Histol Histopath (1990) 5: 407-413

Histology and Histopathology

Haemangioblastoma: Histological and immunohistological study of an enigmatic cerebellar tumour

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Summary. Paraffin-embedded blocks of 36 cerebellar haemangioblastomas were reacted with a panel of antibodies including glial fibrillary acidic protein, vimentin, epithelial membrane antigen, cytokeratin, Factor VIII, a neuroendocrine marker and with *Ulex europaeus. agglutinin* The main histological features, apart from the characteristic large abnormal vessels, were a prominent reticulin network, a cystic architecture and cellular and nuclear polymorphism. Two cell types were identified: endothelial and stromal. Twenty tumours were positive for glial fibrillary acidic protein because of included or reactive astrocytes as well as positive stromal cells. Vimentin was positive in all tumours with a diffuse distribution and a somatic pattern; blood vessels, stromal cells and reactive astrocytes were strongly positive. Factor VIII and *Ulex europaeus* agglutinin reactivity were present in a similar pattern of staining in endothelium and in five cases there were stromal cells that were positive with the latter.

We were not able to ascertain the histogenesis of the stromal cell, which remains enigmatic.

Key words: Cerebellum, Haemangioblastoma, Histology, Immunohistology

Introduction

The origin of haemangioblastoma is an enigma. Histologically this tumour has a highly vascular network and intervascular stromal cells (Rubinstein, 1972; Jeffreys, 1975). Differential diagnostic problems may arise with respect to some primary CNS tumours (McComb et al., 1987) and of secondary tumours such as metastatic renal cell carcinoma (Andrew and Gradwell, 1986; Goldesbrough et al., 1988). In a few cases malignant spread has been reported (Mohan et al., 1976). The origin of the tumour is controversial and several authors, on the basis of ultrastructural studies (Cervós-Navarro, 1971; Kawamura et al., 1973; Spence and Rubinstein, 1973; Chaudrey et al., 1978; Ho, 1984; Shimura et al., 1985) have proposed that haemangioblastoma may have a vascular origin. Immunohistological studies focused on the stromal cells have not led to a definite conclusion. Results of staining with glial fibrillary acidic protein (GFAP) (Kepes et al., 1979; Deck and Rubinstein, 1981; Kochi et al., 1984), Factor VIII/von Willebrand factor and *Ulex europaeus* (UEA-1) (Bohling et al., 1983; Alles et al., 1986; Grant et al., 1988; Ironside et al., 1988) have been controversial. Ismail et al. (1985), in an immunohistological and ultrastructural study have suggested that haemangioblastomas may originate from primitive peptidergic neurons. We have carried out an immuno histological study on a series of haemangioblastomas in an attempt to shed some light on this contentious topic.

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Materials and methods

Paraffin wax-embedded blocks of 36 cerebellar haemangioblastomas were available for study. Of 36 tumours, 14 occurred in 6 patients. Four had one recurrence (2 had Von Hippel Lindau syndrome [VHLS] and recurred at 6 and 7 years and the other two with no VHLS recurred at 1 and 3 years) and 2 had 2 recurrences (1 case had VHLS and recurrence was at 5 and 7 years and the other without VHLS recurred at 4 and 6 years).

There were 10 females and 18 males with a mean age of 43 years and a range from 11 to 70 years. The case aged 11 was the only child and did not have VHLS.

The tumours had been fixed in 10% formaldehyde and 7 μm sections were cut and stained with haematoxylin & eosin (H&E), reticulin stain and with a panel of antibodies including GFAP, vimentin, epithelial membrane antigen (EMA), cytokeratin (LP34 and Cam

5.2), Factor VIII/von Willebrand factor (clone F8/86) and PGP 9.5 (neuroendocrine marker) (Doran et al., 1983; Thompson et al., 1983). UEA-1 binding was also assessed.

The peroxidase anti-peroxidase method (Sternberger, 1979) was used with polyclonal antisera to GFAP and to UEA-1 and the indirect two stage immunoperoxidase technique (Warnke et al., 1983) with the other monoclonal antibodies. Cam 5.2 was obtained from Becton Dickinson, UEA-1 from Serotec, PGP 9.5 from Ultraclone and the other antibodies from Dako.

As positive controls we used 2 astrocytomas (GFAP), 2 adeno and 2 squamous carcinomas (EMA, LP34 & Cam 5.2), 4 renal carcinomas (EMA), a malignant fibrous histiocytoma (vimentin) normal cerebral cortex (PGP 9.5) and a cavernous haemangioma (Factor VIII and UEA-1).

Conventional histological features (Cruz-Sánchez et al., 1988) were used to characterize the tumours.

Results

Histological characterization

All tumours had a prominent reticulo-vascular network (Fig. 1) and 89% featured microcysts. Two main cell types were present; cells related to blood vessels or «endothelial» cells and stromal cells. In 35% stromal cells were more prominent than the reticular component. In 69% there was infiltration of the cerebellum consisting of fine capillary prongs extending into the cerebellar tissue. Meningeal infiltration was present in 50% and consisted of small and large abnormal vessels. Marked nuclear density with cellular polymorphisph was present in 69% and nuclear polymorphism in 100%. Atypia and giant nuclei were seen in 40% (Fig. 2) and mitoses (1-2 mitoses x 25 high power fields) were seen in 25% of cases but not in the remainder even after examining 50 or more fields.

All tumours had a large number of blood vessels including a large number of capillaries and of intermediate-sized vessels. A small number of large vessels were seen especially at the periphery of the tumours. There was evidence of hemorrhage in 100%, proliferation of fibrous stroma in 8% and calcifications in 5%.

Table 1 summarizes the histological characteristics of the 36 haemangioblastomas.

Fig. 1. Haemangioblastoma: prominent reticulo-vascular network. Two main cell types are present, cells related to blood vessels («endothelial») and stromal cells. H & E × 125

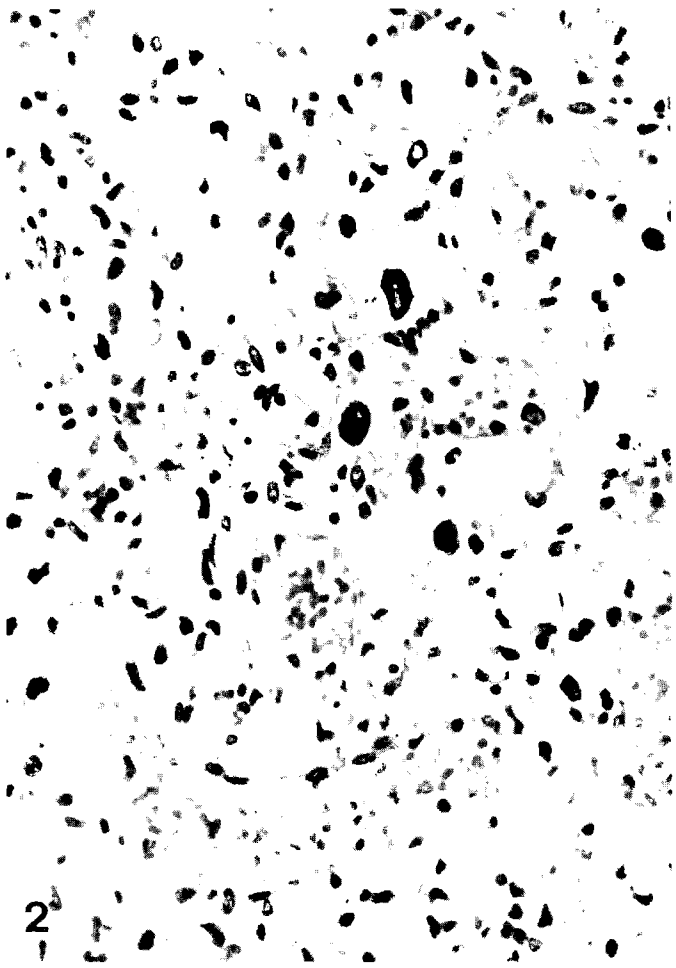

Fig. 2. Haemangioblastoma: area showing stromal cells with atypical nuclei. H & E × 175

Table 1. Summary of the histological features in 36 haemangioblastomas in relation to classical histological characteristics (modified from Cruz-Sánchez et al. 1988).

	N.° of cases	Percentage
HISTOLOGICAL ARCHITECTURE		
Nodularity	3	8
Cystic configuration	32	89
Reticular network	36	100
Whorls	0	0
Perivascular distribution	0	0
Necrosis	3	8
Nervous tissue infiltration	25	69
Meningeal infiltration	17	47
CYTOLOGICAL FEATURES		
High nuclear density	32	89
Anisopoikilocytosis	25	69
Anisopoikilokaryosis	36	100
Mitoses	9	36
VASCULAR COMPONENT		
Marked vascular density	36	100
Vascular abnormalities	36	100
Thrombosis	0	0
DEGENERATIVE CHANGES		
Hemorrhage	34	94
Calcification	2	5
Fibrous stroma	3	8

Table 2. Immunohistological findings in 36 cases of Haemangioblastoma (numbers of positive tumours).

ANTIBODY	VASCULAR COMPONENT	STROMAL CELL	ASTROCYTES (included or reactive)
Factor VIII	36	0	0
UEA-1	36	0	0
Vimentin	36	36	36
GFAP	0	10	36
EMA	0	0	0
LP 34	0	0	0
Cam 5.2	0	0	0
PGP 9.5	0	0	0

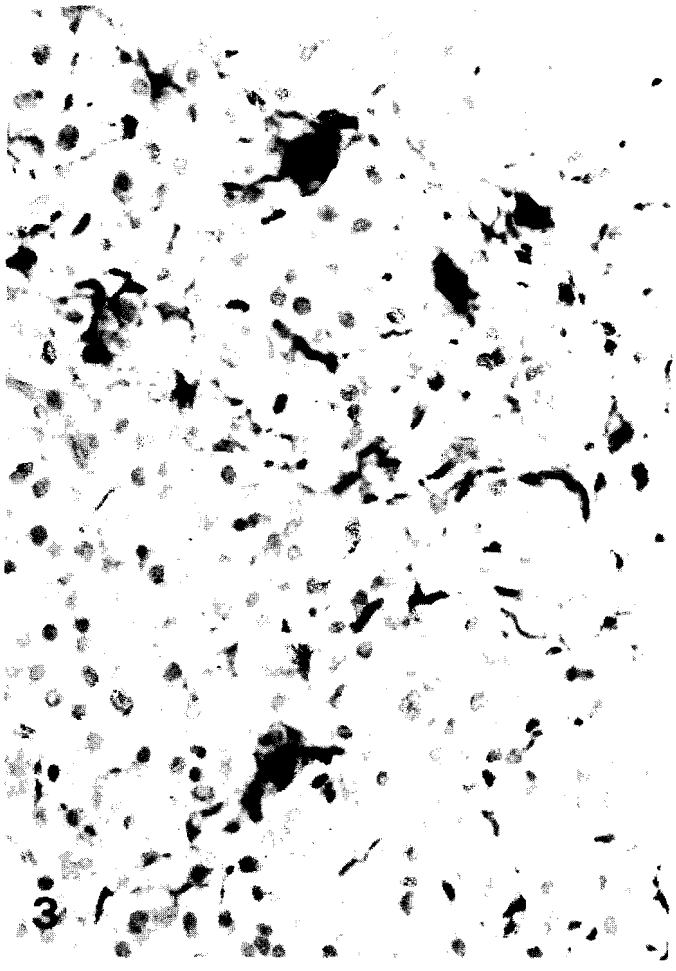

Fig. 3. Haemangioblastoma: GFAP-positive cells with prominent processes thought to be reactive or included astrocytes in the centre of the tumour. GFAP. ×300

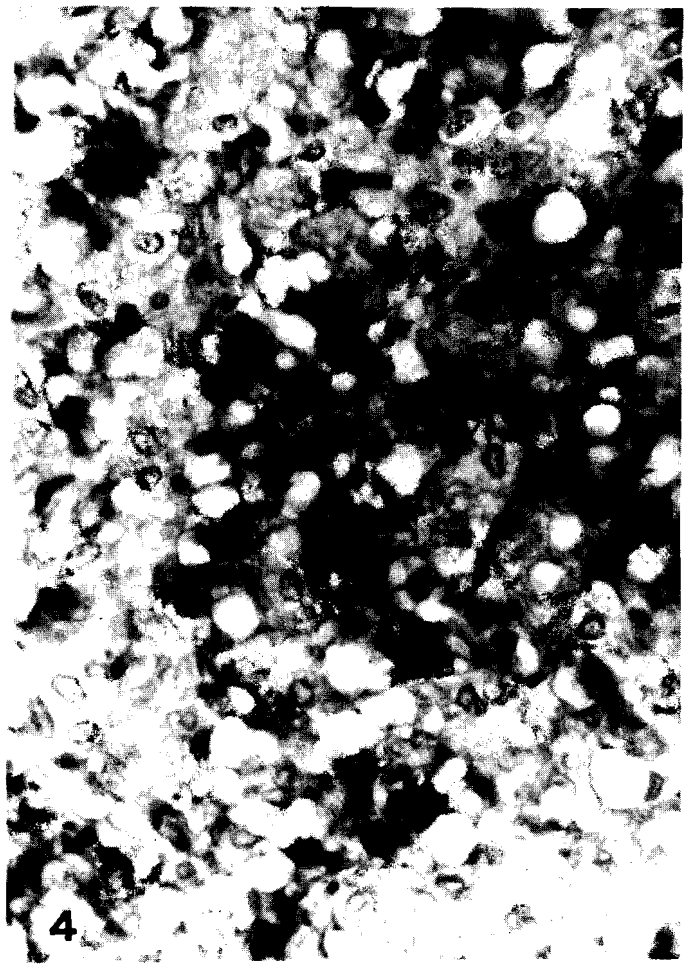

Fig. 4. Haemangioblastoma: GFAP-positive stromal-like cells arranged in islands. GFAP. ×500

Immunohistological findings

The results are detailed in Table 2.

Twenty tumours were positive for GFAP, but the distribution and pattern of positive cells varied. In all cases there was positivity at the periphery of the tumour which we interpreted as being due to included or reactive astrocytes.

In the centre of some tumours, some GFAP-positive cells mimicking reactive astrocytes with prominent processes were also seen (Fig. 3). In 10 tumours (28%) there were some positive cells resembling stromal cells and featuring vacuolated cytoplasm, but clear cellular processes were not present. These stromal-like cells were grouped in islands (Fig. 4), around intermediate-sized vessels and at the edge of cysts. Only in 4 cases more diffuse positivity for GFAP was seen. Tumours with a prominent cellular component (35%) represented by stromal cells were GFAP negative.

Reactivity for vimentin within the cytoplasm was present in all the tumours (Fig. 5). The walls of thin, intermediate and large vessels were also positive. Vacuolated stromal cells showed strong juxta-nuclear positivity. Included or reactive astrocytes at the periphery or at the centre of tumours were positive and the stromal-like cells which were GFAP positive showed co-expression for vimentin. Stromal cells within cellular tumours (35%) were strongly vimentin positive.

Factor VIII reactivity was present in the endothelium of large and intermediate vessels and in proliferated capillaries. Stromal cells were negative in all cases including in the cellular areas.

UEA-1 showed a similar pattern of staining to that of Factor VIII but with stronger positivity (Fig. 6). Five cases also showed stromal cell membrane reactivity and two of them featured cytoplasmic reactivity as well. Positive cells were grouped in islands, around blood vessels and at the edge of cysts or areas evincing degenerative changes.

EMA, Cam 5.2, LP34 and PGP 9.5 were consistently negative in all cases. Renal carcinomas showed strong positivity for EMA.

Histologically and immunohistologically there were

Fig. 5. Haemangioblastoma: vacuolated stromal cells reactive for vimentin with a somatic pattern. Vimentin. ×500

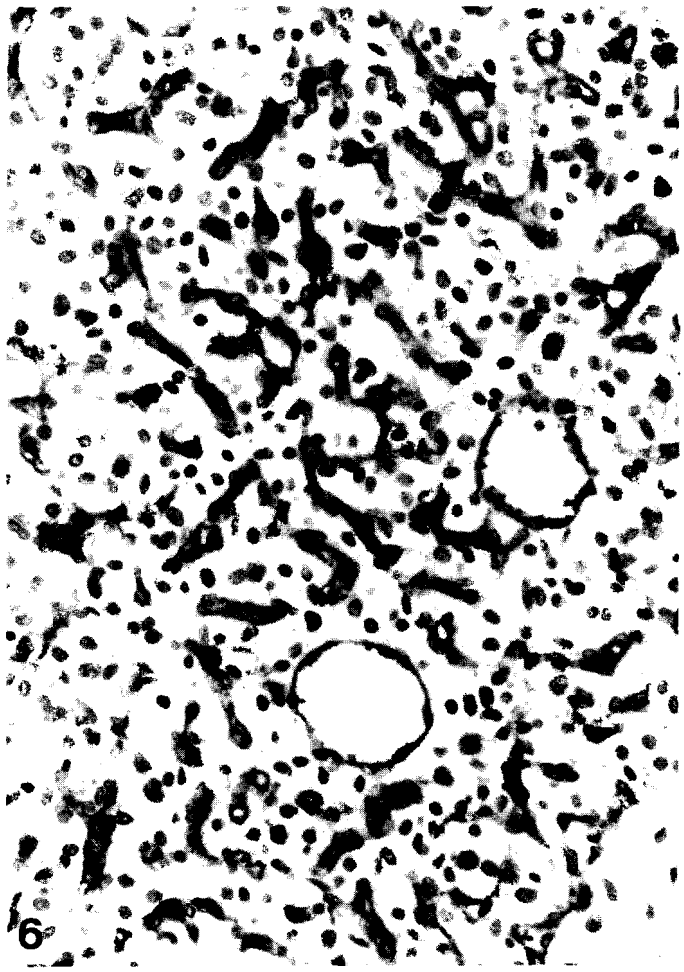

Fig. 6. Haemangioblastoma: strong reactivity for UEA-1 in the endothelium of large and intermediate vessels and in proliferated endothelium. Stromal cells are negative. UEA-1. ×250

no differences between cases with and without von Hippel Lindau syndrome and cases with and without recurrence.

Discussion

Eleven percent of our cases had associated stigmata of VHLS (Horton et al., 1976). All of these tumours «recurred». All of the tumours were negative with epithelial membrane antigen and, therefore, in agreement with other authors (Andrew et al., 1986; Gouldesbrough et al., 1988), the possibility of secondary renal carcinoma was unlikely. Cyst formation and a reticular architecture with infiltration of both cerebellum and meninges were the principal histological features. Cystic changes were both macro and microscopic and this is in agreement with the findings of Jeffreys (1975). The pattern of tumour infiltration within the cerebellum was of particular interest, suggesting that tumour expansion occurs along infiltrating vascular prongs. The meningeal infiltration consisted more in expansion than in infiltration by tumour and it has been suggested that haemangioblastoma may originate from the leptomeninges (Russell and Rubinstein, 1989).

Our tumours showed nuclear pleomorphism and anisopoikilokaryosis, but according to some authors (Rubinstein, 1972; Jeffreys, 1975) these features are not indicative of malignancy. Furthermore, two autopsied cases of haemangioblastoma with malignant spread but no anaplasia have been reported (Mohan, 1976). In our recurrent tumours, increasing degrees of pleomorphism between original tumours and recurrences were not observed.

Anisopoikilokaryosis was evident in both the endothelial and the stromal cell. Anisopoikilocytosis was also present in both cell types but it was more marked in the former. Large to small vessels were always abnormal and their numbers varied between tumours. The relation between the cellular component and the reticulovascular network allowed the separation of the tumours into two groups: cellular and reticular, which is in agreement with Deck and Rubinstein (Deck et al., 1981). The nature of haemangioblastoma has been discussed by several authors (Cervós-Navarro, 1971;

Kepes et al., 1979; Deck et al., 1981; Grant et al., 1988). A vascular origin has been proposed on the basis of histological observations.

Our results, in agreement with Bohling et al. (1983), demonstrate that the vascular component includes capillaries, and intermediate and large vessels which are positive for Factor VIII and UEA-1. According to Grant et al. (1988), UEA-1 appears to be more specific than Factor VIII as an endothelial marker. Ismail et al. (1985) suggested the possibility that haemangioblastoma originates from primitive peptidergic neurons, but staining for PGP 9.5 was negative in our tumours. In our cases both stromal and endothelial cells were strongly positive for vimentin. Schiffer et al. (1986) found similar results and suggested that the stromal cells may be of mesodermal origin. However, they found co-expression with GFAP. In our cellular tumours (35%) we found strong reactivity for vimentin and none for GFAP, which led us also to believe that stromal cells may be of mesodermal origin.

We found two types of GFAP-positive cells: the first was clearly identifiable as an astrocyte-bearing processes, the other being the stromal-like cell featuring polygonal shape and no processes. Both cell types showed co-expression of GFAP with vimentin as judged by adjacent sections.

Several electron microscopic studies have demonstrated the vascular origin of these cells (Kawamura et al., 1973; Chaudry et al., 1978) which is supported by the presence of Weibel-Palade bodies (Ho, 1984). The main feature, however, was the degenerative change observed in some stromal cells (Cervós-Navarro, 1971) which consisted mainly of vacuolation of the cytoplasm and lipid accumulation.

From the immunohistological viewpoint, the interpretation of the staining of degenerating cells is difficult. The presence of an abundant extracellular glycoprotein matrix further contributes to mask the true reactivity of these cells with vascular markers such as Factor VIII and UEA-1 (Rubinstein, 1986). Reactivity with UEA-1 on stromal cells membrane was found in 5 cases and intracytoplasmic positivity in 2 of them; this may be due to the fact that such cells were situated near the areas of degeneration.

We found that the positive stromal-like cells were arranged focally around cysts and blood vessels. Jeffreys (1975) suggested that microcystic degenerative changes may occur as a result of degeneration of stromal cells. These cysts would enlarge by coalescence, but we believe that haemorrhage may contribute and this has been demonstrated in cutaneous haemangiomata (Bailey and Ford, 1932).

Kepes et al. (1979) and Jakobiec et al. (1976) suggested that GFAP-positive stromal cells may be lipidized astrocytes. On the other hand Deck and Rubinstein (1981) proposed that stromal cells may take up GFAP released from reactive astrocytes.

Both theories could explain our findings because all the positive stromal cells we observed were in areas with degenerative changes or close to blood vessels. Large areas of stromal cells, but not associated with cysts or large vessels, were GFAP-negative but vimentin positive. Therefore the theory of Deck and Rubinstein (1981) seems the more plausible to us.

Induction of anaplasia of the astrocytic component could be another possibility, but electron microscopic reports (Cervós-Navarro, 1971; Kawamura et al., 1973; Ho, 1987) have only demonstrated features of reactive astrocytes at the periphery of haemangioblastomas (Cervós-Navarro, 1971).

We have found immunohistological evidence of differences between endothelial and stromal cells because endothelial markers were negative in the latter; however, vimentin (mesodermal marker) reacted with both.

Immunohistology therefore does not clarify whether the stromal cell has a vascular origin, a question for which immunoelectronmicroscopy might provide answers.

Acknowledgements. We wish to acknowledge Dr. R.O. Barnard for his advice, Dr K.C. Gatter, Ms M. Jones, H. Turley and J. Cordell for providing some of the antibodies. Ms. M. Reading, Ms. P. Deacon, and Mr. R.B. Cross for their skilled technical assistance. This study was financed in part by the Oxfordhire District Health Authority and the A. von Humboldt Foundation. S. Rodríguez-Prados was supported by a fellowship from Tucuman National University of Argentina.

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Accepted April 20, 1990