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RESEARCH ARTICLE

Endothelial Expression of Endocan Is Strongly Associated with Tumor Progression in Pituitary Adenoma

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Abstract

Although benign, pituitary adenomas frequently invade adjacent sinuses or recur after first surgery. To date, there is no histological marker predictive of recurrence. Angiogenic factors are candidate markers. Endocan is a proteoglycan secreted by endothelial cells, associated with an aggressive behavior in several tumor types. Endocan expression was investigated by immunohistochemistry and reverse transcription polymerase chain reaction (RT-PCR) in 18 normal post-mortem pituitaries and in 107 patients operated for a pituitary adenoma (with a follow-up of at least 8 years after surgery). In normal pituitaries, endocan was never observed in vessels but was detected in isolated endocrine cells. In adenoma tissue, we found a strong association between endocan immunoreactivity in endothelial cells and progression ($P = 0.0009$), as well as tumor size ($P = 0.0012$), raised mitotic count ($P = 0.02$) and p53 expression ($P = 0.032$). Morphometric analysis of the microvessels showed that the mean vessel area was significantly higher in the subgroup of tumors with an endothelial expression of endocan ($P = 0.028$), coherent with the neoangiogenesis process occurring in the pituitary. The immunolabeling of endocan in endothelial cells may therefore appear to be a relevant marker of aggressive behavior in pituitary tumors.

INTRODUCTION

Pituitary adenomas are the most common tumors of the pituitary, frequently asymptomatic and discovered at autopsy in up to 15% of patients in a meta-analysis (7). Pituitary carcinomas are their malignant counterpart, representing approximately 0.2% of all pituitary tumors. The occurrence of metastasis is today the only criteria of malignancy in such tumors (18). Nevertheless, one-third of all pituitary adenomas invade the cavernous and sphenoid sinuses, and/or recur (9, 35). In order to minimize the rate of recurrence, postoperative treatments may be proposed, such as somatostatin or dopamine analogs in growth hormone (GH) and prolactin adenomas, radiotherapy in nonfunctioning adenomas, and recently temozolomide therapy in carcinomas or aggressive tumors with multiple recurrences (25).

Predictive markers of recurrence are yet to be firmly established. The preoperative maximal diameter and the infiltration of cavernous sinuses are negative prognostic factors, but mostly through impairing the total surgical removal of the tumor. Until now, there are no consensus histopathological criteria of invasiveness or recurrence, which would be useful for organizing the treatment strategy. Raised mitosis count, Ki-67 proliferation index over 3% and p53 immunoreactivity (IR) of tumor cells have been proposed (18). However, new pertinent criteria, including molecular data, are still needed to complete the prognostic score (26, 43, 44).

Normal pituitary and pituitary tumors dramatically differ in their vascular network. Some authors have already pointed out this striking difference (8). It suggests that vascular remodeling may be a key event during pituitary tumorigenesis and should be investigated as a putative prognostic factor in aggressiveness and recurrence.

Endocan, also called endothelial cell specific molecule-1, is a new biomarker associated with angiogenesis and growth in tumors involving lung, kidney, stomach, liver and brain (3, 11, 12, 15, 16, 20). Endocan is a proteoglycan carrying a unique chondroitin sulphate chain that binds and regulates growth factor activities (2, 30).

In vitro, endocan biosynthesis and secretion are controlled by pro-angiogenic molecules such as vascular endothelial growth factor (VEGF) or fibroblast growth factor (FGF-2) (11, 20, 32). Endocan has been recently described as a marker of neoangiogenesis (6, 27, 34). Interestingly, endocan was also shown to be markedly expressed during the switch between dormant to fast-growing phenotype in experimental models of angiogenic tumors (1). Furthermore, vascular endocan expression is correlated with a worse prognosis in pulmonary nonsmall cell carcinomas and in hepatocarcinomas, and its expression is correlated with higher grade in gliomas (11, 12, 20).

We have investigated endocan expression in 18 normal pituitaries and in pituitary tumors obtained in a retrospective series of 107 patients with a postoperative follow-up longer than 8 years after surgery. Endocan's expression was compared with the clinical and radiological criteria (size of the tumor, invasiveness and recurrence), the established histological markers (raised mitotic count, p53 and Ki-67), and was compared with the CD34 expression to analyze microvessel density (MVD) and vessel area in pituitary tumor.

PATIENTS AND METHODS

Normal pituitary

Eighteen pituitaries were obtained at autopsy from people devoid of any endocrine disease. The cause of death was neurodegenerative disease in most cases (Alzheimer disease, vascular dementia, progressive supranuclear palsy, Lewy body disease, corticobasal degeneration, Pick's disease, paraneoplastic limbic encephalitis), infectious disease, cancer or multiple organ failure.

Pituitary tumors

We selected 107 patients (43 men and 64 women; age at first surgery: mean age 45.2 years, extremes 13–75 years, median age 45 ±SD 13.9 years) in whom a surgical treatment was performed between 1987 and 1998 in the University Hospital of Lille. In all these patients, the follow-up was at least 8 years after first surgery (median 12 years ±SD 4.3 years). As a part of the global Hypopronos grant (*PHRC national 27-43: Facteurs pronostiques des tumeurs hypophysaires*), the design of our study fulfilled the criteria of the local ethical committee (Hospices Civils de Lyon).

Tumors were classified according to the tumor size determined on magnetic resonance imaging (MRI) before surgery, as microadenoma (diameter < 1 cm; n = 7), macroadenoma (diameter > 1 cm; n = 100). MRI data were available in 83/107 cases: the average tumor size was 20.8 mm (median: 19 mm ±SD 11.6 mm; extremes: 2–55 mm). Invasion was evaluated on MRI examination. Recorded data included hormone levels. The initial pathological diagnosis was established on the base of cytologic appearance of the tumor under light microscopy and immunohistochemistry. No electron microscopy was performed. There was no discrepancy between clinical data and the pathological diagnosis. Altogether, there were 48 GH, 21 adrenocorticotropic hormone (ACTH), 6 prolactin and 32 nonfunctioning adenomas (including 27 gonadotroph and 5 null-cell adenomas). One prolactin carcinoma with appearance of cervical lymph node metastasis during the follow-up was also studied by immunohistochemistry but not included. There were 16 out of 48 acromegaly patients who received treatment before surgery (ie, four patients received dopamine antagonists alone, nine patients received somatostatin analogs alone and three patients received dopamine antagonists followed by somatostatin analogs). There were three out of six patients with prolactin adenoma who received dopamine antagonists before surgery. Tumor progression was defined as an increase of hormone plasma level and/or radiological evidence of tumor mass after a previous remission or an increase of the size of a tumor remnant during the whole follow-up period. Tumor progression was then observed in 46 patients.

Histology and immunochemistry

One hundred seven pituitary tumors were fixed in Bouin fixative, embedded in paraffin and stained according to Herlant's technique. As controls, 18 normal pituitaries removed at autopsy from different patients were fixed in alcohol—formalin—acetic acid.

For immunohistochemistry, 3 μm thick sections were labeled in a Benchmark XT automat (Ventana Medical System, Tucson, AZ, USA). The following antibodies were used: Ki-67 (1/50, Dako, Glostrup, Denmark), p53 (1/100, Dako), chromogranin (1/200, Dako), PS100 (1/200, Dako), ACTH (1/4500, Dako), prolactin (1/2000, Dako), GH (1/10000, Dako), βFSH (1/100, Biogenex, the Hague, the Netherlands), βLH (1/2, Neomarkers, Astmoot Runcorn, UK), βTSH (1/100, Biogenex), α subunit (1/9000, Immunotech, Marseille, France), CD34 (1/150, Immunotech) and endocan (clone MEP08, 1/200, Lunginnov, Lille, France).

The Ki-67 labeling index was expressed as a maximum percentage on a count of 2000 cells minimum and the mitoses were counted on 10 adjacent high magnification microscopic fields (0.19 mm^2/field).

P53 immunolabeling was considered positive when strong nuclear staining was present in more than five cells.

In order to further characterize the endocan immunoreactive cells, serial sections were tested with endocan and antihormone antibodies on normal pituitaries; a double fluorescent labeling with endocan, anti-hormone and anti-S100 antibodies was performed on normal and tumoral pituitary.

Molecular study

For mRNA analysis, 6 post-mortem pituitaries and 15 pituitary tumors were frozen and stored at −80°C until use. As there was no frozen tissue available among the series of 107 patients with long follow-up, fresh tissue samples suitable for molecular analysis were obtained from 15 adenomas operated in 2008. Tissue blocks from these additional tumors were immunolabeled for endocan, in order to check the occurrence of IR and the staining pattern.

RNA extraction and reverse transcription polymerase chain reaction (RT-PCR): total RNA was isolated from Trizol-treated samples according to the manufacturer's recommendations. Two μg of total RNA was reverse transcribed using SuperScript™ II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). The cDNA was amplified for PCR 35 cycles. Specific primers for endocan were within the exon 1 (5′GAG GCA GCT GGG AAA CAT GAA G3′) and in the exon 3 (5′GCT TTC TCT CAG AAA TCA CAG 3′). RT-PCR products were then resolved on a 1.5% agarose gel.

Determination of the MVD and the mean vessel area (MVA)

For morphometric analysis of the microvessels, sections were immunostained for the CD34 endothelial marker and digitalized using a Nanozoomer scanner (Hamamatsu Photonics, Hamamatsu, Japan). In only 65/107 tumors, the tumor area was sufficient for morphometric analysis (software Vessels designed for vascular morphometry, Tribvn, France).

Statistical analysis

Analysis was performed with SAS software v9.2 (SAS Institute Inc., Cary, NC, USA). Subsets of patients were compared using Fisher exact and χ^2 tests. Variables were compared using Student's and Mann–Whitney tests. Differences with *P*-values of <0.05 were considered significant.

RESULTS

In normal pituitary, endocan is never expressed in the endothelial cells but is observed in few endocrine cells

Endocan immunolabeling was demonstrated in clustered or isolated endocrine cells. The labeling was cytoplasmic and granular. The intensely stained cells were mostly located in the mucoid wedge (Figure 1A,B). on adjacent sections; these cells presented the cytological characteristics of normal ACTH cells such as basophilic cytoplasm containing an enigmatic body. On serial sections, most endocan-immunoreactive clusters, but not all of them, were positive with anti-ACTH antibody (Figure 1C,D). Double fluorescent labeling showed that the endocan-positive cells were also immunoreactive for chromogranin A, but not stained for S100 protein, excluding a folliculostellate lineage (not shown). There was no endothelial IR for endocan. Similarly, when non-neoplastic pituitary tissue was observed around a pituitary adenoma, there was no endocan immunolabeling within vessels. The posterior lobe, when present, was immunonegative for endocan.

Endocan is expressed in endothelial and/or endocrine cells in a subset of pituitary tumors

In pituitary adenoma tissue samples, a cytoplasmic labeling of endothelial cells with endocan antibody was observed in 52/107 cases. The immunolabeling never involved all the capillaries in a field, but only scattered vessels (Figure 2A). Most labeled capillaries exhibited a segmentary positivity, involving not all the endothelial cells of a vessel but only a part of the endothelium lining (Figure 2B). The labeling was not restricted to the luminal or abluminal compartment but diffuse to the whole cytosol. We were also able to study endocan expression by immunohistochemistry in one case of prolactin carcinoma: almost all capillaries displayed strong endocan IR, involving all the endothelial cells on each labeled capillary. In contrast, in this sample, there was no tumor endocrine cell stained (Figure 2C).

The endocan labeling of endocrine tumoral cells was observed in 32/107 cases, involving from 1% to 100% of the tumor cells (Figure 2D,E). Double fluorescent labeling of endocan vs. chromogranin and endocan vs. PS100 (Figure 2F,G) confirmed endocan expression by tumor cells, but not by folliculo-stellate cells.

Considering the adenoma subtype, endocrine cell IR was more often observed in ACTH adenomas (38%: 8 out of 21 tumors) than in nonfunctioning adenomas (25%: 8 out of 32 tumors) or GH adenomas (15%: 7 out of 48 tumors). No IR was observed in prolactinoma (0/6). Several cases displayed endothelial and endocrine cell endocan immunoreactivity (six adenomas, four

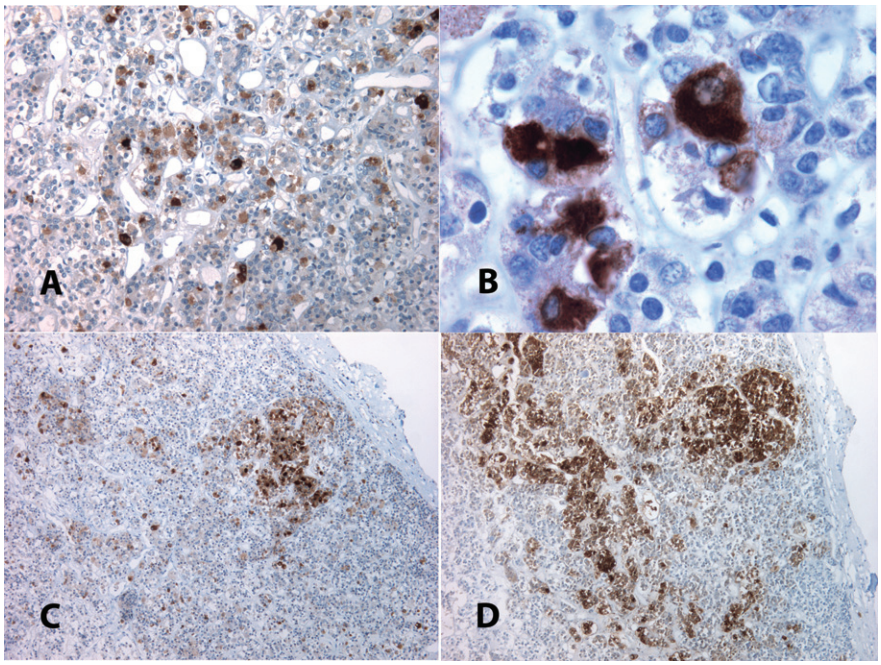

Figure 1. *Immunolabeling of endocan in normal pituitary.* **A and B.** Anti-endocan antibody. Post-mortem pituitary gland showing scattered immunoreactive endocrine cells but no endothelial labeled cell (A); the labeling is cytoplasmic and granular (B). **C and D.** Anti-endocan (C) and anti-ACTH (D) antibodies. Serial sections from a post-mortem pituitary gland show that the endocan-IR cells are also ACTH-IR. But only some ACTH cells express endocan. ACTH = adrenocorticotropic hormone; IR = immunoreactivity.

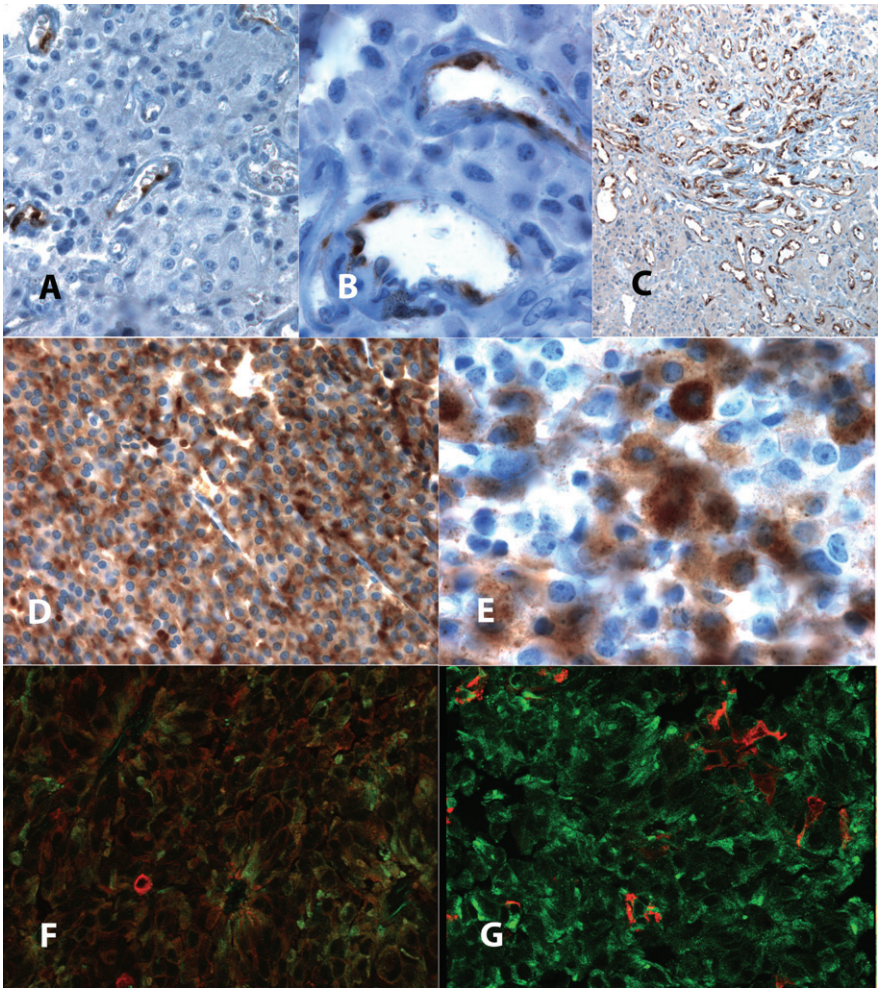

Figure 2. *Immunolabeling of endocan in pituitary tumors.* **A and B.** Labeling of capillaries in pituitary adenoma is not found in all vessels (A: prolactin adenoma) and, even in a labeled vessel, not all endothelial cells are labeled (B: ACTH adenoma). **C.** A case of prolactin carcinoma displaying broad and strong vascular immunoreactivity for endocan. **D and E.** Endocan reactivity is variably intense, from null to strong, as here displayed in a gonadotroph adenoma (D) and an ACTH adenoma (E). **F and G.** Double fluorescence labeling in a gonadotroph adenoma. The endocan-positive cells are also chromogranin-positive (F: endocan stained red, chromogranin green, merge yellow), but PS100-negative (G: endocan stained red, PS100 green, merge yellow).
ACTH = adrenocorticotropic hormone.

ACTH and two nonfunctioning adenomas). Results are summarized in Table 1.

RT-PCR

Endocan mRNA was studied by RT-PCR and was shown in 6 postmortem pituitary gland samples and 15 pituitary adenoma samples (Figure 3).

In 7 out of the 15 adenoma samples, although endocan was detected at mRNA levels, no immunolabeled cells were found within the tumor, but in two out of them, a few endocan-positive cells were detected in the surrounding rim of nontumoral pituitary.

Statistical analysis of the endocan IR as a marker of recurrence and/or aggressiveness

A significant positive association was shown between endothelial endocan expression and recurrence during the follow-up period ($P = 0.0009$). Endothelial endocan expression and p53 IR were also associated ($P = 0.032$; Table 2). In contrast, there was no

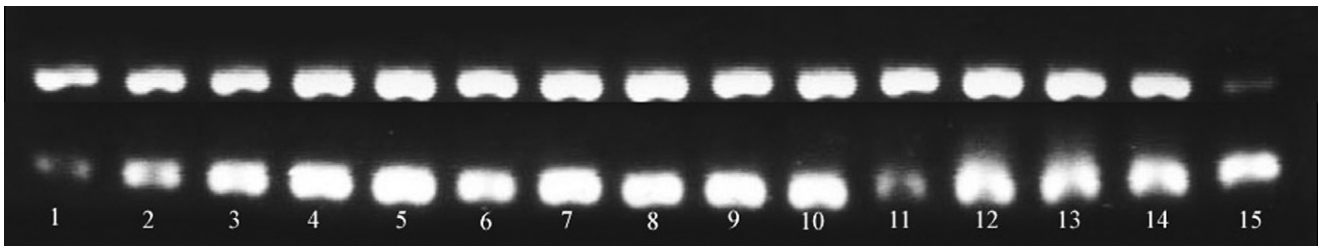

Figure 3. RT-PCR analysis of samples of 15 pituitary adenomas. Endocan is expressed in all tumors. Upper line: endocan; lower line: GAPDH. GAPDH = glyceraldehyde 3-phosphate dehydrogenase; RT-PCR = reverse transcription polymerase chain reaction.

Table 1. Distribution of endocan immunoreactivity (IR) and subtype of pituitary adenoma tissue.

	GH pituitary adenoma	ACTH pituitary adenoma	PRL pituitary adenoma	Nonfunctioning pituitary adenoma
Endothelial IR	15	10	5	22
No endothelial IR	33	11	1	10
Tumor cell IR	7	8	0	8
No tumor cell IR	41	13	6	24

ACTH = adrenocorticotropic hormone; GH = growth hormone; PRL = prolactin.

association between cellular endocan expression and recurrence, p53 IR and tumoral invasion.

A significant positive association was shown between endothelial endocan expression and tumor size ($P = 0.0012$) and average mitosis count ($P = 0.02$; Table 3). In contrast, there was no association between endothelial endocan expression and the Ki-67 labeling index.

There was no association between tumoral cell endocan IR and tumor size, mitosis count and Ki-67. The significant positive associations were found when the tumors were considered altogether. However, there was no significant association between endocan IR, tumor size, mitosis count, Ki-67 index, invasion or recurrence when each adenoma subtype was considered individually (data not shown).

Endocan endothelial IR is associated with a higher MVA in pituitary adenomas

We then compared endocan expression with CD34 expression (as an endothelial marker to assess microvascular density) and using morphometric software. In our series, 32 out of 65 pituitary adenomas showed endothelial endocan IR and 33 out of 65 did not.

We did not show any association between the occurrence of an endocan endothelial IR and the MVD ($P = 0.59$).

In contrast, the MVA was significantly higher in the group of endocan-positive adenomas ($P = 0.028$; Table 4). Interestingly, the MVA was 147.1 μm^2 in the endocan-positive group (SD 91.2 μm^2) and 100.1 μm^2 in the endocan-negative group (SD 77.0 μm^2).

DISCUSSION

In this first study of endocan expression in the normal pituitary and adenomas, we demonstrated that endocan is never expressed in the vessels of normal pituitary gland and that endocan IR may appear in clusters of endocrine cells. The expression of the endocan gene was confirmed at mRNA levels on the whole pituitary tissue. Endocan expression, at mRNA and/or protein level, has already been shown in several normal tissues: lung, kidney, adipose tissue and colorectal mucosa (11, 13, 14, 42, 46), whereas many normal tissues are devoid of any detectable expression, particularly the brain (14, 20). We may suggest that endocan secreted and/or trapped by these endocrine pituitary cells may have a function, for instance, acting in a paracrine way on endothelial cells as VEGF does (4).

We then found an endocan IR in vessels of pituitary tumors. The occurrence of an endocan-endothelial staining in pituitary tissue is probably indicative of a neoplasia, as it has never been observed in any normal pituitary cases. Our results also suggest

Table 2. Association between endocan immunoreactivity (IR) in pituitary adenoma tissue and recurrence before the end of the long-term follow-up, the occurrence of p53 immunoreactive cells and invasion of the surrounding structure on MRI.

	Recurrence	No recurrence	p53 IR	No p53 IR	Invasion	No invasion
Endothelial IR	31/52	21/52	27/52	25/52	31/52	21/52
No endothelial IR	15/55	40/55	17/55	38/55	23/55	32/55
P (Fisher exact test)	**0.0009**		**0.032**		0.08	
Tumor cell IR	9/23	14/23	6/23	17/23	8/23	15/23
No tumor cell IR	37/84	47/84	38/84	46/84	46/84	38/84
P (Fisher exact test)	0.8		0.15		0.1	

A *P*-value under 0.05 is considered as significant (bold).

Table 3. Association between endocan immunoreactivity (IR) in pituitary adenoma tissue and quantified data: the largest axis of the tumor before first surgery, the mitotic count per 10 high power fields and the percentage of Ki-67 immunoreactive tumor cells.

	Size (mm ±SD)	Mitosis	Ki-67 index (% ±SD)
Endothelial IR	24.74 (±12.36)	3.3 (±2.99)	0.82 (±1.59)
No endothelial IR	16.7 (±9.12)	2.04 (±2.68)	0.45 (±0.68)
P (Student's test)	**0.0012**	**0.02**	0.11
Tumor cell IR	17.47 (±10.16)	2.87 (±3.36)	0.99 (±2.11)
No tumor cell IR	21.88 (±11.86)	2.61 (±2.77)	0.54 (±0.81)
P (Student's test)	0.08	0.89	0.5

A *P*-value under 0.05 is considered as significant (bold).

Table 4. Association between endocan endothelial immunoreactivity (IR) in pituitary adenoma tissue and mean vessel area (MVA) and microvessel density (MVD).

	MVA (μm^2 ±SD)	MVD (μm^2 ±SD)
Endothelial IR	147.1 (±91.2)	290.9 (±258.3)
No endothelial IR	100.1 (±77.0)	258.5 (±231.6)
P (Student's test)	**0.028**	0.59

A *P*-value under 0.05 is considered as significant (bold).

that immunohistochemistry is a better method for endocan detection than RT-PCR, because it can identify endothelial expression whereas the latter cannot discriminate between the normal endocrine and abnormal endothelial expression patterns.

In pituitary adenomas, the endothelial IR was not distributed widely, but was clearly restricted to some vessels. The restriction of endothelial labeling uniquely to the vessels located within a tumor has already been observed in several cancers (11, 12, 15, 20, 28) and somehow attributed to focal hypoxia. For example, the immunoreactive vessels in glioma tissue mostly appear in foci of microvascular proliferation, probably linked to hypoxia-induced gene expression such as VEGF (20). As in gliomas, increased expression of HIF-1α has already been shown in pituitary adenomas (45) but might only be indicative of hypoxia. Only experimental data obtained from a relevant animal model would strengthen such a hypothesis but they are not available at the time. Most pituitary adenomas have a poor blood supply, their endothelium appears less fenestrated under electron microscope examination, and the basal lamina seems thicker and fragmented, whereas the normal pituitary gland is supplied by a rich network of fenestrated capillaries (8, 39). The modification of the blood supply may reflect a shift from the fenestrated endothelium (hypohysal-hypothalamic portal meshwork) to a continuous endothelium, lining the arteria-derived emerging capillary network (38).

The labeling of a subset of capillaries in pituitary adenoma tissue might be associated with vascular remodeling. This is suggested by our data, showing an MVA significantly higher in endocan-positive tumors than in endocan-negative tumors. Recently, endocan has been described as a biomarker of the "tip cells" by three independent groups (6, 27, 34). The tip cells are the motile endothelial cells, which mediate the sprouting of developing vessels during the process of neoangiogenesis. They are known to be responsive to VEGF. In their models, Strasser *et al* showed that endocan and CXCR4, the stromal cell-derived factor-1 (SDF-1)/CXCL12 receptor, were both enriched in these specialized tip cells, which are a sign of neoangiogenesis and probably of vascular remodeling in disease (34). Therefore, we may speculate that endothelial cells immunoreactive for endocan in pituitary adenoma might be tip cells and could correspond to a sprouting process of a remodeling capillary, leading to the enlargement of the vessel area.

Furthermore, in our study we clearly show that endocan IR of endothelial cells is strongly associated in pituitary adenomas with recurrence in our series of 107 patients with long follow-up ($P = 0.0009$). Moreover, we demonstrate an association between endocan IR and criteria already stated as indicative of aggressiveness: mitotic count and p53 IR (18). Interestingly, endocan IR is also associated with a larger preoperative tumor size ($P = 0.0012$). The maximal diameter of a pituitary adenoma before surgery is a well-established criterion of recurrence, as a larger adenoma may be less accessible for the surgeon (10, 19). A bigger size may also be linked to angiogenic factors in the adenoma, but it is still a matter of debate whether angiogenesis is associated or not with a higher rate of recurrence and/or aggressiveness. For instance, the angiogenic factor SDF-1/CXCL12 is overexpressed in pituitary macroadenomas when compared with microadenomas (23). The expression of VEGF has been also proposed as a possible negative prognostic factor (17), but no correlation has been found between tumor size, VEGF and VEGF receptors (24, 41). FGF-2, another angiogenic factor, is implicated as well in the growing phase of prolactin adenomas (21) and its IR might be predictive of recurrence in pituitary adenoma (9).

Since its discovery, endocan has been shown *in vitro* and *in vivo* to be highly up-regulated by proangiogenic molecules such as VEGF and FGF-2 that are both mediators involved in angiogenesis and cancer progression (11, 12, 15, 20, 29, 31, 32). Moreover, the dermatan chain of endocan was shown to bind those proangiogenic growth factors and promote their biological activities (2, 5, 30, 33). Furthermore, it has been recently shown that the VEGF-induced endocan secretion by cultured endothelial cells is abolished in the presence of the antiangiogenic drug sunitinib *in vitro*, bringing a rational of further investigations of endocan as a potential biomarker for therapeutic follow-up in antiangiogenic treatment in VEGF-driven cancers.

We show as well that endocan IR is associated with p53 IR and with a higher mitotic count. P53 nuclear staining, Ki-67 index over 3%, elevated mitotic count or related markers such as PCNA are hallmarks of aggressiveness in routine pathology, and they are markers of an aggressive behavior in pituitary adenoma tissue (35–37). To our knowledge, the association between angiogenic factors, p53 and mitotic count has never been documented in pituitary adenoma before. We show here an association between endocan and p53 IR and a higher mitotic count. In contrast, although Ki-67 index over 3% is considered as a prognostic factor in pituitary tumors (37), we have found no association between Ki-67 index and endocan IR. Previous studies concluded that Ki-67 may be not associated with angiogenesis in pituitary tumors, as no association could be demonstrated between VEGF and Ki-67 (4, 22, 24), nor between vessel density and Ki-67 (22, 38, 40).

Finally, we described here an expression of endocan in tumor endocrine cells. In animal models, endocan was recently identified to be one of the genes clearly involved in the switch from dormant to angiogenic tumors (1). However, we failed to show any association between endocan IR in tumor cells and recurrence, invasiveness, tumor size, Ki-67, p53 and mitotic count in pituitary adenomas. In our study, the endocrine cell IR is somehow observed in normal pituitary gland tissue as well as in tumor cells, possibly explaining this lack of association.

To conclude, our results demonstrate for the first time the potential value of endocan as a new and pertinent tissue-based biomarker of aggressive behavior in pituitary adenomas. It would probably be included in a multiparametric scale, in association with p53, Ki-67 and molecular markers. In the future, it might be interesting to investigate the potential of endocan secreted in blood as a biomarker of aggressiveness or recurrence for pituitary tumors.

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