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Histogenesis of Stromal Cells in Cerebellar Hemangioblastomas

An Immunohistochemical Study

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Fifteen cerebellar hemangioblastomas were examined by immunohistochemistry for expression of neuron-specific enolase (NSE) and various neuropeptides using the avidin-biotin-complex peroxidase reaction with the following antibodies: NSE, synaptophysin, serotonin, substance P, vasoactive intestinal peptide (VIP), neuropeptide YY, neurotensin, and leu-enkephalin. In all tumor biopsies most of the stromal cells were positive for NSE. About 30% of the stromal cells showed a weak cytoplasmic synaptophysin positivity. Approximately 25% of the stromal cells were labeled with antibodies against substance P and neuropeptide YY. The partly strong reactivity was localized preferentially in perinuclear regions. These positive cells were mainly distributed in small cell clusters but were also scattered in the tumor parenchyma. In all tumor biopsies scattered cells exhibited strong perinuclear enkephalin positivity, corresponding probably to mast cells, whereas stromal cells were entirely negative. For serotonin, VIP, and neurotensin no specific reaction was seen. On the basis of these findings it is proposed that hemangioblastomas have a neuroendocrine component. (Am J Pathol 1989, 134:271–275)

Hemangioblastomas are rare benign tumors localized predominantly in the cerebellar hemisphere, but they may also occur at other sites in the central nervous system.[1] The association of cerebellar, spinal cord, or retinal hemangioblastomas with cystic deformations or tumors in various abdominal organs (eg, phaeochromocytomas, pancreatic islet cell tumors, renal cell carcinomas, or testicular cystadenomas) is characterized as von Hippel-Lindau syndrome.[2–4] The hemangioblastoma is a distinct nosologic entity with clearly recognized clinical features, biologic behavior, and histologic characteristics. Although many investigations including ultrastructural and immunohistologic studies have been published on the histogenetic origin of this tumor, its origin has not been satisfactorily clarified. The presence of three cell types characteristic of hemangioblastomas, ie, endothelial cells, pericytes, and stromal cells, has been established by electron microscopy.[5–10] The stromal cells have no counterpart among normal cells. It is still unknown whether all three cell types are neoplastic or whether only one type is responsible for the neoplastic proliferation. In any case, elucidation of the origin of stromal cells should help clarify the histogenesis of hemangioblastomas. Most authors suggest that stromal cells originate from vessel walls,[10–16] (ie, endothelium, pericytes, and angiogenic mesenchymal cells) or from histiocytes,[17] or their precursor cells.[6,8,18] Derivation from astrocytes,[19–21] microglia,[22] or meningeal cells[23] also has been proposed.

In view of the endocrine associations of von Hippel-Lindau syndrome, the histologic similarities between some hemangioblastomas and neuroendocrine tumors, as well as the description of neuron-specific enolase (NSE) positivity of stromal cells in single cases, we examined cerebellar hemangioblastomas for expression of NSE and a panel of neuropeptides.

Materials and Methods

Formalin-fixed, paraffin-embedded sections from 15 surgical biopsies of cerebellar hemangioblastomas were studied using immunohistochemical methods. Five micron, nonpredigested sections were incubated with affinity-purified antibodies (Table 1) using the avidin-biotin peroxidase complex reaction (ABC), as modified by Hsu et al.[24] Tissue sections were developed in 3,3′-diaminobenzidine:H_2O_2 (Sigma Chemical Company, St. Louis, MO) and counterstained with hematoxylin. The specificity of the reaction was demonstrated in positive control tissue, known to express high levels of the investigated neu-

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Table 1. *Primary Antibodies, Source, Dilution and Positive Control Tissue*

Antigen	Species	Dilution	Positive control
NSE*	Rabbit	1:2000	Medulla oblongata
Synaptophysin†	Mouse	1:50	Neuroblastoma
Serotonin‡	Rabbit	1:50	Rectal mucosa
Substance P§	Rabbit	1:250	Substantia nigra
VIP§	Rabbit	1:4000	Median eminence
Neuropeptide YY¶	Rabbit	1:4000	Rectal mucosa
Neurotensin§	Rabbit	1:1000	Ant. hypothalamus
Leu-Enkephalin§	Rabbit	1:500	Sub. gelatinosa

* Dako Corporation, Santa Barbara, CA.
† Boehringer Mannheim, FRG.
‡ Bio-Science Products, Emmenbrücke, Switzerland.
§ Amersham International, Buckinghamshire, UK.
¶ Cambridge Research Biochemicals, Cambridge, UK.

ropeptides (Table 1).[25] Negative controls were provided by omitting the primary antibody and replacing it with non-immune serum.

Results

All anatomic structures expressing the corresponding antigen used as positive controls were strongly labeled with the antibodies (Table 1).

Hematoxylin and eosin (H&E)-stained sections of all 15 tumor specimens showed the characteristic histology of hemangioblastomas (Figure 1A). The tumors were composed of thin-walled endothelium-lined vascular channels of varying sizes. Polygonal stromal cells showed some nuclear pleomorphism and abundant, lightly stained, partly foamy cytoplasm. Some stromal cells were small and difficult to differentiate from endothelial or pericytic cells.

In all biopsies, most of the stromal cells showed a variably strong cytoplasmic immunoreactivity to NSE. About one third of the stromal cells (range, 15–40%) expressed synaptophysin diffusely in the cytoplasm (Figure 2). These cells with positive reaction were predominantly localized in small cell clusters; some were scattered throughout the tumor parenchyma. About 10–30% of the stromal cells showed cytoplasmic immunoreactivity for substance P (Figure 3) and neuropeptide YY. In the case of both peptides, the partly strong reactivity was localized preferentially in perinuclear regions. These positive-staining cells were distributed primarily in small cell clusters. In all tumor biopsies, scattered cells (no more than 5%) exhibited strong perinuclear enkephalin positivity, whereas stromal cells were negative (Figure 1B). On serial sections, these cells were identified with alcian blue as mast cells. No immunoreactivity was demonstrated with antibodies against serotonin, VIP, and neurotensin in any part of the tumors.

Discussion

In spite of many immunohistochemical and electron microscopic studies, the histogenesis of cerebellar heman-

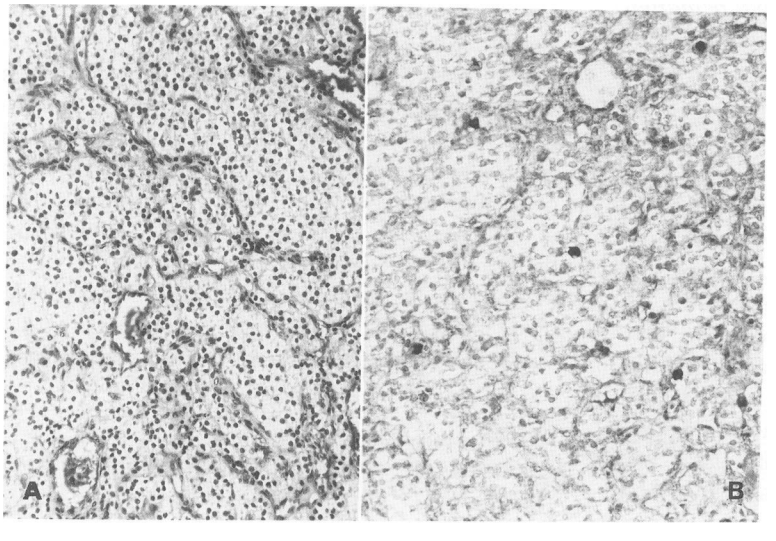

Figure 1A. *The classical appearance of cerebellar hemangioblastomas, composed of thin-walled blood vessels and polygonal stromal cells with lightly stained, partly foamy cytoplasm. H & E, ×100.* **B:** *Scattered cells exhibit strong perinuclear enkephalin positivity, stromal cells are negative. ABC-technique, Leu-enkephalin-antibody, paraffin section, ×150.*

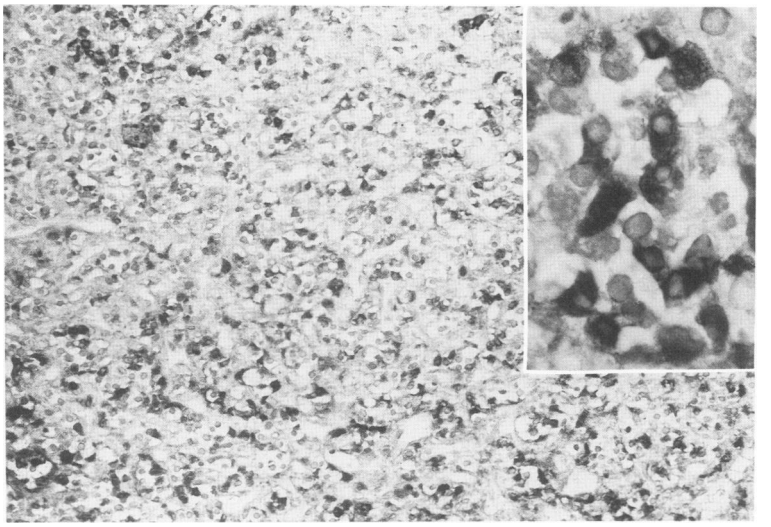

Figure 2. *Some stromal cells express synaptophysin. These positive cells are predominantly localized in small cell clusters. ABC-technique, synaptophysin-antibody, paraffin section, ×150; Inset ×500*

gioblastomas remains controversial. Several theories have been proposed including derivation from astrocytes, angiogenic cells, histiocytes, primitive mesenchymal or meningeal cells. The availability of antibodies against various neuropeptides enabled us to substantiate the hypothesis of a neuroendocrine origin.

We demonstrated cytoplasmic NSE immunoreactivity in the stromal cells of all 15 examined hemangioblastomas. Our results are corroborated by four reports[26–29] describing positive-staining stromal cells for NSE in collectives of one, two, three, and ten tumors. To the best of our knowledge, hemangioblastomas nonreacting for NSE have never been described. NSE, an isomer of the glycolytic enzyme enolase, was initially thought to be present exclusively in neurons. It was later detected in neuroendocrine cells, reactive astrocytes, and several non-neural CNS tumors.[30] Some authors demonstrated NSE in other cells such as platelets[31] or smooth muscle cells.[32]

In addition, we showed variable cytoplasmic positivity to neuropeptides, (ie, substance P, neuropeptide YY [PYY]) in stromal cells. Ismail and coworkers[28] described a granular cytoplasmic staining of stromal cells with antisera to somatostatin and bombesin, sometimes with antisera to pancreatic polypeptides. Pancreatic polypeptide has a chemical structure similar to that of peptide YY (69% sequence homology) and neuropeptide Y. It, therefore, is possible that staining with pancreatic polypeptide is due to a cross-reactivity to PYY or neuropeptide Y of the antibody directed at these peptides.[33,34] Consequently the cells labeled with pancreatic peptides that Ismail and coworkers found, might correspond to our positive-staining cells for PYY. In their recent study, Grant and coworkers[29] examined ten cerebellar hemangioblastomas with a selection of antibodies against peptide hormones and synaptophysin. They found no positive staining for bombesin, pancreatic polypeptide, somatostatin, thyroglobulin, calcitonin, glucagon, insulin and gastrin. Synaptophysin was also totally negative in their tumors; they therefore argued against a neuroendocrine origin of stromal cells. The different reactivity, especially for synap-

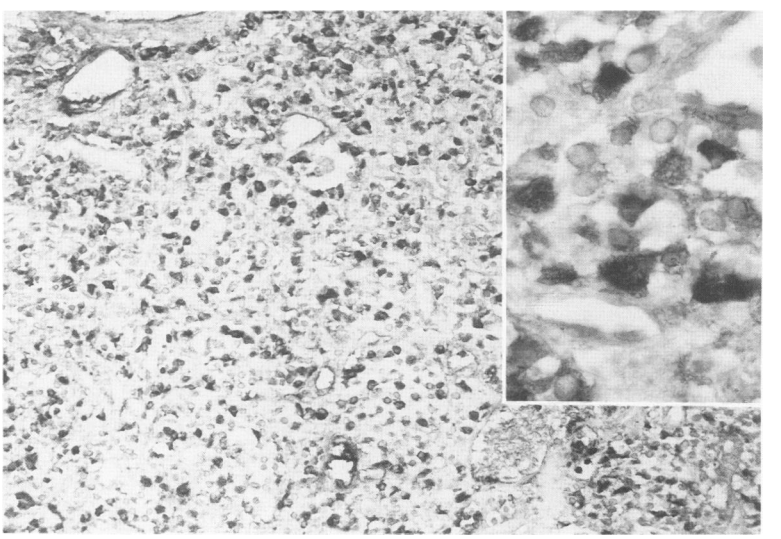

Figure 3. *Some stromal cells show cytoplasmic immunoreactivity for substance P. Note the intermingeling of positive and negative stromal cells. ABC-technique, Substance P-antibody, paraffin section, ×150; Inset ×500*

tophysin, could be method related: Grant and coworkers predigested the paraffin sections for immunostaining using 0.1% trypsin in 0.1% calcium chloride solution at 37 C. They used the same antibody for synaptophysin (clone SY 38) that we did. In our preparation, however, we omitted predigestion, as recommended by the manufacturer, to avoid antigen denaturation. In ultrastructural studies of hemangioblastomas, Ishwar and coworkers,[35] Andrioli and Scarini,[36] and Ismail and coworkers[28] demonstrated the presence of membrane-bound electron-dense granules in stromal cells. Ismail speculated that these secretory granules could contain neuropeptides; Ishwar and Andrioli, however, suggested that they contain erythropoietin. Böhling et al[37] examined ten hemangioblastomas and found small granular cells scattered among stromal cells that showed a positive-staining reaction with anti-erythropoietin and anti-renin. They concluded that hemangioblastomas harbor a fourth cell type with erythropoietin-like immunoreactivity that may be responsible for the secondary polycythemia associated with these tumors.

Our immunohistochemical studies showed enkephalin positive-stained mast cells scattered in hemangioblastomas. The presence of mast cells in hemangioblastomas is a common finding and has been reported by several authors.[13,38,39] Immunoreactivity for endorphins and enkephalins in lymphoid cells was described by Smith et al,[40] and Martin and coworkers[41] found high levels of preproenkephalin mRNA in mast cells.

Our immunohistochemical findings, the histologic appearance of hemangioblastomas containing cells with lightly stained cytoplasm and cell nests surrounded by capillaries reminiscent of a neuroendocrine tumor, and the description of the association of hemangioblastomas and neuroendocrine tumors[4] point toward a neuroendocrine component in hemangioblastomas.

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