Relationship between Co-expression of Vascular Endothelial Growth Factor and its Receptor, Kinase Insert Domain Containing Receptor and Tumor Angiogenesis in Invasive Carcinoma of the Cervix

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OBJECTIVE The aim of the present study was to determine the expression of vascular endothelial growth factor (VEGF) and its receptor, kinase insert domain containing receptor (KDR), and their significance in regulating tumor angiogenesis in the early stages of cervical cancer.

METHODS Using the immunohistochemical SP method, the expression of VEGF and KDR was determined in the cancer cells. In addition, the microvessel density (MVD), labeled by CD34 in the tumor stroma, was examined in 18 cases of cervical intraepithelial neoplasms (CIN), 75 cases of early invasive cervix carcinomas (ICC) and 15 specimens of normal cervical epithelium (NCE).

RESULTS In ICC cases, VEGF and KDR were mainly expressed in the cellular membrane and/or cytoplasm of tumor cells, while expression of CD34 was found mainly in the vascular epithelial cells of the tumor stroma. The positive expression rate of VEGF and KDR, and the MVD increased remarkably from NCE through CIN to ICC ($P<0.01$). For the ICC group, in the patients with positive expression of VEGF and KDR, the MVD was significantly higher than those with negative expression of VEGF and KDR ($P<0.05$). Expression of VEGF in ICC was positively related to KDR expression ($r=0.56$, $P<0.01$). The MVD was also positively related to both the expression of VEGF ($r=0.60$, $P<0.01$), and KDR ($r=0.33$, $P<0.01$). In the cases with both positive expression of VEGF and KDR, the MVD was significantly higher than those in which there was negative expression of both ($P<0.01$).

CONCLUSION Expression of VEGF and its receptor KDR plays a key role in up-regulating tumor angiogenesis in cervical carcinoma. Co-overexpression of VEGF and KDR results in rapid tumor vasculogenesis. Detection of co-expression of VEGF and KDR may be of value in further understanding tumor angiogenesis and in searching for new targets for anti-angiogenesis therapy in invasive carcinoma of the cervix.

KEYWORDS: cervical carcinoma, VEGF, KDR, tumor angiogenesis, immunohistochemistry.

At present, among all the angiogenic stimulators, vascular endothelial growth factor (VEGF) is believed to play the most important role. The kinase insert domain containing receptor (KDR),

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one of the VEGF receptors, can bind with VEGF causing-enhanced cellular chemotaxis, promotion of endothelial mitogenesis, resulting in vascular endothelial differentiation, cellular proliferation and migration.[1] Studies in the past have shown that VEGF and its receptor, KDR, are crucial factors in solid tumor angiogenesis, invasiveness and metastasis.[1] There are few reports in China, however, concerning the relationship between the co-expression of VEGF and KDR, and angiogenesis in cervical cancer. Using the immunohistochemical SP method, we examined VEGF and its receptor KDR expression and tumor microvessel density (MVD, labeled by CD34) in the early stages of cervical carcinoma. The data were used to explore the correlation between co-expression of VEGF and KDR and regional tumor angiogenesis in these tumors in order to provide further understanding of tumor angiogenesis and the potiential for anti-angiogenesis therapy.

MATERIALS AND METHODS

Clinical data

Ninty three patients were selected for our study who had no prior pre-operation chemotherapy, radiotherapy or immunotherapy. The cases consisted of 18 cervical intraepithelial neoplasms (CIN), and 75 invasive carcinomas of the cervix (ICC). The patients had been referred to our department during the period of January, 1998 to February, 2002. All the cases were confirmed by a pathological examination. Mean age of these patients was 42 years (range: 24~72 years). With regard to the histological grade, in the CIN group, 2 cases were staged as Ⅰ, 6 as Ⅱ and 10 as Ⅲ. In the ICC group, 3 were staged as Ⅰ, 27 as Ⅱ and 45 as Ⅲ. For the 75 ICC cases, according to the FIGO staging system, 4 cases were in Ia, 29 in Ib, 41 in IIa and 1 in IIb. The 75 ICC consisted of 66 cases of squamous cell carcinoma and 9 of adenocarcinoma (including adenosquamous cell carcinoma and clear cell carcinoma each one); there were 14 cases of pelvic lymph node metastasis and 22 intravascular invasion. Microscopically, we found that there were 4 cases of early infiltration, 23 of superficial muscularis infiltration, 41 of deep muscularis infiltration and 7 of panmural infiltration. In addition to the cervical carcinoma cases, 15 samples of normal cervical epithelium (NCE) were selected as controls.

Reagents

VEGF monoclonal antibody concentrate (clone No. JH121) was obtained from Neomarkers Co., USA, with a working concentration at 1:25. KDR monoclonal antibody concentrate (clone No. sc6251) was obtained from Santa Cruz Co., USA, with a working concentratioin of 1:75. Instant CD34 monoclonal antibody (clone No. QBEnd/10), streptomycin avidin-peroxidase (SP) immunohistochemistry staining assay kit and an AEC coloration solution etc. were all purchased from Maxim Co., USA.

Methods

All the fresh specimens were fixed in 10% formalin. The tissues were utilized within 48 h of excision, embedded in paraffin and cut into 4~5μm thick serial sections. Based on the kit's instructions, expression of VEGF, KDR and CD34 in the cervical carcinoma specimens was measured by the immunohistochemistry SP method. Using an EDTA buffer solution, microwave antigen retrieval was undertaken for determination of VEGF and KDR, while for CD34, antigen retrieval was unnecessary. Known positive sections were used as positive controls, and for negative controls, PBS was used instead of the first antibody.

Definition of the results

The procedure for reporting the results for expression of VEGF, KDR and CD34 was described by Jiang et al.[2] previously. The degrees of expression for VEGF and KDR were divided into 3 grades: negative (-), positive (+) and intensified positive (≥ ++).

Statistics

All data were analyzed by a SPSS 10.0 software package. For the semi-quantitative data, a χ^2 test or exact probabilities in a 2 × 2 table was used. For quantitative data, analysis of variance (ANOVA) was used and for a relationship between 2 variables, a linear correlation analysis was performed.

Table 1. Expression of VEGF and KDR in NCE, CIN and ICC(%)

Group	Cases	VEGF				KDR			
		-	+	≥ ++	Positive rate	-	+	≥ ++	Positive rate
NCE	15	11(73.33)	4(26.67)	0(0)	(26.67)*	13(86.67)	2(13.33)	0(0)	(13.33)*
CIN	18	7(38.89)	8(44.44)	3(16.67)	(61.11)**	7(38.89)	9(50.00)	2(11.11)	(61.11)**
ICC	75	17(22.67)	34(45.33)	24(32.00)	(77.33)***	23(30.67)	32(42.67)	20(26.67)	(69.33)***

Comparison between NCE and CIN: *P<0.05; CIN and ICC: **P<0.01; ICC and NCE: ***P<0.01.

RESULTS

Expression of VEGF and KDR in NCE, CIN and ICC

There were significant increases in VEGF and KDR expression from NCE to CIN, then to ICC (P<0.01). In the ICC group, VEGF and KDR were mainly expressed in the cellular membrane and/or cytoplasm of the tumor cells, some also were expressed in the vessel endothelium of the tumor stroma. Expression of VEGF and KDR was higher at the edge of the carcinoma nest than that in the central portion, and the highest expression was observed at the site of the most obvious stroma infiltration with the cancer cells. In the CIN group, VEGF and KDR were mainly found in heterocellular membranes and/or cytoplasm, a little also was expressed in the vascular endothelial cells adjacent to the basement membrane. Positive expression rates of VEGF in CIN stage I, II, III, were 0 (0/2), 66.67% (4/6) and 70.00% (7/10), while for KDR, the rates were 0 (0/2), 83.33% (5/6) and 60.00% (6/10), respectively. Further statistical analysis was not performed for the data mentioned above due to insufficient cases. In the NCE group, VEGF and KDR were weakly expressed in the cellular membrane and/or cytoplasm of cells in the basal layer. Results for this portion of the study are shown in Table 1 and Fig. 1.

MVD in NCE, CIN and ICC

A significant increase of average MVD labeled by CD34 was found in the CIN and ICC groups (P<0.01), the results of which are indicated in Table 2 and Fig. 2.

Relation between expression of VEGF and KDR and MVD in CIN and ICC

As Table 3 shows, with the enhancement of VEGF and KDR expression (P<0.05), MVD was significantly increased.

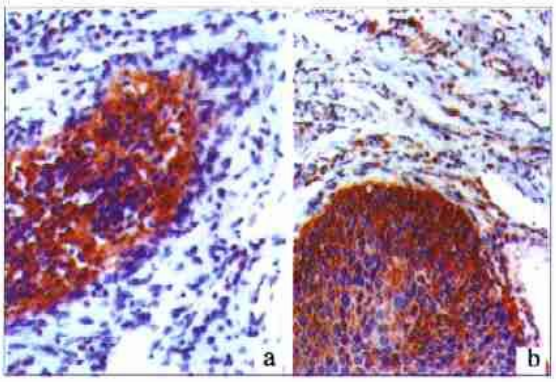

Fig.1. Expression of VEGF and KDR in invasive cervical cancer (SP × 200, AEC coloration, counterstained with hematoxylin).
a: Positive expression of VEGF in cytoplasm and/or cellular membrane of the cancer cells of the cervix.
b: Positive expression of KDR in cytoplasm and/or cellular membrane of the cancer cells of the cervix.

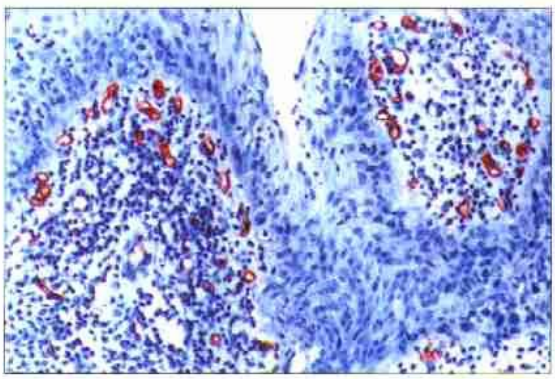

Fig.2. Expression of CD34 in the carcinoma of the cervix (SP × 2 00, AEC coloration, counterstained with hematoxylin.) Vascular endothelial cells in the stroma of the invasive cervix carcinoma were stained in rose.

Table 2. MVD in NCE, CIN and ICC

Group	Cases	MVD
NCE	15	16.36 ± 4.21*
CIN	18	34.02 ± 12.93**
ICC	75	44.21 ± 11.25***

Comparison between NCE and CIN: *P<0.01; CIN and ICC: **P<0.01; ICC and NCE: ***P<0.01.

Correlation of co-expression of VEGF and KDR and MVD

In Table 4, A represents carcinomas where both VEGF and KDR showed negative expression (13 cases, 17.33%), B represents cancers in which either VEGF or KDR expression was positive (14 cases, 18.67%) and C represents cancer in which both of VEGF and KDR were positive (48 cases, 64.00%). In cervical cancer patients in which either one or both VEGF or KDR showed positive expression, MVD was significantly higher than those cancers in which both of them showed negative expression (P<0.01). Linear correlation analysis indicated that VEGF expression was strongly positively related to KDR in ICC (r=0.56, P<0.01) and both of them were also strongly positively related to MVD (for VEGF, r=0.60, P<0.01; for KDR, r=0.33, P<0.01).

Table 4. Relationship of co-expression of VEGF and KDR with MVD

Group	Cases	MVD
A	13	34.45 ± 6.43
B	14	43.79 ± 6.71**
C	48	47.18 ± 11.60**

Comparison between B and A: **P<0.01; C and A: **P<0.01.

DISCUSSION

Past studies have demonstrated that there is over-expression of the VEGF protein and VEGF mRNA in cervical carcinoma and CIN, and that VEGF expression is closely related to cervical cancer initiation and progression.[3,4] Our current study showed that the VEGF positive expression rate was significantly increased in the CIN and ICC groups (P<0.01), which was consistent with the reports cited. VEGF is a homodimeric glycoprotein coded by a single VEGF gene, with a molecular mass in the range of 34~45 kDa. The human VEGF gene is assigned to chromosome 6p21.3 and its coding region spans approximately 14kb. It is organized in 8 exons, separated by 7 introns. Five VEGF isoforms are generated as a result of alternative splicing from a single VEGF gene: VEGF121, VEGF145, VEGF165, VEGF189 and VEGF206. Out of the 5, VEGF121, VEGF145 and VEGF165 mainly participate in angiogenesis, while VEGF189 and VEGF206 mainly are involved in increasing microvessel permeability.[5]

In the past, VEGF-B, C, D etc. also have been identified,[6,7] and it was reported that they might be linked to angiogenesis, increased permeability of blood vessels and lymphatics and tumor cell invasiveness and metastasis etc. Only scant studies have been reported concerning KDR expression in cervical cancer in China and abroad. According to Di et al.,[8] KDR expression was found to be increased in cervical cancer. Di et al. have also indicated that the positive expression rate of KDR is significantly raised in CIN and ICC (P<0.01), which verifies further the high expression of KDR in cervical cancer. There are 3 VEGF receptors: VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1) and

Table 3. Relationship between expression of VEGF and KDR and MVD in ICC

Group	Cases	MVD					
		VEGF(-)	(+)	(≥ ++)	KDR(-)	(+)	(≥ ++)
CIN	18	25.62 ± 10.07	36.67 ± 9.82	46.59 ± 16.31*	24.10 ± 7.45**	37.40 ± 9.43**	53.59 ± 15.44**
ICC	75	35.53 ± 6.07**	42.44 ± 7.27**	53.27 ± 12.13**	39.33 ± 8.64*	45.09 ± 11.20	48.90 ± 11.55*

Comparison between (-) and (+): *P<0.05, **P<0.01; (+) and (≥ ++):**P<0.01; (≥ ++) and (-): *P<0.05, **P<0.01.

VEGFR-3 (Flt-4). All of them are tyrosine kinase receptors.[9] The 5 isoforms of VEGF all can bind to KDR. As a result, their binding promotes endothelial cell differentiation, proliferation and migration, and up-regulates angiogenesis.[1]

Dobbs et al.[4] reported that there were significant increases in VEGF and MVD expression from normal cervix through CIN I to CIN III to invasive squamous cell carcinoma of the cervix. There was a strong correlation between MVD and VEGF expression, both were associated with the histological grade of CIN and they concluded that abnormal epithelium of the cervix promoted VEGF expression. Our present study indicated that the mean MVD significantly increased in CIN and ICC ($P<0.01$) groups. In ICC, when expression of VEGF increased, MVD was remarkably enhanced. In the CIN group, with ascending VEGF expression, MVD gradually increased ($P<0.01$), and a striking increase was observed when VEGF expression was intensely positive ($P<0.05$), results which are consistent with Dobbs et al.[4]

There are a only a few reports concerning the relation between KDR and MVD from China and abroad. We discovered that, with the increasing expression of KDR, MVD was significantly increased in CIN and ICC ($P<0.05$). As a VEGF receptor with high affinity, KDR plays an important role in promoting vasculogenesis. According to Waltenberger et al.,[10] mitogenesis of the vascular endothelium promoted by VEGF mainly is a result of its interaction with KDR, and increased VEGF expression leads to up-regulation of KDR expression. Results above show us that the synthesis and expression of VEGF and KDR are increased in cervical cells, from damaged cervical epithelium to invasive cervical carcinoma. With the enhanced expression of VEGF and KDR, angiogenesis of the tissues is also stimulated. VEGF and KDR participate in positive regulation of tumor angiogenesis in invasive cervical cancer, and this knowledge may be of great value for therapy in regulating tumor angiogenesis.

Because it is complex, tumor angiogenesis is a dynamic process, which involves a series of regulators. So far, VEGF is regarded as the most important angiogenic stimulator, and KDR is the main functional receptor for VEGF. Mediated by KDR, VEGF is a key factor in tumor angiogenesis, and it has been shown that if only the binding of VEDF to KDR is inhibited, tumor angiogenesis will subsequently be suppressed.[10] In ICC cases, expression of VEGF and KDR were both significantly positively related to MVD (for VEGF, $r=0.60$, $P<0.01$; for KDR, $r=0.33$, $P<0.01$) and VEGF expression was also positively related to KDR ($r=0.56$, $P<0.01$). Markedly significant increases in MVD were found in invasive cervical carcinoma patients with both VEGF and KDR positive expression ($P<0.01$). Therefore, through KDR mediations, VEGF can up-regulate tumor angiogenesis in cervical cancer. There is significant increase in tumor angiogenesis in patients with both VEGF and KDR positive expression.

Our current study indicated that from NCE to CIN to ICC, namely, with deeper damage to the cervical epithelium, expression of VEGF and KDR and MVD are accordingly remarkably higher. Expression of VEGF is positively correlated with KDR in ICC, and both of them are also positively related to MVD. VEGF and its receptor are not only participants in tumor angiogenesis in cervical cancer, but also take part in its progression.

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