Epidemiology, Technical University Munich;

⁵Department of Gynecology and Obstetrics, Sankt Gertrauden Hospital Berlin, Berlin;

⁶Department of Gynecology and Obstetrics, University Medicine Berlin, Campus Charité Virchow, Berlin;

⁷Breast Care Unit, Munich, Germany

Abstract. Aim: To study the impact of circulating vascular endothelial growth factors (VEGF) -A, -C and -D and their soluble receptors VEGFR-1/-2 on disease invasion and progression in patients with pre-invasive (CIN), invasive (PCC) and recurrent (RCC) cervical cancer. Patients and Methods: Blood samples were obtained from 125 women, including 50 cases of CIN, 51 of PCC and 24 of RCC, before treatment. Soluble (s) biomarker levels were determined by ELISA and tested for correlation with histopathological factors. Results: With disease progression, sVEGF-A ($p=0.007$) and sVEGFR-2 ($p=0.014$) significantly increased, while sVEGFR-D ($p=0.046$) decreased. sVEGFR-2 levels were increased in node+ patients ($p=0.024$) and in metastatic disease ($p=0.003$). sVEGF-A values were higher in HPV+ cases ($p=0.019$). In detecting disease invasiveness, sensitivity and specificity were 76% and 48% for sVEGF-A, 52% and 32% for sVEGFR-D, 25% and 94% for sVEGF-C, 93% and 6% for sVEGFR-1 and 73% and 34% for sVEGFR-2, respectively. Conclusion: In cervical neoplasia, a switch from a lymphangiogenic phenotype towards a hemangiogenic phenotype occurs with disease invasion and progression. The

sensitivity and specificity values, however, seem not convincing enough to establish these factors as clinical markers for disease invasiveness in cervical cancer.

The formation of new vessels is obligatory for the growth, progression and spread of epithelial tumors. The family of vascular endothelial growth factors (VEGF) are known to be involved in tumor neoangiogenesis. Presently, four VEGF members have been identified: While VEGF-A and VEGF-B are mainly involved in hemangiogenesis (1), VEGF-C and its close homolog VEGF-D take part primarily in the growth of lymphatic vessels (2, 3). In several types of carcinoma, including cervical cancer, a high expression of VEGF has been associated with disease progression and poor outcome (4-8). Circulating VEGFs are regarded as surrogate markers of neoangiogenesis. To date, studies investigating the influence of the various circulating VEGF members on disease progression and their prognostic significance in cervical cancer are limited. A potential clinical use of circulating VEGF-A and -C in cervical cancer has recently been hypothesized based on reports that pre-therapeutic serum (s) VEGF-A and sVEGF-C values increase with advancing stage, decrease after treatment and correlate with disease recurrence or disease persistence after treatment (9). No studies as yet exist regarding the value of sVEGF-D measurements nor of circulating levels of the corresponding VEGF receptors VEGFR-1/-2 in patients with cervical neoplasm.

This study aimed to investigate firstly, if the levels of sVEGF-A, -C, -D and their receptors sVEGFR-1 and -2 change between preinvasive, invasive and recurrent disease;

Correspondence to: Sherko Kuemmel, Department of Gynecology and Obstetrics, University of Duisburg-Essen, Hufelandstr. 55, 45122 Essen, Germany. Tel: +49 2017232346 Fax: +49 2017235663, e-mail: Sherko.Kuemmel@uk-essen.de

Key Words: Soluble VEGF members, soluble VEGF receptors, cervical carcinoma, disease progression, tumor markers, vascular endothelial growth factor.

secondly, if changes occur, whether the markers behave in a similar manner; and thirdly, if the parameters correlate with established clinicopathological factors in cervical cancer.

Patients and Methods

Study population. This prospective study included all patients with newly diagnosed preinvasive and invasive cervical cancer who were evaluated and treated at Charité University Hospital, Berlin, between January 2003 and December 2005. Patients with a history of other cancer were excluded. The study was conducted in accordance with local regulations and with the approval of the local institutional review boards. All patients gave written informed consent for this study. A total of 125 women were enrolled into this study, comprising 50 pre-invasive (CIN), 51 primary invasive (PCC) and 24 recurrent invasive cervical carcinomas (RCC). All patients were staged following the guidelines of the International Federation of Gynecology and Obstetrics (FIGO). Histological diagnosis was established following surgical resection or biopsy. Pathological specimens were centrally reviewed and classified according to the World Health Organization classification.

Sample collection. Serum and plasma samples were collected before initiation of treatment. Blood samples were obtained by peripheral venous puncture, centrifuged immediately at 3000 ×g, aliquoted and immediately frozen at -80°C until further analysis.

Assays. The values of plasma VEGF-A, serum VEGF-C, serum VEGF-D and serum VEGFR-1 /-2 were determined using the Quantikine Human Vascular Endothelial Growth Factor Immunoassay (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's guidelines and as described elsewhere (10).

Statistical analysis. Comparisons between unpaired groups were made using the Mann-Whitney *U*-test. Comparisons between multiple groups were performed using the Kruskal-Wallis test. Correlations between the serum parameters and histopathological factors were calculated using the Spearman correlation coefficient. Receiver operating characteristic (ROC) curves were evaluated and plotted for sensitivity and specificity assessments. For statistical analysis, SPSS Version 13.0 software was used (SPSS, Chicago, IL, USA). Significance was assumed at a value of *p*<0.05.

Results

Study population. The characteristics of the patients at diagnosis are summarized in Table I.

Preinvasive versus invasive and recurrent cervical carcinomas. The results of the biomarker analyses are provided in Table II. A significant increase in mean sVEGF-A values was found comparing CIN (81.9 pg/ml), PCC (139.8 pg/ml) and RCC (152.9 pg/ml; *p*=0.007). Similarly, increasing levels of sVEGF-R2 were detected comparing CIN (10,606 pg/ml) and RCC (11,622.1 pg/ml; *p*=0.014). In contrast, a significant decrease of sVEGF-D levels was found comparing pre-invasive (425.8 pg/ml) with invasive (382.5 pg/ml) and recurrent lesions (376.7 pg/ml; *p*=0.046). Analysing the

Table I. Patient characteristics.

	CIN n (%)	PCC n (%)	RCC n (%)
Total no. patients	50 (100%)	51 (100%)	24 (100%)
Median age (years)	36	47	47
Premenopausal status	44 (88%)	30 (59%)	14 (58%)
Stage (CIN/FIGO)			
I	7 (14%)	22 (43%)	
II	8 (16%)	13 (26%)	
III	35 (70%)	13 (26%)	
IV		3 (6%)	
Histology			
Squamous cell		37 (73%)	21 (88%)
Adenocarcinoma		7 (14%)	1 (4%)
Adenosquamous		4 (8%)	1 (4%)
X		3 (6%)	1 (4%)
Grade			
I		2 (4%)	0 (0%)
II		25 (49%)	13 (54%)
III		21 (41%)	10 (42%)
X		3 (6%)	1 (4%)
N +		19 (37%)	9 (38%)
M +		3 (6%)	14 (58%)
L +		15 (29%)	5 (21%)
HPV +	20 (40%)		

N+, Lymph node-positive; M+, distant metastasis-positive; L+, lymphatic vessel invasion-positive; HPV+, human papilloma virus high-risk-positive; X, unknown; CIN, preinvasive cervical carcinoma; PCC, primary invasive cervical carcinoma; RCC, recurrent invasive cervical carcinoma.

subgroup of patients with pre-invasive lesions, a significant increase in sVEGF-A values was found in HPV high-risk-positive patients (104.3 pg/ml) compared to HPV high-risk-negative patients (60.8 pg/ml; *p*=0.019), while the other biomarkers did not reveal any significant correlation. Serum VEGF-C and sVEGFR-1 did not show any significant correlation with disease invasion or progression.

Correlation with clinicopathological parameters. Correlations between the biomarkers and clinicopathological factors were performed for tumor histology, FIGO stage, lymph node status, presence of distant metastasis, grade, age at primary diagnosis and smoking habits. Higher mean sVEGF-R2 values were found in node-positive cases (11, 206.5 pg/ml) versus node-negative cases (10, 216.5 pg/ml; *p*=0.024) as well as in patients who presented with distant metastases (11, 206.5 pg/ml) compared to those without (10, 360.1 pg/ml; *p*=0.003). Serum VEGF-D levels were lower in cases with lymphatic vessel invasion (302.7 pg/ml) versus cases without (508.5 pg/ml; *p*=0.019). The other biomarkers did not correlate with lymphatic vessel invasion, lymph node involvement, or distant metastasis. None of the biomarkers correlated with tumor histology, FIGO stage, tumor grade, age at primary diagnosis, or smoking habits.

Table II. Serum levels (pg/ml, mean (range)) of the circulating biomarkers in relation to histopathological factors.

	pVEGF	sVEGF-D	sVEGF-C	sVEGF-R1	sVEGF-R2
CIN	81.9 (8-272)	425.8 (181-1002)	9653.0 (3716-19906)	73.4 (19.5-116)	10606.1 (6001-15131)
PCC	139.8 (20.5-893)	382.5 (104-2385)	10682.2 (3631-23722)	83.6 (25-190)	10242.0 (5792-14670)
RCC	152.9 (24.1-529)	376.7 (30-996)	10909.9 (4134-20948)	72.4 (23.9-157)	11622.1 (7455-15050)
<i>p</i> -Value	0.007	0.046	0.427	0.123	0.014
N0	114.4 (26.6-279)	454.0 (133-2385)	12302.1 (3631-23722)	83.0 (43.4-166)	10216.5 (7848-13227)
N1	180.6 (20.5-893)	358.0 (30-996)	10738.9 (4134-19233)	790.0 (23.9-190)	11206.5 (7450-14670)
<i>p</i> -Value*	0.484	0.484	0.277	0.338	0.024
M0	131.0 (20.5-529)	385.5 (30-2385)	10691.2 (3631-23722)	79.5 (23.9-190)	10360.1 (5792-15050)
M1	195.7 (24.1-893)	379.6 (36.8-996)	11103.4 (4134-20948)	75.3 (35.8-157)	11855.2 (8668-14670)
<i>p</i> -Value*	0.140	0.860	0.759	0.360	0.003
L0	117.0 (20.5-279)	508.5 (151-2385)	12175.9 (3631-23722)	82.0 (43.4-166)	10242.5 (7716-13983)
L1	126.0 (26.6-387)	302.7 (104-602)	10992.3 (4134-21861)	67.6 (23.9-157)	10786.1 (7455-13890)
<i>p</i> -Value*	0.978	0.020	0.456	0.074	0.332
Premenop.	100.7 (8-390)	412.0 (36.8-2385)	10595.8 (3716-23722)	78.2 (19.5-190)	10735.7 (6001-15131)
Postmenop.	166.0 (24.1-893)	368.3 (30-996)	9726.5 (3631-16714)	76.6 (25-164)	10522.1 (5792-15050)
<i>p</i> -Value	0.033	0.393	0.436	0.968	0.606
HPV +	60.8 (8-130)	433.6 (192-1002)	10172.3 (3716-19906)	76.3 (38.2-116)	11315.0 (8085-15131)
HPV-	104.3 (27.9-272)	455.1 (248-789)	9067.8 (5918-12227)	67.8 (30.9-90.9)	10378.4 (6675-14155)
<i>p</i> -Value°	0.019	0.539	0.501	0.225	0.231

CIN: Preinvasive cervical cancer; PCC: primary invasive cervical cancer; RCC: recurrent cervical cancer; *invasive lesions only, °preinvasive lesions only.

Evaluation of the circulating biomarkers as clinical markers for disease invasiveness. ROC curves were performed for each biomarker. No satisfactory sensitivity or specificity rates indicating disease invasion for any of the five biomarkers were found: for pVEGF-A, sensitivity was 76% with a specificity of 48%; for sVEGF-D: 52% and 32%, for sVEGF-C: 25% and 94%, for sVEGFR-1: 93% and 6%; and for sVEGFR-2: 73% and 34%.

Discussion

The family of VEGFs plays a crucial role in tumor neoangiogenesis. While VEGF-A is mainly involved in the formation of blood vessels (1), VEGF-C and -D predominate in lymphangiogenesis (2, 3). Recent studies demonstrate that not only the detection of VEGFs in the tumor specimen itself but also the analysis of the levels of circulating VEGFs provide useful information about the neoplasm (11). In several malignancies, including breast (12, 13), ovarian (14) and gastrointestinal cancer (15), elevated levels of sVEGFs have been associated with malignant potential and poor prognosis. It has been postulated that the circulating VEGFs might be clinically valuable as tumor markers. In cervical cancer, data on the significance of circulating VEGF-A and -C are limited (9, 16). Studies on the influence of sVEGF-D and sVEGFR 1/-2 are lacking overall. In this study, we aimed to investigate the influence of the different sVEGF members as well sVEGFR-1 and -2 on disease invasion and progression in cervical carcinoma. We found increasing

sVEGF-A levels in invasive and recurrent cervical cancer compared to preinvasive lesions. These data confirm the observation of Lebrecht *et al.* (16), who described increased circulating sVEGF-A levels in invasive cervical cancer compared to CIN. The data are in accordance with Mitsuhashi *et al.* (9), who detected elevated levels of sVEGF-A in recurrent cancer. We did not find a correlation with FIGO stage, lymph node involvement (17), tumor histology or further pathohistological factors as described by these authors (9, 16). VEGF-C and sVEGF-D are thought to be involved in the formation of lymphatic vessels (18, 19). In cervical cancer, sVEGF-C has been associated with lymph node metastasis, lymphatic tumor spread and advanced or persistent cancer (9, 20). Moreover, an up-regulation of sVEGF-C by carcinogenic nicotine has been described in cell lines (21). In our study, sVEGF-C did not correlate with any parameter analysed, not even with the lymph node status nor with smoking habits. Our study, therefore, does not confirm sVEGF-C as a prognostic parameter in cervical cancer.

Like VEGF-C, the close homolog VEGF-D is thought to promote lymphangiogenesis (22). Data on the role of circulating VEGF-D in human carcinomas are extremely limited. A study on pretherapeutic sVEGF-D levels in colorectal cancer did not find any association with clinicopathological parameters (14). Similarly, a study in breast cancer did not find any differences in sVEGF-D levels in carcinomas cases as compared to controls (12). This study is the first to analyse circulating VEGF-D in cervical cancer. Interestingly, we found higher levels of sVEGF-D in CIN compared to cases with

invasive and recurrent lesions. In cervical cancer specimens, Van Trappen *et al.* (23) detected higher levels of VEGF-D in CIN III compared to CIN I/II. The levels in CIN III lesions were similar to those in invasive carcinomas. The authors concluded that in CIN III, a lymphangiogenic phenotype becomes predominant compared to earlier stages. In colorectal cancer specimens, Hanrahan *et al.* (8) discovered higher levels of sVEGF-D in normal tissue compared to adenomas and carcinomas. Higher levels were also detectable in the normal tissue distant from the primary tumor. The authors concluded that higher levels of VEGF-D in normal tissue indicate changes that facilitate a subsequent tumor spread. In lung adenocarcinoma specimens, Niki *et al.* (24) found elevated VEGF-D levels in normal lung tissue and in patients without lymph node metastases. In node-positive patients, VEGF-D levels were significantly lower. The authors speculated that VEGF-D might represent an antimetastatic factor. Finally, Kopfstein *et al.* were able to demonstrate in a transgenic mouse model that VEGF-D induced lymphangiogenesis but yet repressed hemangiogenesis and tumor outgrowth (25). The observation of our study on soluble VEGF D in cervical cancer confirm these results. The high levels of sVEGF-D in CIN rather than in invasive cervical cancer indicate that the lymphangiogenic pathway predominates in preinvasive lesions. In invasive and recurrent lesions, a shift towards a hemangiogenic phenotype seems to occur, characterized by reduced sVEGF levels and increased levels of sVEGF-A in advanced carcinomas.

The different VEGFs act through activation of the corresponding receptor (26, 27): VEGF-A promotes vascular growth by activating VEGFR-1 and -2, primarily localized on endothelial cells (28). VEGF-D primarily activates VEGFR-3, a receptor mainly localized on lymphatic vessels (29). In the analyses of the soluble receptors sVEGFR-1 and sVEGFR-2, only sVEGFR-2 proved to be correlated with histopathological factors in this study. Serum levels of VEGFR-2 were higher in recurrent compared to pre-invasive and primary invasive carcinoma, in node-positive disease and in patients with distant metastases. These results confirm the proangiogenic status of advanced invasive carcinomas as indicated by the elevated serum levels of VEGF-A in advanced tumors.

For serum VEGF-A and serum VEGF-C, their potential clinical use as tumor markers has been proposed based on observations that serum levels are elevated prior to treatment, decrease after treatment and correlate with disease persistence, recurrence and treatment failure (9, 11, 30). The design of our study does not allow conclusions regarding this matter. However, due to the wide range of the serum levels, a huge overlap of the serum level range is observed between invasive and advanced carcinomas compared to preinvasive lesions, early tumor stages or even healthy controls in most studies, including our series (9, 15). This is reflected in the disappointing sensitivity and specificity rates demonstrated in this study. From

these data we, so far, cannot postulate that these factors might serve as useful clinical tools for differentiation between preinvasive and invasive lesions and disease surveillance in cervical cancer. From these data, we conclude that the VEGF family plays a distinct role in cervical cancer. In preinvasive tumors, a lymphangiogenic phenotype seems characteristic. With disease progression, a hemangiogenic phenotype predominates. At present, however, the differences in the serum levels do not seem clear enough to support the value of circulating VEGFs as tumor markers in cervical cancer.

References

- Olofsson B, Pajusola K, Kaipainen A, von Euler G, Joukov V, Saksela O, Orpana A, Pettersson RF, Alitalo K and Eriksson U: Vascular endothelial growth factor B, a novel growth factor for endothelial cells. *Proc Natl Acad Sci USA* 93: 2576-2581, 1996.
- Wang TB, Deng MH, Qiu WS and Dong WG: Association of serum vascular endothelial growth factor-C and lymphatic vessel density with lymph node metastasis and prognosis of patients with gastric cancer. *World J Gastroenterol* 13: 1794-1798, 2007.
- Kukk E, Lymboussaki A, Taira S, Kaipainen A, Jeltsch M, Joukov V and Alitalo K: VEGF-C receptor binding and pattern of expression with VEGFR-3 suggests a role in lymphatic vascular development. *Development* 122: 3829-3837, 1996.
- Cheng WF, Chen CA, Lee CN, Wei LH, Hsieh FJ and Hsieh CY: Vascular endothelial growth factor and prognosis of cervical carcinoma. *Obstet Gynecol* 96: 721-726, 2000.
- Tamura M, Oda M, Tsuneyuka Y, Matsumoto I, Kawakami K and Watanabe G: Vascular endothelial growth factor expression in metastatic pulmonary tumor from colorectal carcinoma: utility as a prognostic factor. *J Thorac Cardiovasc Surg* 128: 517-522, 2004.
- Dai Y, Zhang X, Peng Y and Wang Z: The expression of cyclooxygenase-2, VEGF and PGs in CIN and cervical carcinoma. *Gynecol Oncol* 97: 96-103, 2005.
- Kaushal V, Mukunyadzi P, Dennis RA, Siegel ER, Johnson DE and Kohli M: Stage-specific characterization of the vascular endothelial growth factor axis in prostate cancer: expression of lymphangiogenic markers is associated with advanced-stage disease. *Clin Cancer Res* 11: 584-593, 2005.
- Hanrahan V, Currie MJ, Cunningham SP, Morrin HR, Scott PA, Robinson BA and Fox SB: The angiogenic switch for vascular endothelial growth factor (VEGF)-A, VEGF-B, VEGF-C, and VEGF-D in the adenoma-carcinoma sequence during colorectal cancer progression. *J Pathol* 200: 183-194, 2003.
- Mitsuhashi A, Suzuka K, Yamazawa K, Matsui H, Seki K and Sekiya S: Serum vascular endothelial growth factor (VEGF) and VEGF-C levels as tumor markers in patients with cervical carcinoma. *Cancer* 103: 724-730, 2005.
- Obermair A, Bancher-Todesca D, Bilgi S, Kaider A, Kohlberger P, Müllauer-Ertl S, Leodolter S and Gitsch G: Correlation of vascular endothelial growth factor expression and microvessel density in cervical intraepithelial neoplasia. *J Natl Cancer Inst* 89: 1212-1217, 1997.
- Yamamoto Y, Toi M, Kondo S, Matsumoto T, Suzuki H, Kitamura M, Tsuruta K, Taniguchi T, Okamoto A, Mori T, Yoshida M, Ikeda T and Tominaga T: Concentrations of vascular endothelial growth factor in the sera of normal controls and cancer patients. *Clin Cancer Res* 2: 821-826, 1996.

- 12 Kuemmel S, Eggemann H, Luftner D, Thomas A, Jeschke S, Zerfel N, Heilmann V, Emmons G, Zeiser T, Ulm K, Kobl M, Korlach S, Schmid P, Sehouli J, Elling D and Blohmer JU: Changes in the circulating plasma levels of VEGF and VEGF-D after adjuvant chemotherapy in patients with breast cancer and 1 to 3 positive lymph nodes. *Anticancer Res* 26: 1719-1726, 2006.
- 13 Hoar FJ, Lip GY, Belgore F and Stonelake PS: Circulating levels of VEGF-A, VEGF-D and soluble VEGF-A receptor (sFlt-1) in human breast cancer. *Int J Biol Markers* 19: 229-235, 2004.
- 14 Cooper BC, Ritchie JM, Broghammer CL, Coffin J, Sorosky JI, Buller RE, Hendrix MJ and Sood AK: Preoperative serum vascular endothelial growth factor levels: significance in ovarian cancer. *Clin Cancer Res* 8: 3193-197, 2002.
- 15 Duff SE, Saunders M, McCredie V, Kumar S, O'Dwyer ST and Jayson GC: Pre-operative plasma levels of vascular endothelial growth factor A, C and D in patients with colorectal cancer. *Clin Oncol (R Coll Radiol)* 17: 367-371, 2005.
- 16 Lebrecht A, Ludwig E, Huber A, Klein M, Schneeberger C, Tempfer C, Koelbl H and Hefler L: Serum vascular endothelial growth factor and serum leptin in patients with cervical cancer. *Gynecol Oncol* 85: 32-35, 2002.
- 17 Hashimoto I, Kodama J, Seki N, Hongo A, Yoshinouchi M, Okuda H and Kudo T: Vascular endothelial growth factor-C expression and its relationship to pelvic lymph node status in invasive cervical cancer. *Br J Cancer* 85: 93-97, 2001.
- 18 Thelen A, Scholz A, Benckert C, von Marschall Z, Schröder M, Wiedenmann B, Neuhaus P, Rosewicz S and Jonas S: VEGF-D promotes tumor growth and lymphatic spread in a mouse model of hepatocellular carcinoma. *Int J Cancer* 122: 2471-2481, 2008.
- 19 Wong SY, Haack H, Crowley D, Barry M, Bronson RT and Hynes RO: Tumor-secreted vascular endothelial growth factor-C is necessary for prostate cancer lymphangiogenesis, but lymphangiogenesis is unnecessary for lymph node metastasis. *Cancer Res* 65: 9789-9798, 2005.
- 20 Mathur SP, Mathur RS, Gray EA, Lane D, Underwood PG, Kohler M and Creasman WT: Serum vascular endothelial growth factor C (VEGF-C) as a specific biomarker for advanced cervical cancer: Relationship to insulin-like growth factor II (IGF-II), IGF binding protein 3 (IGF-BP3) and VEGF-A [corrected] *Gynecol Oncol* 98: 467-483, 2005. Erratum in: *Gynecol Oncol* 101: 186, 2006.
- 21 Lane D, Gray EA, Mathur RS and Mathur SP: Up-regulation of vascular endothelial growth factor-C by nicotine in cervical cancer cell lines. *Am J Reprod Immunol* 53: 153-158, 2005.
- 22 Juttner S, Wissmann C, Jons T, Vieth M, Hertel J, Gretschel S, Schlag PM, Kemmler W and Höcker M: Vascular endothelial growth factor-D and its receptor VEGFR-3: two novel independent prognostic markers in gastric adenocarcinoma. *J Clin Oncol* 24: 228-240, 2006.
- 23 Van Trappen PO, Steele D, Lowe DG, Baithun S, Beasley N, Thiele W, Weich H, Krishnan J, Shepherd JH, Pepper MS, Jackson DG, Sleeman JP and Jacobs JJ: Expression of vascular endothelial growth factor (VEGF)-C and VEGF-D, and their receptor VEGFR-3, during different stages of cervical carcinogenesis. *J Pathol* 201: 544-554, 2003.
- 24 Niki T, Iba S, Tokunou M, Yamada T, Matsuno Y and Hirohashi S: Expression of vascular endothelial growth factors A, B, C, and D and their relationships to lymph node status in lung adenocarcinoma. *Clin Cancer Res* 6: 2431-2439, 2000.
- 25 Kopfstein L, Veikkola T, Djonov VG, Baeriswyl V, Schomber T, Strittmatter K, Stacker SA, Achen MG, Alitalo K and Christofori G: Distinct roles of vascular endothelial growth factor-D in lymphangiogenesis and metastasis. *Am J Pathol* 170: 1348-1361, 2007.
- 26 Shibuya M: Differential roles of vascular endothelial growth factor receptor-1 and receptor-2 in angiogenesis. *J Biochem Mol Biol* 39: 469-478, 2006.
- 27 Hiratsuka S, Maru Y, Okada A, Seiki M, Noda T and Shibuya M: Involvement of Flt-1 tyrosine kinase (vascular endothelial growth factor receptor-1) in pathological angiogenesis. *Cancer Res* 61: 1207-1213, 2001.
- 28 Shibuya M: Vascular endothelial growth factor receptor-1 (VEGFR-1/Flt-1): a dual regulator for angiogenesis. *Angiogenesis* 9: 225-230, 2006; discussion 231.
- 29 Laakkonen P, Waltari M, Holopainen T, Takahashi T, Pytowski B, Steiner P, Hicklin D, Persaud K, Tonra JR, Witte L and Alitalo K: Vascular endothelial growth factor receptor 3 is involved in tumor angiogenesis and growth. *Cancer Res* 67: 593-599, 2007.
- 30 Bachtiary B, Selzer E, Knocke TH, Potter R and Obermair A: Serum VEGF levels in patients undergoing primary radiotherapy for cervical cancer: impact on progression-free survival. *Cancer Lett* 179: 197-203, 2002.

*Received April 11, 2008**Revised November 3, 2008**Accepted November 21, 2008*