Frequent Overexpression of Vascular Endothelial Growth Factor Gene in Human Renal Cell Carcinoma

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Departments of Urology, *Biochemistry and †Pathology, Akita University School of Medicine, Akita 010, ‡Department of Pathology, Institute of Medical Science, University of Tokyo, Tokyo 108, and §Department of Urology, Sendai Shakai Hoken Hospital, Sendai 981

SATO, K., TERADA, K., SUGIYAMA, T., TAKAHASHI, S., SAITO, M., MORIYAMA, M., KAKINUMA, H., SUZUKI, Y., KATO, M. and KATO, T. Frequent overexpression of Vascular Endothelial Growth Factor Gene in Human Renal Cell Carcinoma. Tohoku J. Exp. Med., 1994, 173 (3), 355-360 —— Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen and an inducer of angiogenesis. Expression of the VEGF gene was investigated in 20 patients with renal cell carcinoma by Northern blot hybridization analysis. Of the 20 tumors, 12 (60%) overexpressed the gene 3.0 times more than in normal renal tissues. No significant correlation was found between overexpression of the VEGF gene and the histopathological data such as grade, cellular and structural subtypes, stage and size of tumor. These results suggest that VEGF is produced by the tumor cells and is responsible for development of this hypervascular tumor. —— vascular endothelial growth factor; renal cell carcinoma; angiogenesis

Angiogenesis plays a crucial role in tumor growth and metastasis, and may be regulated by growth factors secreted by tumor cells. Several endothelial growth factors with angiogenic activity have been described (Folkman and Klagsbrun 1987; Risau 1991). These include fibroblast growth factors, platelet-derived growth factor and vascular endothelial growth factor (VEGF). VEGF is a heparin-binding dimeric glycoprotein purified from media conditioned by bovine pituitary follicular or folliculostellate cells (Ferrara and Henzel 1989). This factor has also been termed vascular permeability factor (VPF) because of its ability to induce vascular leakage in vivo (Senger et al. 1983; Keck et al. 1989).

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VEGF has a hydrophobic signal sequence and exerts a potent mitogenic activity on vascular endothelial cells isolated from both small and large vessels, but does not affect the growth of other types of cells such as fibroblasts, lens epithelial cells, corneal endothelial cells, keratinocytes, or adrenal cortex cells (Ferrara and Henzel 1989; Leung et al. 1989).

Two receptors for VEGF, flt (Shibuya et al. 1990; De Vries et al. 1992) and flk-1 (Matthews et al. 1991; Millauer et al. 1993), have been identified restrictively on vascular endothelial cells in vivo. Recently, it was demonstrated that an insufficient vascular supply and a resultant reduction in tissue oxygen tension stimulate glioblastoma multiforme, a rapid growing tumor, to secrete VEGF so as to cause angiogenesis (Plate et al. 1992; Shweiki et al. 1992). These findings suggest that VEGF is an endothelial cell-specific mitogen and an angiogenesis inducer in normal as well as neoplastic tissues.

Renal cell carcinoma is defined as a rapid growing tumor associated with necrosis and prominent vascularization with endothelial cell proliferation. These characteristics of renal cell carcinoma would be attributable, at least in part, to the function of VEGF which may be secreted by this tumor. In the light of glioblastoma multiforme, we evaluated expression of VEGF gene in the renal cell

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aOverexpression, >3 in T/N ratio;
bA predominant component was listed.
VEGF Gene Overexpression in Renal Cell Carcinoma

MATERIALS AND METHODS

Tumor and corresponding normal renal tissue specimens were obtained at surgery from 20 consecutive patients with renal cell carcinoma (Table 1). A part of each tissue specimen was stored at −80°C for molecular biological study and the remainder was fixed with formaldehyde for routine histological study. White blood cells were obtained from peripheral blood of patients and also subjected to molecular biological study. For preparation of a DNA probe identical to human VEGF gene (Tischer et al. 1991), 0.4 μg of cDNA, synthesized from total RNA extracted from human normal kidney with Moloney murine leukemia virus (MMLV) reverse transcriptase (Gibco BRL, Gaitherburg, MD, USA), was subjected to 35 rounds of amplification by polymerase chain reaction (PCR). Cycles were 5 sec at 94°C, 10 sec at 55°C and 15 sec at 72°C in a thermal cycler (Air Thermo-Cycler, Idaho Technology Co., Ltd., Idahofall, ID, USA). The nucleotide sequence of the primers used in the PCR was as follows; sense strand, 5′-GAGGAGTCCACCACCATGCAG-3′ (VEGFUP) and antisense strand, 5′-GGCTCACCGCCTCGGCTTGTAACA-3′ (VEGFDW). This PCR yielded three sizes of product spanning 156 base pair (bp), 288 bp and 360 bp of coding region, corresponding to VEGF<sub>121</sub>, VEGF<sub>165</sub> and VEGF<sub>189</sub> subunits (Tischer et al. 1991), respectively. Of the three, a product of 156 bp was isolated from an agarose gel, purified and provided as VEGF probe, because DNA sequence of this...
probe was common in these 3 subunits. Identification of the probe to VEGF gene was confirmed by direct sequencing technique using Sequi-Therme™ cycle sequencing kit (Epicentre Technologies, Madison, WI, USA) (data not shown). Denatured 10 μg of total RNAs extracted from frozen tissue samples by guanidium acid phenol method were electrophoretically fractionated on a 0.8% agarose gel and transferred on to a nylon membrane (Gene Screen Plus filter, NEN, London) (Maniatis et al. 1989). The membrane was hybridized with 50 ng of 32P-labeled VEGF probe described above (specific activity, at least 10⁶ cpm/μgDNA), washed and autoradiographed at −80°C (Maniatis et al. 1989). As the internal control for signal alignment, a β-actin probe was used. Photodensitometry was carried out in a comparative approach.

RESULTS AND DISCUSSION

The Northern blot hybridization analysis of the 20 tumor/normal kidney pairs was shown in Table 1 and Fig. 1. Tischer et al. have found VEGF transcripts of 5.5 kilobase (kb), 4.4 kb and 3.7 kb in cultured human vascular smooth muscle cells (Tischer et al. 1991), whereas expression of a transcript of 5.5 kb was very low. In the present study, a major 3.7 kb VEGF transcript was observed in all of the tissue samples examined, although the signal intensity varies from sample to sample even in the normal kidneys. The level of the VEGF gene expression was divided by that of the β-actin gene expression for each specimen to adjust differences in the amount of RNA loaded on each lane. The VEGF gene expression in the tumor relative to that in the corresponding normal kidney was calculated as the T/N ratio. A T/N ratio of VEGF expression more than 3 and over was judged as overexpression. With this criteria, overexpression of VEGF gene with a range of 3.0 to 38.0 was detected in 12 (60%) out of the 20 cases. A 4.4 kb VEGF transcript was observed only in the tumors having overexpression of 3.7 kb VEGF transcript and a 5.5 kb transcript not detected in the present study. VEGF gene was not expressed in total RNAs isolated from the peripheral blood leucocytes of the patients, indicating that overexpression of VEGF gene observed in renal cell carcinoma was not influenced by contaminated leucocytes in the tissues. No significant correlation was found between the overexpression of VEGF gene and the histopathological data such as grade, predominant cellular and structural subtypes, stage and size of tumor (Table 1). However, it may be noteworthy that no renal cell carcinomas with cystic subtype have overexpressed the gene.

Our preliminary results has demonstrated the expression of VEGF gene in human renal cell carcinoma, the frequency of overexpression of the gene being as high as 60%. Recently, Brown et al. (1993) investigated expression of VEGF gene in renal cell carcinoma by in situ hybridization and demonstrated that, of the 12 renal cell carcinoma examined, 11 (91.7%) expressed higher levels of VEGF gene compared to normal renal tissue (Brown et al. 1993). Although there was a
difference between overexpression rates of the 2 studies, which possibly came from
detection method employed and criteria for judgment of overexpression, these
studies will indicate that VEGF is frequently overexpressed in renal cell car-
cinoma and responsible for the development of this hypervascular tumor as it is
in the glioblastoma (Plate et al. 1992; Shweiki et al. 1992; Kim et al. 1993).

In a number of specific tumor types, including neoplasms of the bladder
(Chodak et al. 1980), cervix (Sillman et al. 1981) and cutaneous melanoma
(Srivastava et al. 1988), there is a growing body of experimental evidence suggest-
ing that vascularity may be an important prognostic indicator of aggressive
behavior. Especially in invasive breast carcinoma, it has been postulated that a
degree of angiogenesis correlates well with metastatic ability of the tumor (Weid-
nner et al. 1991). Follow-up study on our patients will clarify whether the degree
of VEGF gene expression correlates with the malignant potential of renal cell
carcinoma.

References

and Senger, D.R. (1993) Increased expression of vascular permeability factor (vascu-
lar endothelial growth factor) and its receptor in kidney and bladder carcinomas.
Am. J. Pathol., 143, 1255-1262.
activity as a marker of neoplastic and preneoplastic lesions of the human bladder.
The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor.
Science, 255, 989-991.
4) Ferrara, N. & Henzel, W.J. (1989) Pituitary follicular cells secrete a novel heparin-
Commun., 161, 851-858.
(1989) Vascular permeability factor, an endothelial cell mitogen related to PDGF.
Science, 246, 1309-1312.
(1993) Inhibition of vascular endothelial growth factor-induced angiogenesis sup-
endothelial growth factor is a secreted angiogenic mitogen. Science, 246, 1306-1312.
10) Matthews, W., Jordan, C.T., Gavin, M., Jenkins, N.A., Copeland, N.G. & Lemischka,
primitive hematopoietic cells and exhibiting close genetic linkage to c-kit. Proc.
Natl. Acad. Sci. USA, 88, 9026-9030.
11) Millauer, B., Wizigmann-Voos, S., Schnurch, H., Martinez, R., Moller, N.P.H., Risau,
suggest flk-1 as a major regulator of vasculogenesis and angiogenesis. Cell, 72, 835-
846.


