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Relationship between Intratumoral Hemorrhage and Overexpression of Vascular Endothelial Growth Factor (VEGF) in Pituitary Adenoma

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ABSTRACT

The authors investigated the relationship between hemorrhage and the expression of vascular endothelial growth factor (VEGF) in pituitary adenomas. The subjects were 39 patients with pituitary adenomas. Surgically obtained tumor tissue was immunohistochemically stained using antibodies against VEGF, CD-34, Ki-67, and anterior pituitary hormones. The expression of VEGF was graded as 0, 1+, and 2+. The relationship between intratumoral hemorrhage and factors such as tumor size, Ki-67 labeling indices, number of CD-34 positive vessels, and VEGF expression was examined by multivariate analysis. High-grade VEGF expression was the sole independent factor correlated with intratumoral hemorrhage. The number of CD-34 positive vessels had no effect on the incidence of hemorrhage in patients with pituitary adenomas. In conclusion, a positive relationship between VEGF expression and hemorrhage in pituitary adenoma was observed. The patho-mechanical significance of this correlation is under investigation.

Key words: *Vascular endothelial growth factor, Pituitary adenoma, Hemorrhage*

Some patients with pituitary adenoma suffer hemorrhagic infarction and exhibit a symptom complex known as pituitary apoplexy. Frequently these tumors harbor cysts containing new or old hematoma in the absence of overt clinical manifestations[16,30]. Vascular endothelial growth factor (VEGF) is a 32 to 46 kD glycoprotein; alternative splicing of the VEGF exon resulted in the production of 5 isoforms[20]. VEGF has been implicated in vasculogenesis[7,22], vascular permeability[6,23], and stromal degradation[8]. In several kinds of brain tumor, the overexpression of VEGF is known to induce cyst formation[25,27,28], peritumoral brain edema[25,29], and tumor-associated hemorrhage[5]. In normal pituitary, VEGF is expressed only in folliculo-satellite cells[7,10]. On the other hand, most human pituitary adenomas secrete measurable levels of VEGF[14].

The role of VEGF in pituitary adenomas remains poorly understood. We assayed VEGF expression in neoplastic cells from hemorrhagic, cystic, and homogeneously solid adenomas to elucidate the relationship between the level of VEGF expression and these hemorrhagic and cystic changes.

MATERIALS AND METHODS

The specimens included 39 adenomas removed by transsphenoidal surgery. Of these, 6 (15.4%) were from patients in the acute stage of intratumoral hemorrhage; 21 (53.8%) were cystic-, and 12 (30.8%) were solid adenomas. Patients in the acute stage of hemorrhage manifested apoplectic symptoms such as headache, vomiting, and other neurologic symptoms within 7 days of undergoing surgery. In the 21 patients with cystic adenomas, intraoperative inspection revealed that the cysts contained fluid hematoma (n=7, 33.3%) and xanthochromic- or clear watery fluid (n=14, 66.7%). The largest diameter of the tumors ranged from 6–52 mm (mean ± SD, 24.1 ± 8.6 mm). The adenomas were classified based on the hormones excessively secreted to the serum: 23 (59.0%) were clinically nonfunctioning adenomas, most of them

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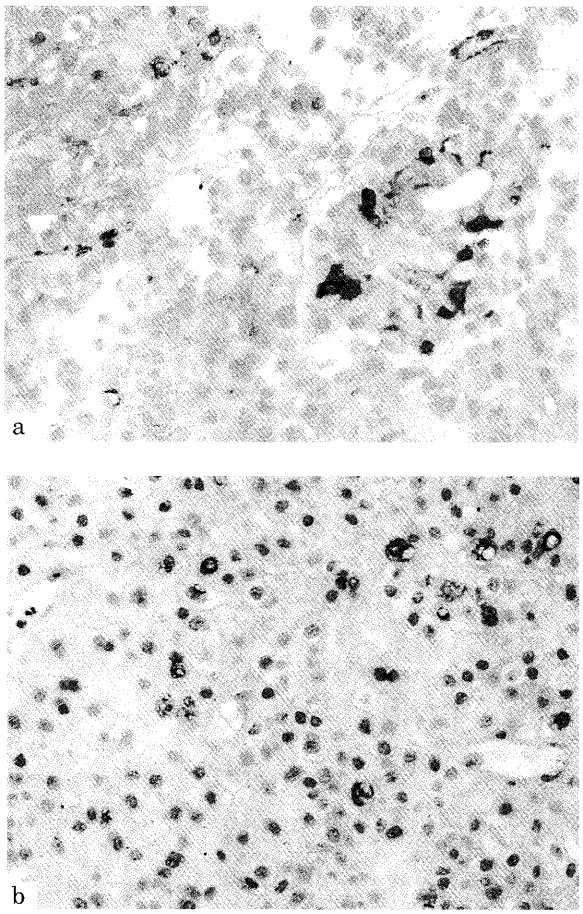

Fig. 1. Photomicrographs of VEGF immunostaining of pituitary adenomas in representative cases.
a. Case 2. VEGF is strongly expressed (2+) in viable part of adenoma in a patient at the acute stage of pituitary apoplexy.
b. Case 11. Positive (1+) VEGF expression in adenoma associated with cyst containing fluid hematoma.
c. Case 30. This homogeneously solid adenoma was negative for VEGF expression (–).

were immunohistochemically identified as gonadotropinomas; 5 (12.8%) were growth hormone (GH)-producing, 9 (23.1%) prolactin-producing, and 2 (5.1%) were adrenocorticotropin-producing adenomas.

To assay the expression of VEGF, we used formalin-fixed, paraffin-embedded tumor samples and the immunoperoxidase method described elsewhere[29]. We employed a goat polyclonal antibody (Santa Cruz Biotechnology) at a 1:200 dilution to detect VEGF. The tumor portion representing the negative control was not exposed to the primary antibody (VEGF expression grade 0); sections from paraffin-embedded anaplastic astrocytoma served as the positive control (VEGF expression grade 2+). VEGF expression was assessed by an investigator (S.H.) blinded to all clinical data and magnetic resonance imaging results. A semi quantitative grade from 0 through 2+ was assigned under a x 200 field (Fig. 1). At least three different areas in a tumor sample were examined for the assessment. The grade was assigned to an area which seemed to be average in the expression of VEGF in the sample.

In addition, tumor tissues were immunohistochemically stained with anti CD-34 and Ki-67 antibody using the immunoperoxidase technique. The number of CD-34 immunopositive vessels in a 0.28 mm^2 area was counted by another author (Y.K.) blinded to all pathological diagnoses and radiological data. In all samples, the number of microvessels was counted in three different areas, and the average counts were recorded.

Intratumoral hemorrhage, including acute-stage hematoma and chronic fluid hematoma, was the variable analyzed by logistic multivariate analysis against tumor size, the Ki-67 labeling index, the number of CD-34 positive vessels, and the level of VEGF expression (dependent variables). Values were expressed as the mean ± standard deviation. Differences of $p<0.05$ were regarded as statistically significant.

RESULTS

Histologic examination confirmed hemorrhagic infarction in all 6 cases in the acute phase of intratumoral hemorrhage. The neoplastic cells in different parts of their adenomas were judged to be strongly immunopositive (2+) for VEGF in 4 cases and positive (1+) in the other 2 (Table 1). Among the 21 cystic adenomas, 5 of 7 whose cysts contained fluid hematomas were graded as 2+, 2 as 1+. Of the other 14 cystic adenomas whose cysts contained xanthochromic or clear watery fluid, 3 were grade 2+, 9 were 1+, and 2 were negative for VEGF. Our samples included 12 homogeneously solid adenomas that contained neither cysts nor hematomas; 5 of these were graded as 1+, the remaining 7 were negative for VEGF.

The mean ± SD of CD-34 positive vessels was

Table 1. Relationship between tumor characteristics, VEGF positivity, MIB-1 index, and number of CD-34 positive vessels

Tumors	Case	Age	Sex	Hormone Secreted	Tumor Size	Positivity for VEGF	MIB-1 Index	No. of CD-34 Positive Vessels
Acute stage of apoplexy*	1	50	M	NF	25	+	0.1	60.0
	2	44	M	NF	29	++	0.8	131.0
	3	37	F	GH	28	++	0.2	53.0
	4	62	F	NF	31	+	0.1	18.0
	5	56	M	NF	23	++	0.5	80.0
	6	65	M	NF	32	++	0.1	107.0
mean ± SD								74.8 ± 40.4
Cysts containing fluid hematoma*	7	12	M	PRL	25	+	0.3	52.0
	8	51	M	GH	12	++	0.1	117.0
	9	64	F	NF	38	++	0.2	72.0
	10	63	F	NF	25	++	0.1	57.0
	11	22	F	PRL	20	+	0.2	72.0
	12	22	F	PRL	23	++	0.4	163.0
	13	22	F	PRL	22	++	0.4	25.0
mean ± SD								79.7 ± 46.0
Cysts containing xanthochromic or clear watery fluid	14	27	F	PRL	13	++	0.1	121.0
	15	44	F	ACTH	16	+	0.2	27.0
	16	16	F	PRL	32	++	1.1	175.0
	17	23	M	PRL	25	+	0.1	245.0
	18	46	F	NF	52	+	0.8	38.0
	19	18	M	GH	6	+	0.9	91.0
	20	45	F	NF	20	+	0.6	106.0
	21	46	F	GH	11	++	2.4	145.0
	22	31	M	GH	20	–	0.1	101.5
	23	33	F	PRL	14	+	0.2	130.0
	24	80	M	NF	30	–	0.1	100.0
	25	18	F	PRL	15	+	0.4	126.0
	26	68	F	NF	20	+	0.1	62.0
	27	68	F	ACTH	20	+	0.2	36.0
mean ± SD								107.4 ± 58.7
Homogeneously solid	28	32	F	NF	27	–	0.2	57.0
	29	55	F	NF	23	–	0.1	182.5
	30	60	F	NF	23	+	0.5	261.5
	31	56	M	NF	29	–	0.2	172.0
	32	58	M	NF	25	–	0.1	162.0
	33	60	M	NF	15	+	0.1	57.6
	34	63	M	NF	20	+	0.3	110.5
	35	55	F	NF	30	–	0.1	165.0
	36	52	M	NF	24	–	0.3	180.0
	37	31	M	NF	40	+	0.7	84.0
	38	49	F	NF	30	–	0.1	247.0
	39	40	F	NF	26	+	2.3	174.0
mean ± SD								154.4 ± 66.1

NF: nonfunctioning, GH: growth hormone-producing, PRL: prolactin-producing,
ACTH: adrenocorticotropine-producing adenoma
Positivity for VEGF: –, negative, +, positive, ++, strongly positive (positivity similar to GII astrocytomas)
*intratumoral hemorrhage in the text includes these 2 categories

74.8 ± 40.4 in the apoplexy group, 79.7 ± 46.0 in adenomas with cysts containing fluid hematomas, 107.4 ± 58.7 in those with cysts containing xanthochromic or clear watery fluid, and 154.4 ± 66.1 in homogeneously solid adenomas (Table 1). The differences were not statistically significant.

Multivariate analysis showed that only the expression of VEGF was an independent, significant factor for pituitary apoplexy and post-hemorrhagic cyst formation, i.e. cysts containing fluid hematomas (p=0.0079). None of the other factors we analyzed, i.e., tumor size (p=0.1995), the Ki-67 labeling index (p=0.1401), or the number of CD-34 positive vessels (p=0.2618) played a significant role with respect to pituitary apoplexy and post-hemorrhagic cyst formation. In addition, there was no correlation between VEGF expression and the number of CD-34 positive vessels (p=0.0961) in

the adenomas we analyzed by multivariate analysis.

DISCUSSION

According to previous reports, all types of human pituitary adenoma exhibit immunoreactivity for VEGF[13,18]. The degree of positivity was stronger in some subtypes such as GH-producing and corticotrophic adenomas[13]. In the rat pituitary, the overexpression of VEGF and its receptor (VEGFR-2) seems to play an important early role in estrogen-induced tumor angiogenesis[2,3]. However, in humans, the significance of VEGF expression remains to be elucidated.

Among brain tumors, pituitary adenomas have the highest incidence of spontaneous intratumoral hemorrhage[30]. In addition, around 50% of patients with pituitary adenomas manifest intratumoral cysts whose etiology is thought to be attributable to necrosis, exudation, or asymptomatic hemorrhage[16,21]. In our series, 4 of 6 adenomas with hemorrhagic infarction, and 5 of 7 adenomas with cysts that contained fluid hematomas were strongly positive (2+) for VEGF. The presence of VEGF induces neovascularization by promoting the proliferation, migration, and vasculogenesis of endothelial cells. In malignant neoplasms located in other organs, the CD-34 positive vessel count was positively correlated with the expression of VEGF[11,15,26]. In glioblastoma patients, tumor-associated intracerebral hemorrhage was associated with VEGF overexpression by glioblastoma cells[5]. Although VEGF overexpression may promote neovascularization in pituitary adenomas and result in the hemorrhage of immature vasculatures, in our series the level of VEGF positivity was not correlated with the number of CD-34 positive vessels.

Alternative splicing of the VEGF exon resulted in the production of 5 isoforms ($VEGF_{121, 145, 165, 189, 206}$)[20]. While $VEGF_{189}$ and $VEGF_{206}$ possess no endothelial proliferation effect, they promote vascular permeability[9]. In addition, 2 VEGF receptors, Flt-1 (VEGFR-1) and KDR/Flk-1 (VEGFR-2), have been identified[17]. The former primarily transmits signals for endothelial proliferation while the latter appears to play a role in vascular permeability[19]. Based on these considerations we posit that $VEGF_{189}$ and/or $VEGF_{206}$ are the predominant isoforms in pituitary adenomas. The down-regulation of Flt-1 and/or the up-regulation of KDR/Flk-1 may explain the discrepancy we observed between VEGF expression and CD-34 positive vessels. Increased permeability induced by VEGF overexpression may lead to exudation and cyst formation, which in turn may increase tissue pressure in adenomas. Pituitary adenomas are reportedly irrigated at least partially through the pituitary portal system[4,31]. Thus, even a small increase in tissue pressure within adenomas may suffice to overwhelm the inherently low perfusion pressure and result in tumor tissue necrosis and consequent intratumoral hemorrhage.

Alternatively, we can account for the positive correlation between the level of VEGF positivity and hemorrhage when we consider that VEGF overexpression may be the result, rather than the cause of hemorrhage. VEGF gene expression is stimulated by ischemia[1,12,24] which may be brought about by increased intratumoral pressure induced by intratumoral hemorrhage.

We are testing these hypotheses by studying the status of the VGEF-receptors and messenger RNA of VEGF isoforms and the receptors.

CONCLUSION

We noted a positive relationship between intratumoral hemorrhage and VEGF expression in human pituitary adenomas. The number of CD-34 positive vessels was not correlated with the degree of VEGF expression.

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