SHORT REPORT

THE ROLE OF VEGF165B IN PITUITARY ADENOMAS PATHOGENESIS

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ABSTRACT

Purpose. Inhibitory fraction of vascular endothelial growth factor (VEGF165b) in pituitary adenomas is also overexpressed, as a possible explanation of low microvascular density (MVD). **Design and methods.** For 86 retrospective cases of pituitary adenomas we established the histopathologic diagnosis by using morphological stains, followed by immunoprofile case selection and VEGF165b assessment. **Results.** A percentage of 16.66% of the studied cases had positive reaction to VEGF165b. The reaction was of low intensity in most of the cases, noted as +1 and in 7 cases we recorded moderate intensity reaction +2. Tumor cells that presented a granular cytoplasmic heterogeneous reaction, were noted as +1,+ 2 and +3 in relation to the number of tumor cells and the reaction intensity. In the areas of VEGF165b tumor positive, blood vessels were not observed. Instead, strongly positive cells for VEGF inhibitory fraction tended to enclose mature vessels with patent lumen. **Conclusions.** VEGF165b might have a role of angiogenesis inhibition in pituitary adenomas. The reduced percentage of VEGF165b positive cases also suggests the involvment of other mechanisms of angiogenesis inhibition.

Key Words: VEGF165b, pituitary adenomas, angiogenesis inhibition.

INTRODUCTION

Vascular endothelial growth factor (VEGF) described as a vascular permeability factor isolated from bovine pituitary has been intensively studied in pituitary adenomas. VEGF is a therapeutic target for which there already developed a therapy with humanized monoclonal antibodies type bevacizumab in colon cancer metastasis [1] and attempts to use it in metastatic breast cancer, but the results of clinical trials are controversial [2, 3].

VEGF has been extensively studied in pituitary adenomas regarding its involvement in the angiogenic process, and less examined as a therapeutic target. Increased VEGF expression in most pituitary adenomas correlated with the low vascular density quantified in previous studies on the pituitary gland forced us to identify if VEGF inhibiting variant respectively VEGF165b in pituitary adenomas, is also overexpressed, as a possible explanation of low MVD. For this reason, the quantification of VEGF165b has been realized by immunohistochemical method. Interpretation of immunohistochemical reaction was carried out similar to that of VEGF.

MATERIAL AND METHODS

A retrospective study has been designed by randomly choosing of 86 cases of pituitary adenomas, which were collected by open surgery or transsphenoidal approach and processed in routine standard for paraffin embedding for the feature immunohistochemical and molecular method.

For immunohistochemistry we used VEGF165b (dilution 1:25, Reliatech, Germany) from Santa Cruz Biotehnology (USA). Microscopic evaluation was made using Nikon Eclipse E600 microscope (Nikon Corporation Japan). The images were captured and processed using Lucia G system.

RESULTS

A percentage of 16.66% of the studied cases had positive reaction to VEGF165b. The reaction was of a low intensity in most of the cases, noted by +1 and in 7 cases we recorded moderate intensity reaction +2. The expression pattern was predominantly granular cytoplasmic, and in rare cases membranous combined with the cytoplasmic one. Compared to the histopathological type, all VEGF165b positive cases were of acidophilic type or included acidophilic cells areas in case of mixed pituitary adenomas.

Immunohistochemical reaction was not restricted to tumor cells. In cases where the tumor cells were VEGF165b negative, we noticed positive isolated cells with granular cytoplasm most likely macrophages (Fig.1) or mast cells (fig.1b) included in tumor areas. Rare cells with morphology similar to follicular stellate cells morphology were also noted as VEGF165b positive (fig.1c).

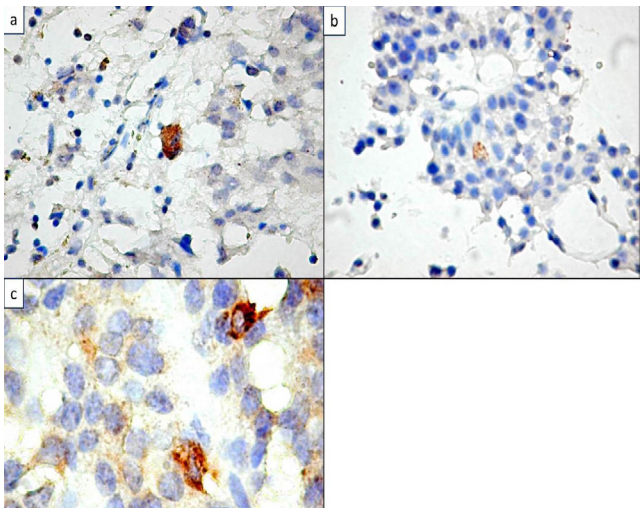

Fig.1. *VEGF165b positive cells types of macrophage* **(a)**, *mast cells* **(b)** *and follicular stellate cells morphology* **(c)**.

Tumor cells that presented a granular cytoplasmic heterogeneous reaction, were noted as +1,+ 2 and +3 in relation to the number of tumor cells and the reaction intensity (Fig. 2 a, b and c).

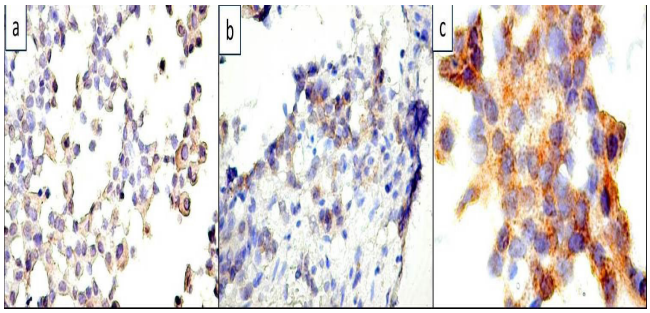

Fig.2. *Tumor cells positive reaction marked with +1* **(a)**, *+2* **(b)** *and +3* **(c)**. *The expression was cytoplasmic of a granular pattern.*

In the areas of tumor positive VEGF165b, blood vessels were not observed (3a). Instead, strongly positive cells for VEGF inhibitory fraction tended to enclose mature vessels with patent lumen (Fig. 3b).

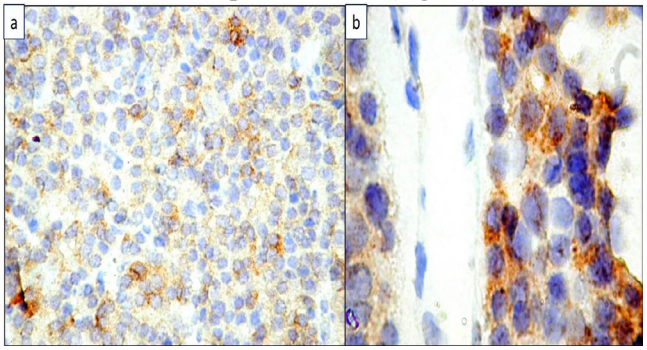

Fig.3. *VEGF165b positive tumor area, in which there are no blood vessels of visible lumen* **(a)**. *VEGF165b strongly positive tumor cells addicted to mature blood vessels with patent lumen.***(b)**

Due to the discrepancy between VEGF overexpression, low number of neovessels and poorly elucidated angiogenesis mechanism in in pituitary adenomas, we differentially quantified VEGF165b expression. Paradoxically, inhibiting VEGF165b in pituitary adenomas has been downregulated in pituitary adenomas. However, in cases where VEGF165b was overexpressed, the positive areas were completely devoided of blood vessels, suggesting the partial involvement of VEGF165b overexpression in tumor angiogenesis inhibition, especially in acidophilic pituitary adenomas cases that have registered an overexpression of VEGF165b.

DISCUSSIONS

A feature of VEGF165b expression in pituitary adenomas was the perivascular specific distribution of VEGF165b positive tumor cells around pre-existing vessels. This indirectly suggests inhibition of endothelial cell activation and initiation of tumor angiogenesis as a possible explanation of the low vascular density in pituitary adenomas. The low amount of VEGF165b positive cases suggests the presence of other tumor angiogenesis inhibitors mechanisms, most likely by a significant Endostatin quantity as has already been described in the literature [4]. Currently, there is one article in which Woolard et. al mention the presence of VEGF 165b in pituitary tissue without giving information about its expression in pituitary adenomas [5].

The present study describes for the first time the expression of VEGF165b also in other pituitary adenomas cellular structure. Thus, stromal cells with macrophage morphology or mast cells overexpressed VEGF 165b in the pituitary adenomas immunohistochemicaly negative for VEGF 165b. Another possible tumor angiogenesis inhibiting mechanism in pituitary adenomas with cells that do not overexpress VEGF 165b, may be explained by the possible accumulation of VEGF 165b positive macrophages or tumor infiltration with VEGF positive 165b mast cells.

The present results even in the preliminary stages are original, representing the first report of the VEGF 165b involvement in the pituitary adenomas pathogenesis.

REFERENCES

1. Köhne C.H. Successes and limitations of targeted cancer therapy in colon cancer. Prog Tumor Res. 2014; 41:36-50.
2. de Haas S., Delmar P., Bansal A.T., Moisse M., Miles D.W., Leighl N., Escudier B., Van Cutsem E., Carmeliet P., Scherer S.J., Pallaud C., Lambrechts D. Genetic variability of VEGF pathway genes in six randomized phase III trials assessing the addition of bevacizumab to standard therapy. Angiogenesis. 2014 Jul 11.
3. Kümler I., Christiansen O.G., Nielsen D.L. A systematic review of bevacizumab efficacy in breast cancer. Cancer Treat Rev. 2014 May 22.
4. Gruszka A., Kunert-Radek J., Pawlikowski M., Stepien H. Serum endostatin levels are elevated and correlate with serum vascular endothelial growth factor levels in patients with pituitary adenomas. Pituitary. 2005; 8(2):163-8.
5. Woolard J., Wang W.Y., Bevan H.S., Qiu Y., Morbidelli L., Pritchard-Jones R.O., Cui T.G., Sugiono M., Waine E., Perrin R., Foster R., Digby-Bell J., Shields J.D., Whittles C.E., Mushens R.E., Gillatt D.A., Ziche M., Harper S.J., Bates D.O. VEGF165b, an inhibitory vascular endothelial growth factor splice variant: mechanism of action, in vivo effect on angiogenesis and endogenous protein expression. Cancer Res. 2004 Nov 1: 64(21):7822-35.