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Endoglin and CD-34 immunoreactivity in the assessment of microvessel density in normal pituitary and adenoma subtypes

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Vascularization is a prerequisite of tumor growth, invasion and metastasis. In the present work, microvessel density was assessed by quantitating using two different endothelial cell biomarkers, endoglin (CD-105) and CD-34. Fifty endocrinologically active and 36 clinically nonfunctioning pituitary adenomas, all surgically resected, as well as 10 autopsy-derived normal adenohypophyses were investigated by immunohistochemistry. The results showed that in every pituitary adenoma type endoglin, an assumed biomarker of proliferating endothelial cells, immunostained fewer vessels than CD-34 which revealed immunopositivity in all capillaries. Differences in endoglin versus CD-34 immunoexpression indicate varying degrees of vascularity in pituitary adenoma subtypes. The low levels of endoglin immunoexpression in pituitary tumors exposed to long-acting somatostatin analogs and dopamine agonists are consistent with the view that these agents inhibit angiogenesis.

Keywords: immunohistochemistry, endoglin, CD34, microvascular density, angiogenesis, pituitary

Angiogenesis plays a key role in supporting tumor cell proliferation and facilitating both invasion and metastasis. If blood supply is insufficient, tumors fail to grow, their cells undergoing apoptosis/necrosis. Neoformation of vessels has been the subject of extensive investigation over the past two decades, numerous neoplasms, including pituitary tumors, having been studied [1–8]. Microvessel density can be quantitated in histologic sections immunohistochemically demonstrating various endothelial markers, such as von Willebrand factor, CD-31 and CD-34. Recently, a new endothelial cell biomarker, endoglin or CD-105, was introduced [9–13]. Studies of endoglin expression suggest it is present only in endothelial cells of newly formed vessels. If correct, its quantification would permit more meaningful measurement of angiogenesis.

Herein, we evaluate microvessel densities of various pituitary adenoma subtypes using endoglin expression in comparison with the results of CD-34 immunoreactivity, their proportions being a reflection of newly formed versus all vessels.

Materials and methods

Fifty endocrinologically active and 36 clinically non-functioning pituitary adenomas were obtained from the archives of St. Michael's Hospital and Mayo Clinic. Nonfunctioning adenomas included gonadotroph and null cell adenomas of nononcocytic as well as oncocytic type. Ten autopsy-obtained, normal anterior pituitaries were also investigated. All adenomas were obtained by transsphenoidal surgery, fixed in 10% buffered formalin, routinely processed, paraffin embedded, cut at 5 μm and stained by the hematoxylin-eosin (H&E), periodic acid-Schiff (PAS) and, in some cases, the Gordon-Sweet silver method for reticulin fibers. In addition, all tumors were immunostained (streptavidin-biotin-peroxidase-complex method) for growth hormone (GH), prolactin (PRL), adrenocorticotrophin (ACTH), thyrotrophin (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and alpha subunit (α SU) of glycoprotein hormones. Antibody sources, clonality, dilutions as well as control procedures have been previously described [14]. In addition, a majority of tumors were fixed in 2.5% glutaraldehyde, osmicated and processed for transmission electron microscopy [15, 16]. Thus, all lesions were accurately classified according to the World Health Organization classification of endocrine neoplasms [16].

For the immunohistochemical demonstration of endoglin and CD-34, the same method was applied using an monoclonal mouse-anti-human endoglin (CD-105) antibody (Dakocytomation, Carpinteria, CA, USA) and a rabbit polyclonal antibody directed against human CD-34 (Immunotech,

France), diluted 1:50 and 1:100, respectively, in antibody diluent reagent solution (Zymed, San Francisco, CA, USA). Microsections of normal human skin and pancreas served as positive controls, one section being included in each staining run. Replacement of the primary antibody with PBS served as a negative control.

Quantitation of immunopositively stained vessels was performed by counting stained contours of any configuration in 10 randomly selected fields under low magnification (X100). Scores were expressed in terms of mean ± SEM (standard error of the mean).

Results

In all the various pituitary adenoma subtypes, vasculature was easily recognized. Compared to nontumoral adenohypophyses in H&E- and PAS-stained slides, several vessels are irregular in size and shape. Smaller and larger capillaries were seen, some dilated and blood-filled. As a reflection of surgical intervention, small acute hemorrhages were also identified. Based upon histologic features of the vasculature, no distinction of various pituitary adenoma subtypes could be made.

Endoglin staining showed that the endothelial cells of scattered small or large, somewhat irregular and dilated vessels were immunopositive. Not all vessels, particularly capillaries were labeled, whereas the CD-34 preparation demonstrated staining of endothelial cells in all visible capillaries. A definite difference in the number of endoglin and CD-34 stained vessels was noted in all adenoma subtypes. Based upon our histologic findings (Figs. 1-6) and quantitations of microvessel densities (Table 1), it was evident that the latter differed considerably among the various tumor subtypes. In the sections immunostained for endoglin, the highest microvessel densities were noted in TSH cell, silent subtype 3, and untreated PRL cell adenomas. The lowest microvessel densities are in untreated and treated GH cell as well as in ACTH cell adenomas.

In CD-34 immunostained sections, the highest microvessel densities were noted in treated PRL cell, TSH cell and in treated GH cell adenomas; the lowest densities were seen in untreated GH cell, silent subtype 3 and ACTH cell adenomas. Studies of these same cases clearly indicated that microvessel densities were lower in endoglin immunostained sections than in the CD-34 preparations. These differences are summarized in Table 2 and are most noticeable in treated PRL cell, treated GH cell and TSH cell adenomas. They are seen to be smallest in silent subtype 3, untreated GH cell and ACTH cell adenomas.

Results were evaluated also between adenomas exposed to long-acting somatostatin analogs and dopamine agonists as compared to adenomas not treated with these agents. Table 3 shows differences in microvessel densities between untreated and treated GH as well as PRL cell adenomas to be smaller in endoglin as compared to CD-34 immunostained sections.

Comparisons made between adenomas and normal pituitaries are summarized in Table 1. Analyzing microvessel densities in endoglin immunostained slides, microvessel densities were lower in normal pituitary tissue. However, in the slides immunostained for CD-34, microvessel densities were higher in all tumors.

Discussion

Endoglin, a component of the TGF receptor complex, is a 180 kDa hypoxia-inducible, disulfide-linked, homodimeric transmembrane glycoprotein. It binds to TGF β-1 and TGF β-3 and modulates TGH β signaling; its gene is situated on chromosome 9q34 [17–21]. Mutation of the gene results in hereditary hemorrhagic telangiectasia, an autosomal dominant disease characterized by arterial/venous malformations and multi-organ hemorrhages [22]. Endoglin-null mice die in utero due to inability to form vessels. Endoglin, a proliferation associated antigen abundantly expressed in angiogenic endothelial cells and in endothelial precursor cells, is regarded as a biomarker of neovasculature [17, 23]. Its expression as summarized in a review by Dallas et al. [24] has been investigated in several tissues and in various tumor types.

To date, endoglin expression in pituitary tumors by immunohistochemistry has been reported only by Pizarro et al. [23]. These authors found no differences between endoglin expression and age, clinical presentation, tumor size, immunohistophenotype and MIB-1 labeling indices. The only difference was noted between male and female patients. According to the findings of these authors, microvessel densities using the endoglin antibody were significantly higher in adenomas of male patients.

Our present study showed microvessel densities based upon endoglin labeling were lower in all adenoma subtypes than those obtained by the application of CD-34. This finding is consistent with the view that endoglin is expressed primarily in the endothelial cells of newly formed vessels, whereas CD-34 labeled endothelial cells of all vessels.

In previous studies from our group, compared to nontumoral adenohypophyses, decreased microvessel densities were noted in slow-growing pituitary adenoma subtypes [3, 4, 5, 8]. In the present work, this decrease was not apparent on CD-34 preparations, a finding perhaps due to differences in immunostaining method and/or quantitation of staining. Microvessel densities based on endoglin staining were, however, very low in the nontumoral adenohypophyses, indicating that vascularization is limited in nontumoral adenohypophysis.

It was not unexpected that, CD-34-based microvessel densities were relatively high in GH cell adenomas exposed to long-acting somatostatin analogues and in PRL cell adenomas exposed to dopamine agonists. This finding was likely due to tumoral shrinkage secondary to decrease in size of tumor cells and their loss, the vessels remaining intact. [25, 26] The very low endoglin-based microvessel densities observed in the two treated adenoma groups indicates that neovascularity is suppressed due to somatostatin analogue and dopamine agonist therapy.

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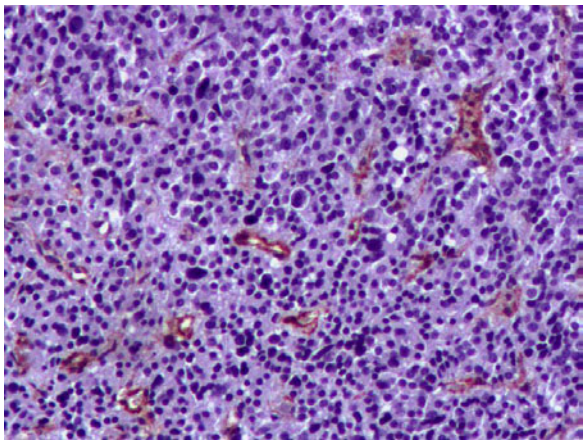


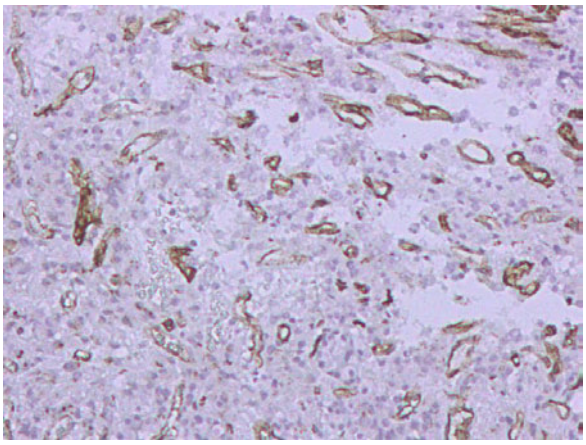
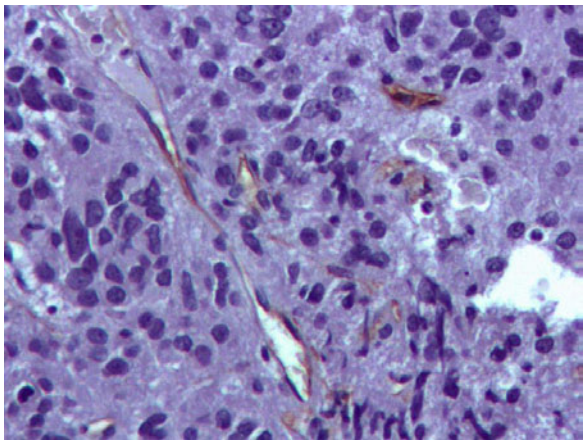


Fig. 1 & 2: TSH adenoma showing vessels immunostained for endoglin and CD-34. Magnification: x100



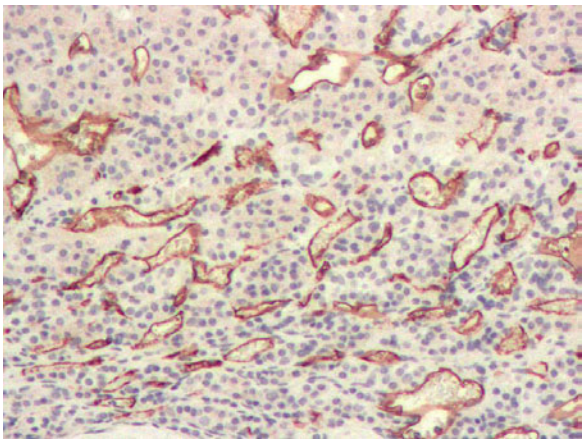
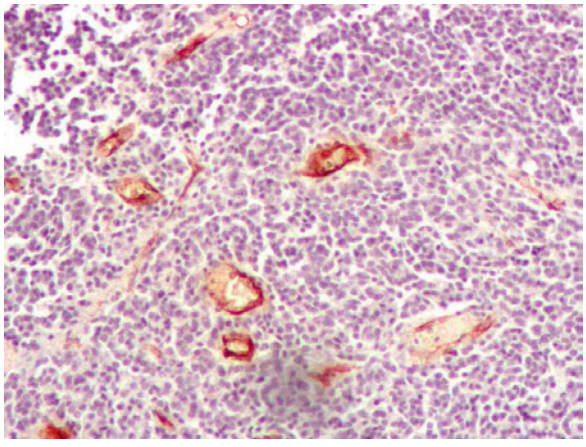


Fig. 3 & 4: Treated PRL cell adenoma showing the absence of neovascularization with the endoglin immunostaining as compared to the CD-34. Magnification: x200 and x100, respectively



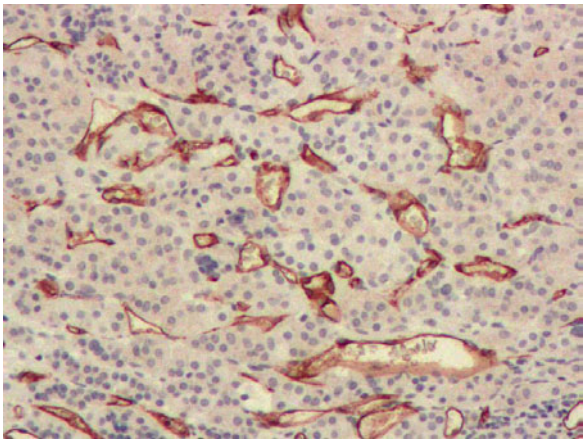


Fig. 5 & 6: Untreated PRL cell adenoma showing immunopositive vessels in both endoglin and CD-34. Magnification: x100

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