Immunohistochemical Evidence for the Presence of S-100 Protein in Stromal Cells of Cerebellar Hemangioblastomas

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Abstract

The histogenesis of the stromal cells in cerebellar hemangioblastomas is still a subject of controversy.

In this study, 13 cerebellar hemangioblastomas were examined immunohistochemically for S-100 protein, glial fibrillary acidic protein (GFAP), and human factor VIII-related antigen in an attempt to elucidate the histogenesis of the stromal cells in these tumors. An unequivocally positive immunoperoxidase reaction for S-100 protein was observed in most of the stromal cells in 10 of the 13 cerebellar hemangioblastomas examined, whereas GFAP and human factor VIII-related antigen assays were essentially negative. The positive GFAP reaction occurred sporadically, in what we thought to be astrocytes trapped within the tumor. Human factor VIII-related antigen was positive exclusively in the capillary endothelial cells of the tumor. Thus, our results suggest that the stromal cells are derived from either vestigial cells of the neural crest or histiocytic cells of the reticuloendothelial system, which potentially produce S-100 protein.

Our results, considered in light of certain previous observations, imply that the stromal cells are the principal neoplastic cells of hemangioblastoma and are associated with the proliferation of vascular channels, possibly as a secondary effect of this tumor.

Key words: hemangioblastoma, stromal cells, S-100 protein, immunohistochemistry, histogenesis

Introduction

Hemangioblastoma is a benign vascular tumor arising in the cerebellum. It is composed of endothelial cells, pericytes, and stromal cells. The precise nature and origin of the stromal cells of hemangioblastoma has been a matter of controversy. Angiogenic mesenchyme,^{2,4,5,13,23)} histiocytic cells of the reticuloendothelial system,^{3,17)} and astrocytes¹²⁾ have each been proposed as possible sources of the stromal cells.

It is useful, in the current diagnosis of brain tumors, to immunohistochemically determine the presence or absence of glial fibrillary acidic protein (GFAP) and S-100 protein. GFAP is a constituent of astrocytic glial filament.⁹⁾ S-100 protein is a highly acidic protein existing mainly in glia of the central nervous system; however, the biological role of this

protein is still unknown, despite the fact that its physicochemical properties have been well defined. To determine the histogenesis of the stromal cells of cerebellar hemangioblastomas, we felt it would be helpful to ascertain whether or not S-100 protein and GFAP exist in these cells. We will describe and discuss the immunohistochemical demonstration of S-100 protein in most of the stromal cells in 10 of the 13 cerebellar hemangioblastomas we examined.

Materials and Methods

Thirteen cerebellar hemangioblastomas that had been operated on in our clinic in the past 5 years were examined. The tumor tissues obtained at surgery were fixed in 10% neutral formalin and embedded in paraffin. Serial 8 μ m sections were deparaffinized and immunohistochemically stained by a minimally modified peroxidase-antiperoxidase (PAP) method of Sternberger²⁵⁾ using monospecific rabbit antisera

Fig. 1 Cerebellar hemangioblastoma, cellular type (Case No. 8). HE stain, $\times 250$.

for S-100 protein, GFAP, and human factor VIII-related antigen. Other sections were processed for staining by hematoxylin and eosin, and by silver impregnation for reticulin. Antiserum to bovine brain S-100 protein was prepared in New Zealand albino rabbits, as described elsewhere.²⁶⁾ GFAP antiserum was a gift from Dr. D.G.T. Thomas (The National Hospital, Queen Square, London). Antiserum to human factor VIII-related antigen was commercially purchased (DAKO, Denmark).

The protocol for immunoperoxidase staining is as follows. 1) Normal goat serum (1 : 5), 10 minutes at room temperature. 2) S-100 protein, GFAP, or factor VIII antiserum (1 : 1,000), overnight at 4°C. 3) Anti-rabbit IgG goat serum (1 : 30) (DAKO, Denmark), 30 minutes at room temperature. 4) PAP reagent (1 : 100) (DAKO, Denmark), 30 minutes at room temperature.

Preimmune rabbit serum and the anti-S-100 antiserum premixed with purified bovine S-100 protein were also used as control sera. The cross-reactivity of bovine and human S-100 proteins has been confirm-

ed, as described previously.^{27,28)}

Results

Table 1 summarizes the clinical and immunohistochemical data from the 13 cerebellar hemangioblastomas examined. In 10 cases, a positive immunoperoxidase reaction for S-100 protein, a dark brown precipitate of diaminobenzidine, was demonstrated in most of the stromal cells (Figs. 1 and 2). The positive reaction was observed predominantly in the cytoplasm, and there was occasional staining of nuclei. No correlation was found between the presence of S-100 protein in stromal cells and the type (reticular or cellular) of tumor.

In three tumors, positive reactions for both S-100 protein and GFAP were observed only in certain cells, which were distributed sporadically and which we considered to be astrocytes trapped within the tumor (Fig. 3). The cells in the periphery of the tumor that showed an intensely positive reaction for S-100 protein were also positive for GFAP and were thought to be non-neoplastic astrocytes. Thus, the

Table 1 Summary of 13 hemangioblastomas processed for immunohistochemistry

Case No.	Age & Sex	Location of tumor	Cyst	Stromal cells		Others
				S-100	GFAP	
1	41F	cerebellar hemisphere	+	—	—	poly-cythemia
2	63M	cerebellar hemisphere	+	—	—	poly-cythemia
3	28M	cerebellar vermis	+	+	—	von Hippel-Lindau's disease
4	35F	cerebellar hemisphere	+	+	—	familial
5	31M	fourth ventricle	—	+	—	familial
6	21F	fourth ventricle	—	+	—	multiple
7	57F	cerebellar hemisphere	+	—	—	
8	51F	cerebellar vermis	+	+	—	
9	36M	fourth ventricle	+	+	—	poly-cythemia
10	40M	cerebellar hemisphere	+	+	—	
11	27M	fourth ventricle	—	+	—	
12	35M	cerebellar vermis	+	+	—	poly-cythemia
13	28F	cerebellar hemisphere	+	+	—

Fig. 2 Unequivocally positive immunoperoxidase staining for S-100 protein, a dark precipitate of diaminobenzidine, is seen in the stromal cells in varying intensities (without counterstain) (Case No. 8). $\times 250$.

Fig. 3 Positive reaction for GFAP is seen exclusively in the dispersed cells that seem to be trapped astrocytes (without counterstain) (Case No. 8). $\times 250$.

positive immunoperoxidase reaction for GFAP was restricted to reactive or trapped astrocytes that were also positive for S-100 protein.

Positive immunoperoxidase staining for human factor VIII-related antigen was observed exclusively in the capillary endothelial cells of all the cerebellar hemangioblastomas examined (Fig. 4), although, the intensity of staining was variable among the tumors.

Discussion

Intracranial hemangioblastoma is a benign vascular tumor. The cerebellum is the most common site; supratentorial hemangioblastoma is extremely rare.^{11,20} A similar tumor sometimes occurs in the retina, and other systemic abnormalities, such as cysts in the abdominal viscera and polycythemia, are occasionally observed.²¹ Histologically this tumor is composed of well-developed channels of capillary structures, with stromal cells in the intervascular

spaces. The histogenesis of the stromal cells has been debated since this tumor was first described.

Bailey and Ford¹⁾ considered the stromal cells to be endothelial cells that had become xanthomatous by phagocytizing plasma lipids. On the other hand, histiocytic cells of the reticuloendothelial system were postulated by some investigators^{3,17)} as the origin of the stromal cells. However, Russell and Rubinstein²²⁾ disagree with this hypothesis because of the absence of metallic impregnation in those cells. They proposed that the stromal cells of cerebellar hemangioblastomas are derived from mesenchymal cells of pia mater, based on the occasional continuity of this tumor with pia mater.

Inspection of the fine structure of the stromal cells suggests that the stromal cells are derived from angiogenic mesenchyme.^{2,4,5,13,23)} Cancilla and Zimmerman²⁾ reported that cerebellar hemangioblastomas consist of a single cell type characterized by numerous intracytoplasmic fibrils, dilated endoplas-

Fig. 4 Intense staining for human factor VIII-related antigen is restricted to the endothelial cells, which are clearly distinguishable from the stromal cells (without counterstain) (Case No. 8). $\times 250$.

mic reticulum containing dense granular material, and lipid bodies. Kawamura *et al.*¹³⁾ observed that this tumor is composed of endothelial cells, pericytes, and stromal cells, that much of the capillary component is of the embryonal type, and that the stromal cells share several morphological features with endothelial cells and pericytes. Therefore, they regard the endothelial cells as the principal cells of this tumor and the stromal cells as the vestige of either endothelial cells or pericytes. By cultivating hemangioblastoma, Spence and Rubinstein²³⁾ confirmed the structural similarities among the three types of cells but did not observe interconversion among these cells. They feel that all three cells are neoplastic in nature.

The presence of astrocytes in cerebellar hemangioblastoma has been noted previously. Bailey and Ford¹⁾ reported that some glial cells are trapped and then become the stromal cells of this tumor. In a study of retinal hemangioblastoma, Jacobiec *et al.*¹²⁾

found structural similarities between the stromal cells and lipid astrocytes trapped within the tumor. Therefore they concluded that the stromal cells are fibrillary astrocytes that have been transformed into lipid, xanthomatous cells by passive imbibition of plasma lipids.

GFAP is a nervous system-specific protein originally discovered by Eng *et al.*⁹⁾ This protein is commonly believed to be a constituent of the glial filament of astrocyte.⁸⁾ Kepes *et al.*¹⁴⁾ immunohistochemically examined the localization of GFAP in cerebellar hemangioblastoma and observed varying degrees of GFAP positive reaction in both the stromal cells and the trapped astrocytes. Therefore, they regard the stromal cells as a heterogeneous group that includes non-neoplastic astrocytes. Deck and Rubinstein⁷⁾ also reported the existence of GFAP in some stromal cells but, because of the close distribution of these cells and reactive astrocytes, they postulated that those stromal cells had taken up extracellular GFAP.

S-100 protein is a highly acidic, calcium-binding protein first discovered by Moore.¹⁸⁾ This protein has been shown to exist in mammalian brain, at higher concentrations in glial cells. However, its neurobiological function is still speculative. Immunohistochemical examination of S-100 protein revealed that this protein is present in astrocytes, oligodendrocytes, ependymal cells, and some neurons in the central nervous system. Recent publications have reported that S-100 protein is also present in melanocytes, human melanoma cell lines, Langerhans cells of skin, satellite cells of the adrenal gland, chondrocytes, interdigitating reticulum cells, and histiocytosis X cells.^{6,10,19,24)}

In this study, most of the stromal cells in 10 of 13 cerebellar hemangioblastomas were S-100 protein-positive, whereas GFAP staining was negative in these stromal cells. This is consistent with the results of McComb *et al.*¹⁶⁾ and Mannoji *et al.*,¹⁵⁾ but partly inconsistent with other observations.^{7,14)} Although we cannot readily explain this discrepancy, it may be due to differences in the GFAP antisera employed. Uptake of extracellular S-100 protein only by stromal cells is less likely to occur *in situ*, in view of the fact that stromal cells lack GFAP. The absence of positive staining for S-100 protein in three of the 13 tumors may represent a quantitative difference or a relative loss of antigenicity by the S-100 protein due to inadequate tissue fixation.

We did not find any relationship between the presence of S-100 protein in the stromal cells and location of the tumor, cyst formation, or degree of reactive gliosis. Our immunohistochemical evidence for human factor VIII-related antigen is consistent

with the results of McComb *et al.*¹⁶⁾ The lack of expression of both factor VIII-related antigen and GFAP by the stromal cells indicates that they are antigenically distinct from both endothelial cells and astrocytes.

Therefore, vestigial neural crest cells may give rise to the stromal cells. Alternatively, the stromal cells may originate from histiocytic cells in the reticulo-endothelial system; S-100 protein has been found in certain histiocytes.¹⁹⁾

The stromal cells have been characterized as neoplastic by some investigators.^{2,3)} On the basis of our results and those of other investigators, we postulate that the stromal cells are the principal neoplastic cells of cerebellar hemangioblastomas, which are tumors of either the neural crest or the reticuloendothelial system and are associated with the proliferation of vascular tissue, possibly as a secondary effect.

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