Endocrine Gland Derived-VEGF Is Down-regulated in Human Pituitary Adenoma

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Abstract. *Background. Endocrine gland-derived vascular endothelial growth factor (EG-VEGF) is an angiogenic molecule restricted to endocrine glands and, particularly, to steroid-secreting cells. The expression of EG-VEGF and its significance in human adenohypophysis in physiological and pathological conditions is still unknown. Materials and Methods: In this study, we investigated by immunohistochemistry the expression of EG-VEGF in 2 samples of normal adenohypophysis and 43 bioptic samples of pituitary adenoma. Moreover, the expression of growth hormone (GH), prolactin (PRL), follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH) and adrenocorticoprophic hormone (ACTH) were also estimated. Results: The results of this study for the first time demonstrate a down-regulation of EG-VEGF expression in human pituitary adenoma as compared to normal adenohypophysis, suggesting an impaired function of the neoplastic cells in terms of hormone release in the blood stream, as a consequence of impaired tumor angiogenesis in the tumor. Conclusion: On the basis of our data showing a marked decrease in the expression of EG-VEGF in pituitary adenoma, with the exception of LH-secreting adenomas, we suggest that LH might be involved in the induction of EG-VEGF secretion.*

In 2001, LeCouter *et al.* (1) described for the first time the isolation and characterization of endocrine gland-derived vascular endothelial growth factor (EG-VEGF). EG-VEGF induces proliferation, migration and fenestration in capillary endothelial cells associated with endocrine glands, while it has little or no effect on other endothelial cells. EG-VEGF does not belong to the VEGF family, but is a member of a new protein family with multiple regulatory functions (2). EG-VEGF has been detected in the adrenal cortex, in the ovary, testis and placenta, and low levels of EG-VEGF mRNA have been also demonstrated in the brain, colon, small intestine, liver, spleen and thymus (3).

The presence of EG-VEGF has also been demonstrated in pathological conditions, such as human cancer, including ovarian carcinoma (3), colorectal cancer (4), pancreatic adenocarcinoma (5) neuroblastoma (6), Leydig cell tumors (7) and benign lesions, such as polycystic ovaries (8). The angiogenic role of EG-VEGF is supported by the correlation found between its expression and microvascular density in Leydig cell tumors (7). The prognostic value of EG-VEGF expression by tumor cells is still a matter of debate, as no significant differences were reported between patients with high and low levels, in terms of overall survival.

There is no literature data to date concerning the expression of EG-VEGF in human adenohypophysis in normal and pathological conditions, even though the presence of VEGF-A and VEGF receptors has been demonstrated in pituitary adenoma (9, 10).

In this study, the expression of EG-VEGF in 2 sample of normal adenohypophysis and 43 bioptic samples of pituitary adenoma was investigated.

Materials and Methods

Forty-three pituitary adenoma and two normal adenohypophysis bioptic samples were processed by fixation in buffered formalin and embedded in paraffin using routine procedures. Slides were stained with hematoxylin-eosin and Gordon-Sweet silver staining for the pathological diagnosis in order to discriminate between pituitary adenoma and normal adenohypophysis. Slides were processed for

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Key Words: EG-VEGF, hypophysis, immunohistochemistry, pituitary adenoma.

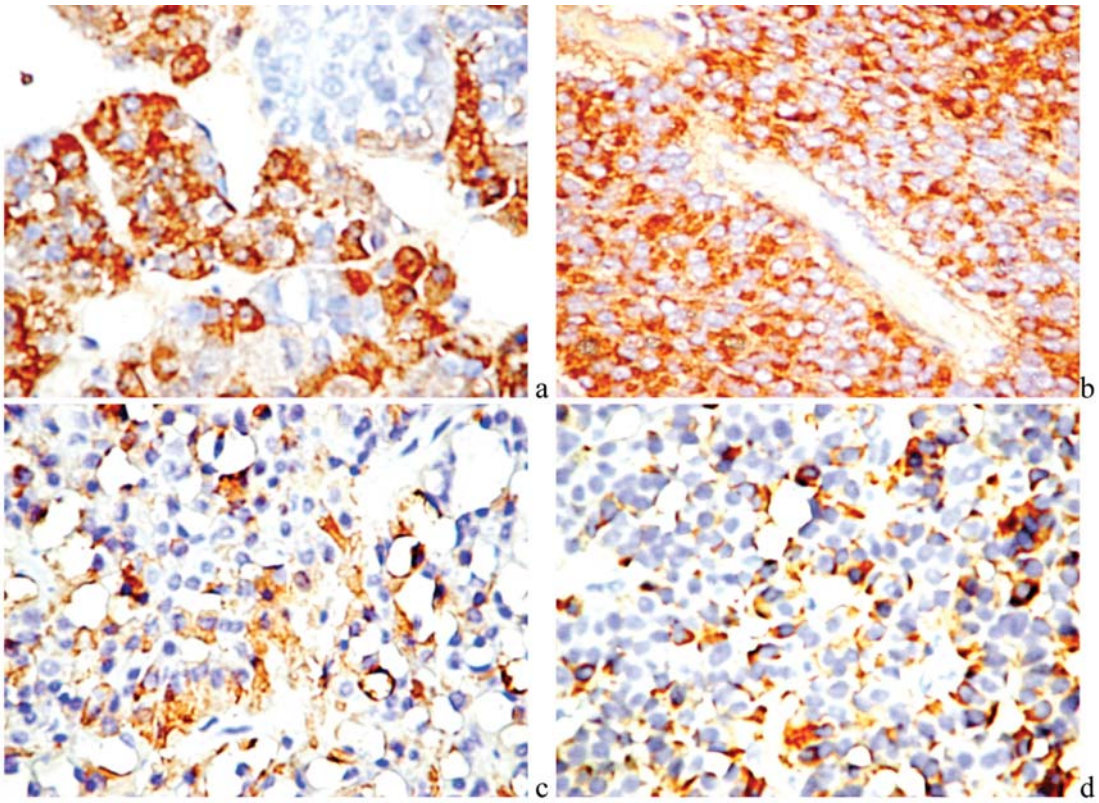

Figure 1. *Immunohistochemical expression of hypophyseal hormones (a) growth hormone, (b) prolactin, (c) luteinizing hormone and (d) follicle-stimulating hormone in pituitary adenoma. Original magnification: ×400.*

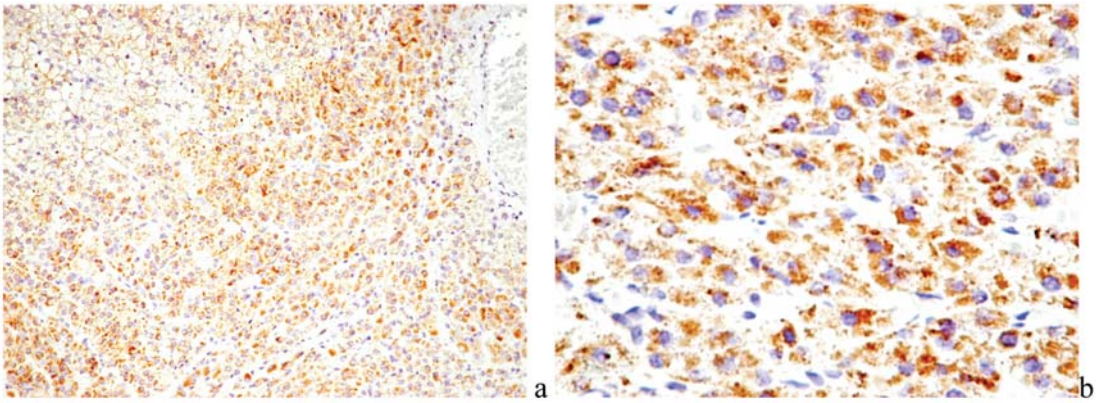

Figure 2. *Immunohistochemical expression of EG-VEGF in normal human adrenal cortex (a), and at higher magnification detail of cytoplasmic granular pattern in epithelial cells (b). Original magnification: (a) ×100; (b) ×400.*

immunohistochemical detection of EG-VEGF, growth hormone (GH), prolactin (PRL), follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and adrenocorticoprophic hormone (ACTH). Details concerning the antibodies, dilution, antigen retrieval and working systems are summarized in Table I.

EG-VEGF immunoreactivity was estimated as the percentage of positive cells according to this score: 0 (0% positive cells); 1 (<10%); 2 (10-50%); 3 (>50%). EG-VEGF immunoreactivity was also evaluated in terms of intensity of reaction, by using a scale from 0 to 3 for a negative, weak, moderate or strong reaction, accordingly. A final score was attributed ranging between 0 and 6, and samples that scored between 0-2 (+1) were considered negative, while samples that scored between 3-6 (+2 and +3) were considered positive. An average score for each group of immunohistochemically detected hormones was also performed. Slides

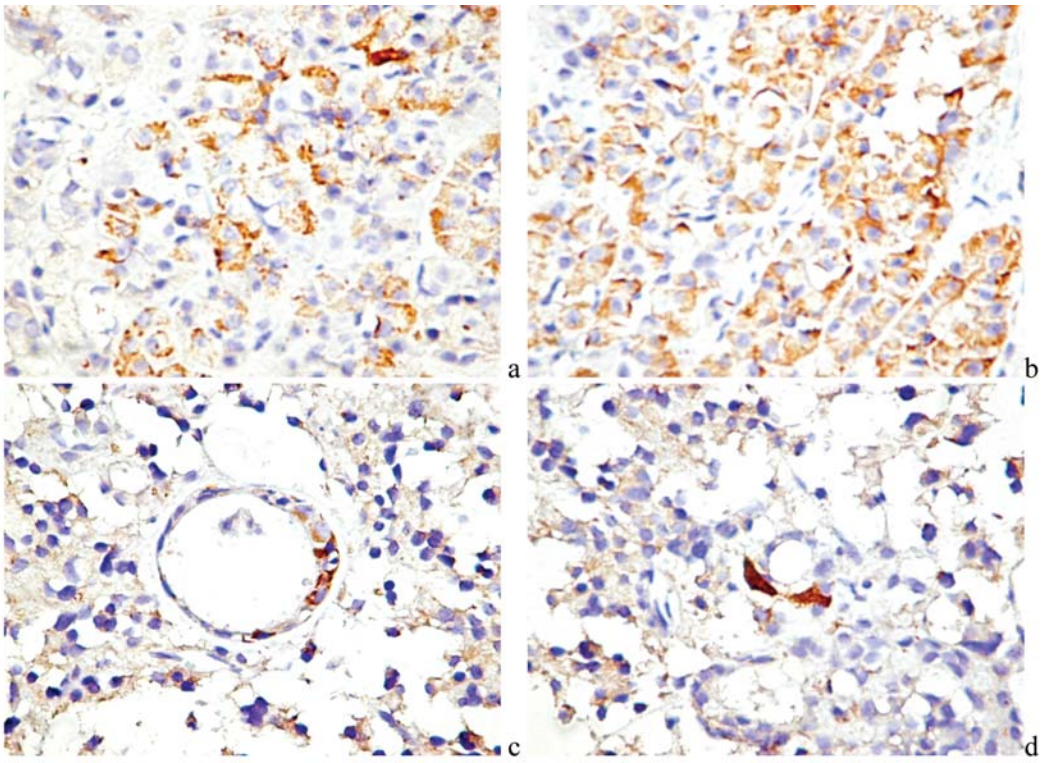

Figure 3. *Immunohistochemical expression of EG-VEGF in normal hypophysis (a). A positive reaction can be seen in the majority of endocrine cells (b), in the follicle of the intermediate hypophysis (c) and in isolated cells with strong cytoplasmic reaction (d). Original magnification: ×400.*

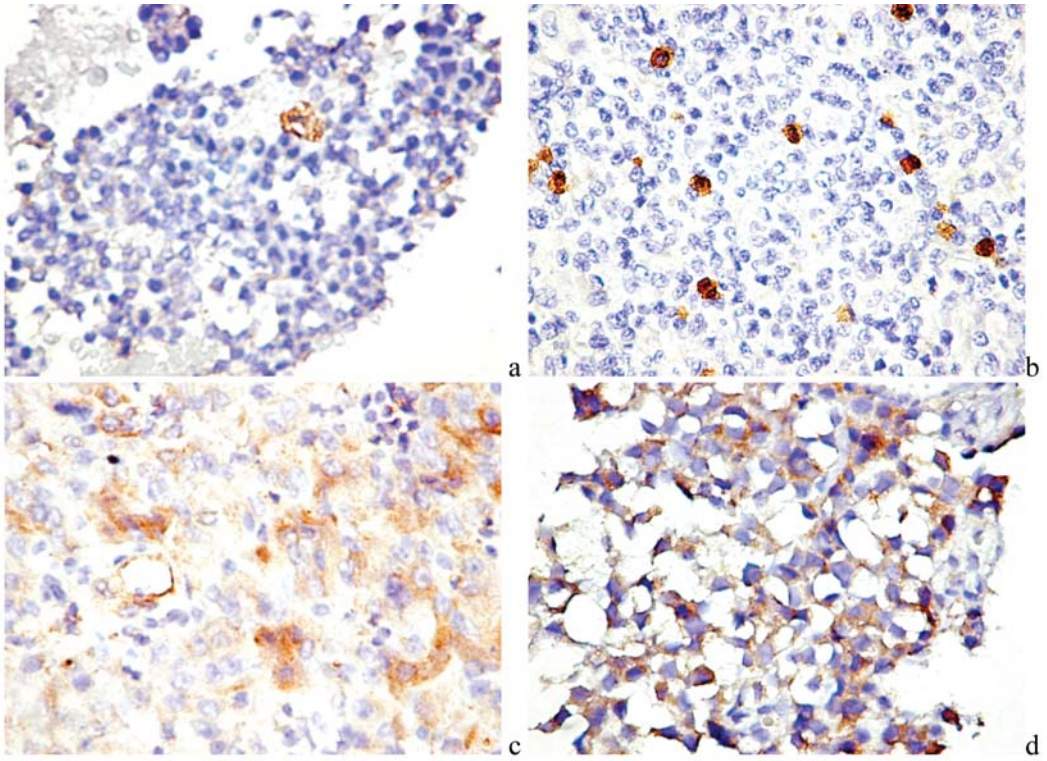

Figure 4. *Immunohistochemical expression of EG-VEGF in pituitary adenoma with staining score +1 (a), +2 (b) and +3 (c). The majority of neoplastic cells are intensely stained in a LH-secreting adenoma (d). Original magnification: ×400.*

Table I. *Reagents and technical details for immunohistochemistry.*

Antibody	Clone	Source	Dilution	HIER	WS
GH	Polyclonal, rabbit anti-human	Dako, Glostrup, Denmark	1:400	No	LSAB+, HRP
PRL	Polyclonal, rabbit anti-human	Dako, Glostrup, Denmark	1:250	No	LSAB+, HRP
FSH	Monoclonal, mouse anti-human, C10	Dako, Glostrup, Denmark	1:50	MW, pH 6, 5 minutes	LSAB+, HRP
LH	Monoclonal, mouse anti-human, C93	Dako, Glostrup, Denmark	1:50	MW, pH 6, 5 minutes	LSAB+, HRP
TSH	Monoclonal, mouse anti-human, 0042	Dako, Glostrup, Denmark	1:50	MW, pH 6, 5 minutes	LSAB+, HRP
ACTH	Monoclonal, mouse anti-human, 02A3	Dako, Glostrup, Denmark	1:50	MW, pH 6, 5 minutes	LSAB+, HRP
EG-VEGF	Polyclonal, goat anti-human, T-16	Santa Cruz Biotechnology, Inc, Santa Cruz, USA	1:100	MW, pH 6, 30 minutes	LSAB+, HRP

HIER: Heat-induced epitope retrieval; WS: working system; MW: microwave; GH: growth hormone; PRL: prolactin; FSH: follicle-stimulating hormone; LH: luteinizing hormone; TSH: thyroid-stimulating hormone; ACTH: adrenocorticoprophic hormone; EG-VEGF: endocrine gland-vascular endothelial growth factor.

obtained from normal deep human adrenal gland, which strongly express EG-VEGF with high intensity (the score was estimated as +3) were used as positive controls.

Results

On the basis of the immunohistochemical expression of hormones, tumors were stratified as follows: 7 GH-secreting tumors; 11 PRL-secreting; 1 LH-secreting; 1 ACTH-secreting. Pluri-hormone expression was demonstrated in 9 samples and the most frequent associations were found between GH and PRL, and between GH and ACTH (Figure 1a-f). No expression was found in 14 samples.

Control slides of normal human adrenal gland stained with anti-EG-VEGF antibody showed a strong positivity in the deep cortex but not in the medulla (Figure 2 a), with cytoplasmic granular pattern restricted to the endocrine epithelial cells (Figure 2 b).

In normal adenohypophysis samples, EG-VEGF stained the epithelial glandular cells (Figure 3 a) and the intensity of reactivity was significantly stronger as compared to adenoma cells, where EG-VEGF showed a cytoplasmic granular pattern (Figure 3 b-d).

In adenoma samples, positivity of reaction to EG-VEGF was detectable in 19 out of 43 samples (44.18%). The distribution of positive cells in the tumor mass was heterogeneous and the intensity of reaction ranged from weak to strong. Capillary endothelial cells within the tumors were also stained by the anti-EG-VEGF antibody in only 2 samples. Nine samples scored +1 (Figure 4a), 6 samples +2 (Figure 4b) and 4 cases +3 (Figure 4c), to indicate significant down-regulation of EG-VEGF expression in pituitary adenoma as compared to normal adenohypophysis.

Categorization of the samples based on the immunohistochemical profile of specific hormones showed that the highest incidence of positive samples was found in non-secreting adenomas (32.55%), followed by PRL-secreting adenomas (25.58%). On the other hand, the average score for EG-VEGF was highest in the samples with LH secretion. Results on the distribution of EG-VEGF-positive cases and average scores are summarized in Table II.

Table II. *Average score according to the hormone expression profile.*

Hormone expression (n=43)	%	EG-VEGF average score
GH (n=7)	16.27	1
PRL (n=11)	25.58	0.81
GH+PRL (n=4)	9.3	0
GH+ACTH (n=3)	6.97	1.33
GH+LH (n=1)	2.32	2
LH (n=1)	2.32	2
ACTH (n=1)	2.32	0
ACTH+LH+FSH (n=1)	2.32	2
No expression (n=14)	32.55	0.57

Discussion

Many growth factors are expressed by the normal adenohypophysis and pituitary tumors, and their major roles are related to gene expression for pituitary hormones and to cell proliferation (reviewed in 11). The expression of these factors is different in the neoplastic pituitary in comparison with normal pituitary tissue. It has been shown that VEGF expression is decreased in adenoma and increased in carcinoma as compared with normal adenohypophysis (12). This finding suggests that growth factors, and particularly VEGF, might be involved in tumor progression by stimulating angiogenesis (13, 14).

EG-VEGF is the first tissue-specific angiogenic molecule that selectively induces proliferation and survival of endothelial cells of endocrine glands and it might favor the entry of hormones in the vascular system. EG-VEGF induces

proliferation, migration and survival of endothelial cells in the adrenal, testis, ovary and placenta. In the ovary, EG-VEGF induces a strong angiogenesis and cysts formations and a strong expression of EG-VEGF has been found in the early pregnancy, in parallel with the highest rate of proliferation of placenta blood vessels (15). This effect has been further supported by a recent study demonstrating a stimulatory effect of EG-VEGF on human uterine microvascular endothelial cells (16). Mouse EG-VEGF has been shown to stimulate the growth of endothelial cells within distinct capillary beds (17). In colorectal cancer, EG-VEGF expression has been associated with an increased hematogenous metastatic potential (18) and an up-regulation of EG-VEGF has been reported in prostate carcinoma, suggesting that EG-VEGF could be involved in prostate carcinogenesis through the regulation of angiogenesis (19).

The results of this study, which for the first time demonstrate a down-regulation of EG-VEGF expression in human pituitary adenoma as compared with normal adenohypophysis, suggest an impaired function of the neoplastic cells in terms of hormone release in the blood stream, as a consequence of an impaired tumor angiogenesis in the tumor. Moreover, on the basis of our data showing a marked decrease in the expression of EG-VEGF in pituitary adenoma, with the exception of LH-secreting adenomas, we suggest that LH might be involved in the induction of EG-VEGF secretion.

The presence of steroidogenic factor-1 in the promoter region upstream of the EG-VEGF gene argues in the favor of the hypothesis that hormonal factors regulate EG-VEGF (7). The best candidate for hormonal regulation in EG-VEGF secretion is LH, which is well known to regulate genes involved in the synthesis of testosterone. This is in accordance with our findings, which showed a marked decrease in the expression of EG-VEGF in pituitary adenoma, with the exception of LH-secreting adenomas. In our series, there were only three LH-secreting cases, but all scored +3. Based on these findings, we can hypothesize that LH can induce EG-VEGF secretion.

In summary, we demonstrate for the first time a significant decrease in the immunohistochemical expression of EG-VEGF in pituitary adenoma in comparison with the normal adenohypophysis. This finding supports the possibility of an impaired release of pituitary hormones in the bloodstream in patients with pituitary adenoma, due to impaired angiogenesis.

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