

## CASE REPORT

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## Progesterone receptor expression in orbital cavernous hemangiomas

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**Abstract** Orbital cavernous hemangiomas (OCH) have thick and highly cellular vascular walls. Ultrastructural studies have demonstrated the smooth muscle nature of these cells. Vascular neoplasms can modify their morphological and clinical features under hormonal stimulation. The purpose of the present study was to investigate the presence of smooth muscle markers and sex steroid receptors in 12 cases of OCH. Orbital cases were compared with cutaneous hemangiomas and subcutaneous angioleiomyomas. Smooth muscle actin (SMA) and desmin were localized in spindle cells of the vascular walls of all 12 cases studied. OCH showed immunohistochemical positivity with progesterone receptor (PR) antibody both in smooth muscular and in endothelial cells. For comparison, sex steroid receptors were studied in 10 cases of cutaneous cavernous hemangioma and in 10 cases of subcutaneous angioleiomyoma. PR was found in smooth muscle and endothelial cells of 6 out of 10 cases of subcutaneous angioleiomyoma and in none of the cases of cutaneous cavernous hemangioma. No positivity was obtained with estrogen receptor (ER) antibody in any of the cases tested. The present data suggest that OCH share morphological and immunohistochemical features with subcutaneous angioleiomyomas. Furthermore, immunohistochemical positivity with PR antibody indicates that OCH have to be added to the list of mesenchymal lesions that express sex steroid receptors.

**Key words** Cavernous hemangiomas · Angioleiomyomas · Orbit · Sex steroid receptors · Progesterone

### Introduction

Orbital cavernous hemangiomas (OCH) are lesions of middle age (mean age at onset of symptoms is 40 years), mainly affecting women [1, 5, 20, 24, 31, 40]. They constitute 7–16% of orbital lesions according to different authors and have a typical clinical presentation characterized by slowly developing proptosis, diplopia, reduction of visual acuity and restriction of the visual field [5, 20, 31]. Histologically, cavernous hemangiomas of the orbit differ from cutaneous and intracranial cavernous hemangiomas in having thicker cellular walls [12, 20, 31, 49]. Ultrastructural studies have shown that the vascular channels are surrounded by one to five layers of smooth muscle cells [31]. Thus, the morphology of OCH is reminiscent of subcutaneous angioleiomyomas. These latter lesions are characterized by smooth muscle bundles that surround blood vessels [9, 16, 18, 22, 29, 32, 42]. Subcutaneous angioleiomyomas can belong to any of three histological types [32], capillary, cavernous and venous, according to their vascular features (closely compact; dilated with thin muscular walls; dilated with thick muscular walls).

In recent years steroid receptors have been identified in several types of human neoplasms. In some cases, such as in breast cancer, these provide useful information on the likelihood of response to hormonal therapy. In contrast, in malignant melanoma [46], renal cell carcinoma [7], pancreatic tumors [15, 26, 45, 50], meningiomas [30, 44], pulmonary [2, 3] and hepatic neoplasms [21] the significance of these findings is not certain [41, 46, 48]. Preliminary reports also indicate the presence of steroid receptors in mesenchymal tissues and in their neoplastic counterparts [14, 48]. Although the significance of steroid receptors in mesenchymal tumors is unclear, it has been suggested that the growth of some of these tu-

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**Table 1** Antibodies used (*SMA* smooth muscle actin, *M* monoclonal, *P* polyclonal)

Antibody	M/P	Source	Clone	Type	Dilution
SMA	M	DAKO (Glostrup, Denmark)	1A4		1.100
Desmin	P	BIOMEDA (Foster City, Calif.)		A69 rabbit	1.50
CD 31	M	DAKO	JC/70A		1.20
Estrogen receptor	M	DAKO	1D5		1.100
Progesterone receptor	M	YLEM (Rome, Italy)	1A6		1.40
S100 protein	P	DAKO		Z311 rabbit anti-cow	1.1500
HMB45	M	DAKO	HMB45		1.50

mors may be hormone dependent [14, 48]. Cases of hemangioma [8, 12, 27, 33, 34, 37, 39] and angioleiomyoma [22] in which pain presented or increased in intensity during pregnancy or in the course of the menstrual cycle have been reported in several sites, one of which is the orbit [24, 25, 51].

The purpose of the present study was to show that the elongated cells present in the vascular walls of orbital cavernous hemangiomas are smooth muscular elements that express PR. In addition, cases were studied with HMB45 and S100 protein to exclude a possible relationship with angiomylipomas and ancient schwannoma.

## Materials and methods

Twelve cases of orbital hemangioma were selected from 126 cases of endo-orbital lesions seen between January 1980 and September 1996 in the Department of Pathology of the University of Bologna at Bellaria Hospital [5]. In addition, the presence of PR and ER was studied in 10 cases of cutaneous cavernous hemangioma and 10 cases of subcutaneous angioleiomyoma. Orbital cavernous hemangiomas were diagnosed according to the classification of orbital tumors [31]. Diagnoses of subcutaneous angioleiomyomas and cutaneous cavernous hemangiomas were considered when all morphological criteria were met [18, 42].

Specimens were formalin fixed and routinely processed to paraffin. From each case serial sections were cut, stained with hematoxylin-eosin (H-E) and the Weigert-van Gieson method for elastic fibers. Appropriate tissue blocks were selected and, after dewaxing in xylene and rehydrating through graded alcohols, sections were immersed in citrate buffer (0.01 M, pH 6), treated in a microwave oven (four cycles for 5 min each at 750 W) and cooled at room temperature. Then sequential tissue sections were incubated with antibodies listed in Table 1. The immunostaining was carried out using the indirect immunoperoxidase avidin-biotin complex method (Vector laboratories, Burlingame, Calif.). Anti-ER and anti-PR antibodies were tested twice: with 1 h incubation at room temperature and with overnight incubation at 4°C. Positive and negative controls were added to each batch of slides.

## Results

Clinical features of OCH, cutaneous cavernous hemangiomas and subcutaneous angioleiomyomas are reported in Table 2.

On histology OCH were characterized by large vessels filled with blood, lined with flattened endothelium.

**Table 2** Clinical data (*F* female, *M* male, OCH orbital cavernous hemangioma, *SA* subcutaneous angioleiomyoma, *CCH* cutaneous cavernous hemangioma)

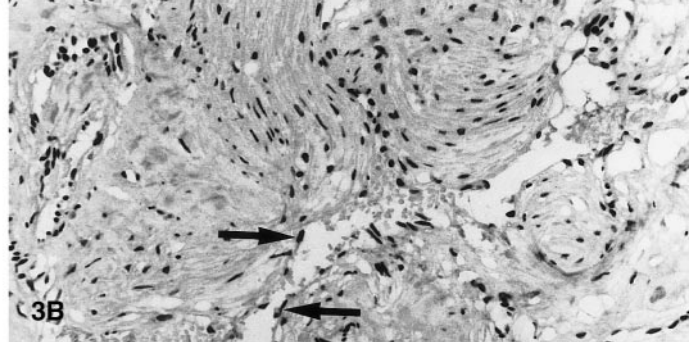
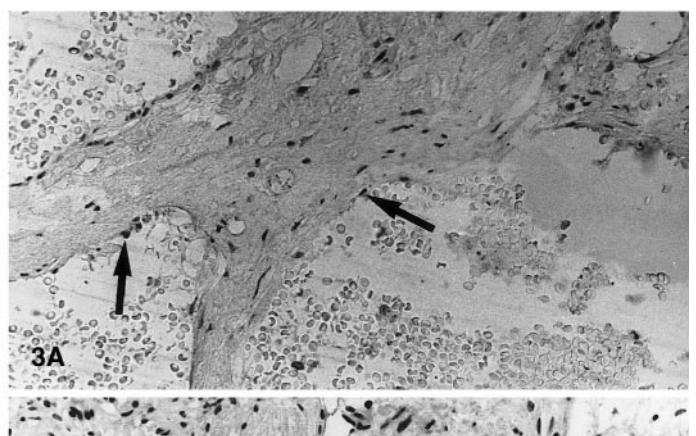
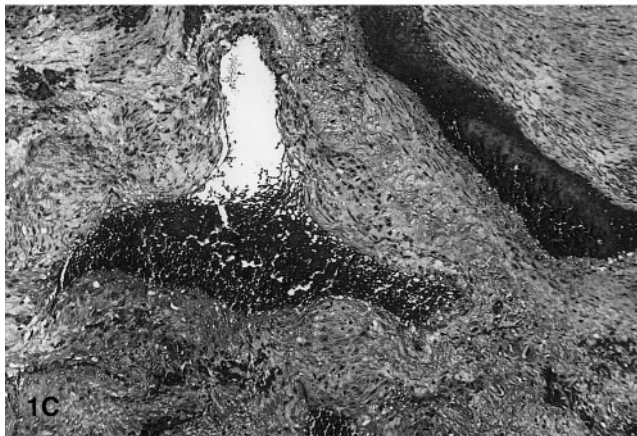
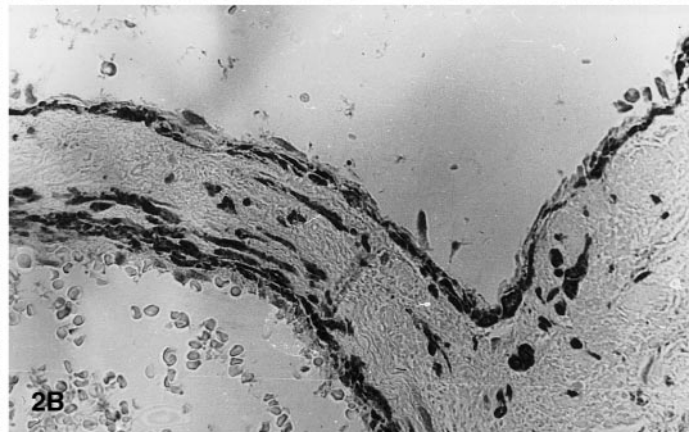
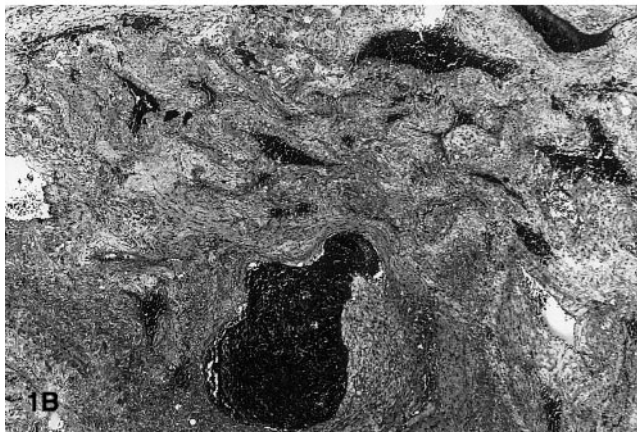
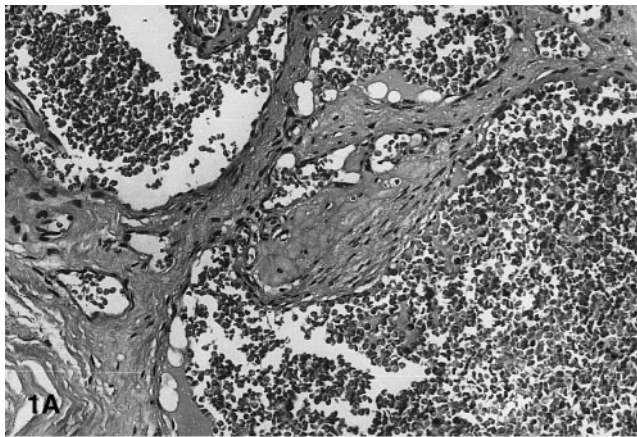
	OCH	SA	CCH
F:M	9:3	3:7	5:5
Age (years)	35–73 (mean 52)	33–78 (mean 59)	23–80 (mean 59)

The vascular walls were of variable thickness, containing spindle cells with elongated nuclei and eosinophilic cytoplasm (Fig. 1a–c). Spindle cells were located around the vascular lumina and arranged in concentric layers. A layer of elastic fibers, located between the endothelial layer and smooth muscle layer, was evidenced by the Weigert-van Gieson method. In four cases the vessel walls were highly cellular (Fig. 1b, c). CD31 antibody strongly stained almost all the endothelial cells. Spindle cells composing vascular walls showed a strong cytoplasmic positivity for desmin and SMA (Fig. 2a, b). Desmin stained between 5% and 50% of the spindle cells and SMA, more than 90% of the same cells. PR antibody demonstrated nuclear positivity in the spindle cells inside the vascular walls and in the endothelial cells. The number of positive cells and the staining intensity increased after overnight incubation. After overnight incubation PR positivity was observed in all 12 cases of OCH. In 8 cases the positivity was intense and diffuse (Fig. 3a, b), comparable to that observed in PR-positive breast carcinomas. In the remaining 4 cases it was limited to 10% of the spindle cells.

ER antibody was negative in all cases.

Six out of 10 cases of subcutaneous angioleiomyoma presented focal positivity with PR antibody in both endothelial and smooth muscle cells. All these cases were negative for ER. All cases of cutaneous cavernous hemangiomas were negative with both ER and PR antibodies. HMB45 and S100 antibodies were negative in all the cases under study.





**Fig. 1A–C** Orbital cavernous hemangiomas (OCH) show vascular walls of variable thickness. **A** In some cases few layers of spindle cells were present; **B**, **C** other cases presented thicker and higher cellular walls. H-E, **A** 40 $\times$ , **B** 25 $\times$ , **C** 40 $\times$

**Fig. 2A, B** OCH: SMA strongly stained the spindle cells inside vascular walls. ABC, **A** 40 $\times$ , **B** 25 $\times$

**Fig. 3A, B** PR antibody showed nuclear positivity both in muscular and endothelial cells in OCH with **A** thin and **B** thick vascular walls. Arrows indicate endothelial cells. ABC, **A** 125 $\times$ , **B** 125 $\times$



## Discussion

Cavernous hemangiomas of the orbit differ from cutaneous and intracranial hemangiomas in both clinical and morphological features [12, 20, 31]. OCH occur in middle-aged adults with typical signs and symptoms of space-occupying lesions [20, 31], while classic cutaneous hemangiomas are lesions that occur at any age [18]. Histologically the presence of two to five layers of spindle cells in the vascular walls of OCH is a distinctive feature. It has been demonstrated that these cells have ultrastructural features of smooth muscle cells [31], suggesting that OCH are similar to subcutaneous angioleiomyomas.

In order to clarify this aspect we studied 12 cases of OCH immunohistochemically. Histologically, all of them were circumscribed lesions composed of dilated vessels filled with blood and lined with flattened endothelium. Vascular walls were of variable thickness and cellularity. The spindle cells located in the walls showed strong immunohistochemical positivity to SMA and desmin antibodies, confirming their smooth muscle cell phenotype. Therefore, it appears more consistent to regard these lesions as similar to angioleiomyomas.

Immunohistochemistry for PR revealed nuclear positivity in both muscular and endothelial cells of all the orbital cases. The immunostaining was diffuse in 8 cases and focal in 4. In addition, focal positivity for PR was detected in 6 out of 10 cases of subcutaneous angioleiomyoma. The same cases showed no positivity for ER. No immunostaining for sex steroid receptors was demonstrated in cutaneous cavernous hemangiomas. The presence of sex steroid receptors in normal vessels and vascular neoplasms has been investigated by several authors, with differing results [6, 7, 28, 35, 38, 47]. Immunohistochemical detection of sex steroid receptors in hemangiomas was obtained by Lui et al. [28], while PR were demonstrated in cerebral hemangioblastoma [7]. Most probably the discrepancies are due to different immunohistochemical techniques used in the various studies. In our cases the number of positive cells increased after overnight incubation. This feature suggests that sex steroid receptors are probably expressed in a small number of copies in vascular neoplasms and require more sensitive methods to be revealed. This might explain the negative results sometimes obtained in vascular tumors [6, 35].

PR were also present in smooth muscle cells. They have been found in uterine leiomyosarcomas, in smooth muscle cells of lymphangioleiomyomatosis and in smooth muscle cells of renal angiomyolipomas related to tuberous sclerosis [11, 13, 23, 38]. In addition, Fiju et al. [19] have produced experimental evidence that progesterone induces differentiation toward smooth muscle cells. Similarly, it may be that in orbital and peripheral angioleiomyomas PR play a part in the development of the lesions.

PR activity is usually dependent upon estrogen [10, 11]. In the present cases no ER were detected. This might

be a result of the methods used, which may not be sensitive enough to demonstrate ER. Nevertheless, the presence of PR in the absence of ER has been described in several tumors [3, 41, 45]. This fact could suggest that PR are expressed but they have no functional role. Alternatively, in specific cases PR could be activated by an alternative pathway, which is still unknown. PR positivity could explain such symptoms as pain arising during pregnancy and during the menstrual cycle. The reason why not all angioleiomyomas are painful during pregnancy or the menstrual cycle might be related to the size of the lesion and to the total number of cells expressing PR.

The immunohistochemical pattern and the histological features suggest a link between angioleiomyomas and pulmonary lymphangioleiomyomatosis, a lesion related to tuberous sclerosis [4, 13, 17, 36, 38, 43]. Pulmonary lymphangioleiomyomatosis is frequently positive with HMB45 [13, 17, 36], while our cases were HMB45 negative and none of them was associated with tuberous sclerosis. Thus, even if they share similar morphological features, these lesions are probably different pathologic processes.

Our data indicate that OCH, the entity studied here, show morphological features that can be superimposed on those of subcutaneous angioleiomyomas. Histological and immunohistochemical features, i.e., the presence of elongated spindle cells immunostained by SMA and desmin within vascular walls, strongly suggest the former explanation. Moreover, these cases, when tested for steroid receptors, revealed marked positivity for PR coupled with ER negativity. These data allow the addition of angioleiomyomas of the orbit to the group of mesenchymal lesions that express PR.

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