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Immunohistochemical demonstration of glial markers in retinoblastomas

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Summary. Twenty retinoblastomas were studied immunohistochemically in order to visualize glial cells. In the retina, the glial cells in the ganglion cell layer and the Müller cells were GFAP positive, while only the glial cells of the ganglion cell layer expressed S-100 reactivity. In the tumours S-100/GFAP positive glial cells were found in areas near the retina and along many tumour vessels. Some S-100 reactive cells previously interpreted as tumour cells were refound in a few tumours. In areas with Flexner-Winterstein rosettes and in areas with light cells showing photoreceptor-like differentiation, glial cells reactive for both S-100 and GFAP were demonstrated. The latter findings may represent differentiation in a glial direction in the more mature parts of retinoblastoma.

Key words: S-100 protein – GFAP – Glia – Immunohistochemistry – Retinoblastoma

Introduction

The histogenesis of the retinoblastoma has often been debated and both a glial and a neuronal origin has been proposed (Dunphy 1964; Tso 1980). The histology of this tumour presents a varying degree of differentiation ranging from areas with photoreceptor-like differentiation to entirely anaplastic areas, even within a single tumour. Immunohistochemical studies have contributed to further characterization of the tumour. Neuronal markers have been demonstrated in the bulk of cells (Terenghi et al. 1984; Molnar et al. 1984; Messmer et al. 1985) and recently the photoreceptor specific antigen S has been visualized in these tumours (Mirshahi et al. 1986). However, populations of cells with a glial character have also been described (Tegenghi et al. 1984; Molnar et al. 1984; Messmer et al. 1985; Lane and Klintworth 1983).

The presence of glial elements in the retinoblastomas has prompted a discussion whether these cells are part of the tumour or a proliferation of glial cells from the retina. With regard to the glial fibrillary acidic protein (GFAP) containing cells, a non neoplastic nature has generally been suggested (Tegrenghi et al. 1984; Molnar et al. 1984; Messmer et al. 1985; Lane and Klintworth 1983). Among the S-100 reactive cells, however, a population of primitive, neoplastic cells have been found (Terenghi et al. 1984).

Our results are of interest in the discussion about the existence of more mature glial elements as part of retinoblastomas.

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Materials and methods

Paraffin embedded material from 20 eyes with retinoblastomas was investigated. Sections were stained with haematoxylin and eosin and immunohistochemically with the PAP technique (Sternberger 1979) for the presence of GFAP (antibody supplied by Dr. Elisabeth Bock) and S 100 (DAKO patts Z 311). Controls included cerebral tissue and peripheral nerves. As previous investigations (Schrøder and Johannsen 1986) have shown that enzyme digestion did not improve the immunohistochemical results, this step was not included in the staining procedure.

Results

In most of the studied cases areas of tumour free retina were present. The GFAP-stained sections showed positive reaction in the glial cell in the ganglion cell layer and around vessels as well as in the Müller cells (Fig. 1c). S-100 immunoreactivity was restricted to the glial cells in the ganglion cell layer and around vessels (Fig. 1b).

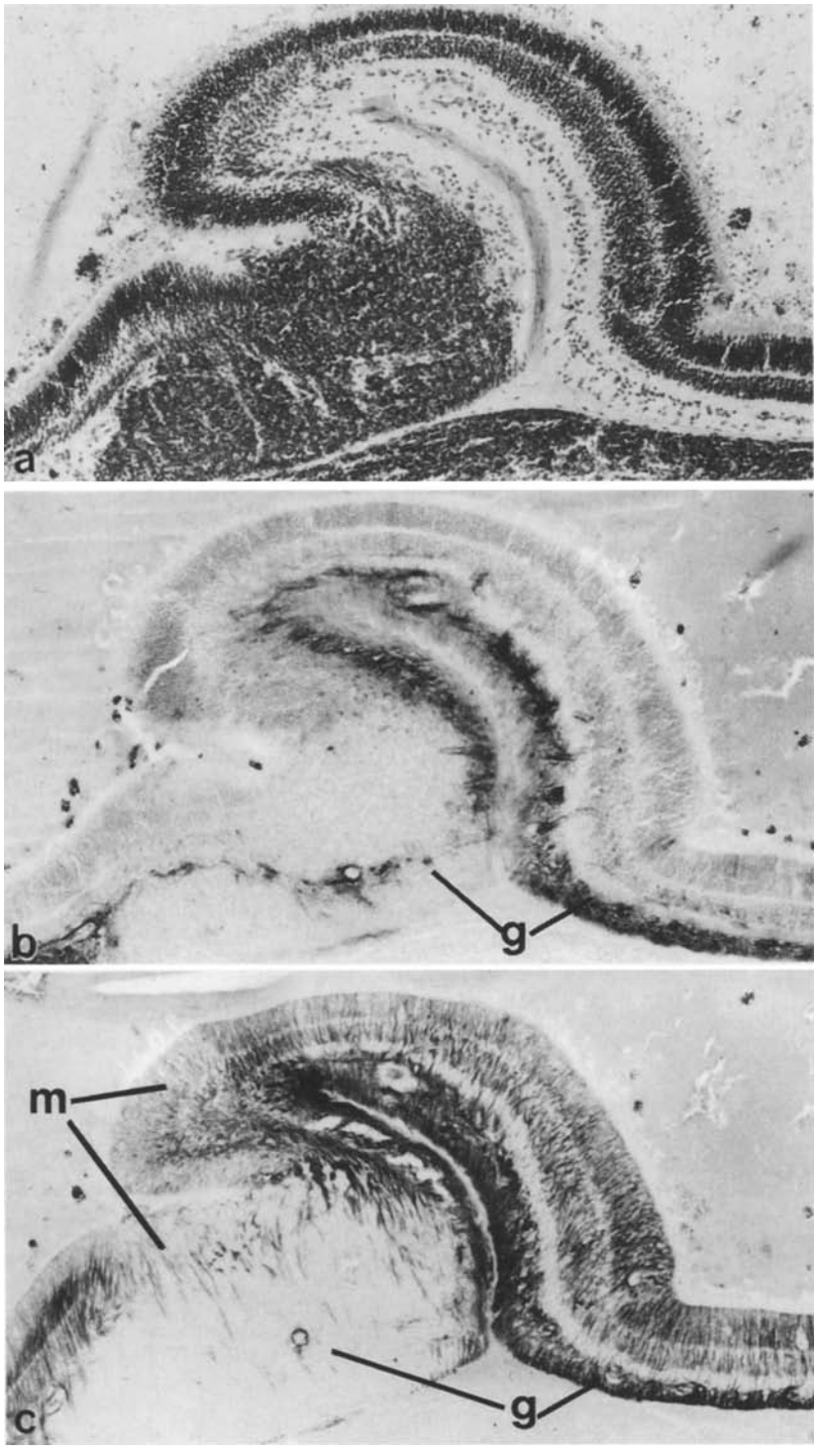


Fig. 1. Loop of retina, partly invaded by tumour. **a** Haematoxylin-eosin stained section. **b** S-100 distribution in adjacent section. S-100 reactivity is found corresponding to the glia (g) in the ganglion cell layer. **c** GFAP. This compound is found in glia (g) in the ganglion cell layer and in the Müller cells m. Note the remnants of Müller cells in the tumour. ×87,5

In the parts of retina invaded by tumour both GFAP positive Müller cells and S-100 and GFAP containing astroglial cells were seen (Fig. 1). In several areas where no remnants of the retina were observed in haematoxylin and eosin stained sections immunohistochemistry visualized remnants at the glial framework. Moreover, the parts of the tumours close to normal retina contained stellate cells of astroglial type with no apparent organization except for perivascular clustering. This population was characterized immunohistochemically by strong S-100 reactivity and moderate GFAP

positivity. These GFAP/S-100 positive cells were also found surrounding vessels at some distance from the retina and in a few cases they appeared on the surface of finger-like tumour projections.

A cell population present in the tumour showed some similarities to the perivascular and retina-near astrocyte-like cells in being GFAP and S-100 positive. The distribution pattern of these cells appeared to reflect the variation in tumour differentiation (Figs. 2 and 3). Thus in anaplastic areas with large tumour cells dominated by nuclei practically no cells of this type were found and they were also absent in relation to Homer Wright rosettes. The GFAP/S-100 positive cells were, however, present in areas with Flexner-Winterstein rosettes and particularly in the light areas of the tumours consisting of cylindrical cells with small nuclei and abundant eosinophilic cytoplasma. Thirteen tumours contained one or more areas of the latter differentiated types: 10 tumours contained Flexner rosettes and 5 tumours demonstrated light areas. All the light areas presented GFAP/S-100 positive cells and only two of the areas with Flexner-Winterstein rosettes lacked this cell type.

The differentiated areas appeared to be unassociated with localisation near to retina and most parts of the periretinal tumour tissue appeared without signs of differentiation, in spite of the content of astrocytes.

There was no evidence for the existence of an exclusively GFAP positive cell type.

Within some tumours scattered S-100 positive/GFAP negative cells were found (Fig. 4). These cells were similar to unstained tumour cells in size and shape.

Discussion

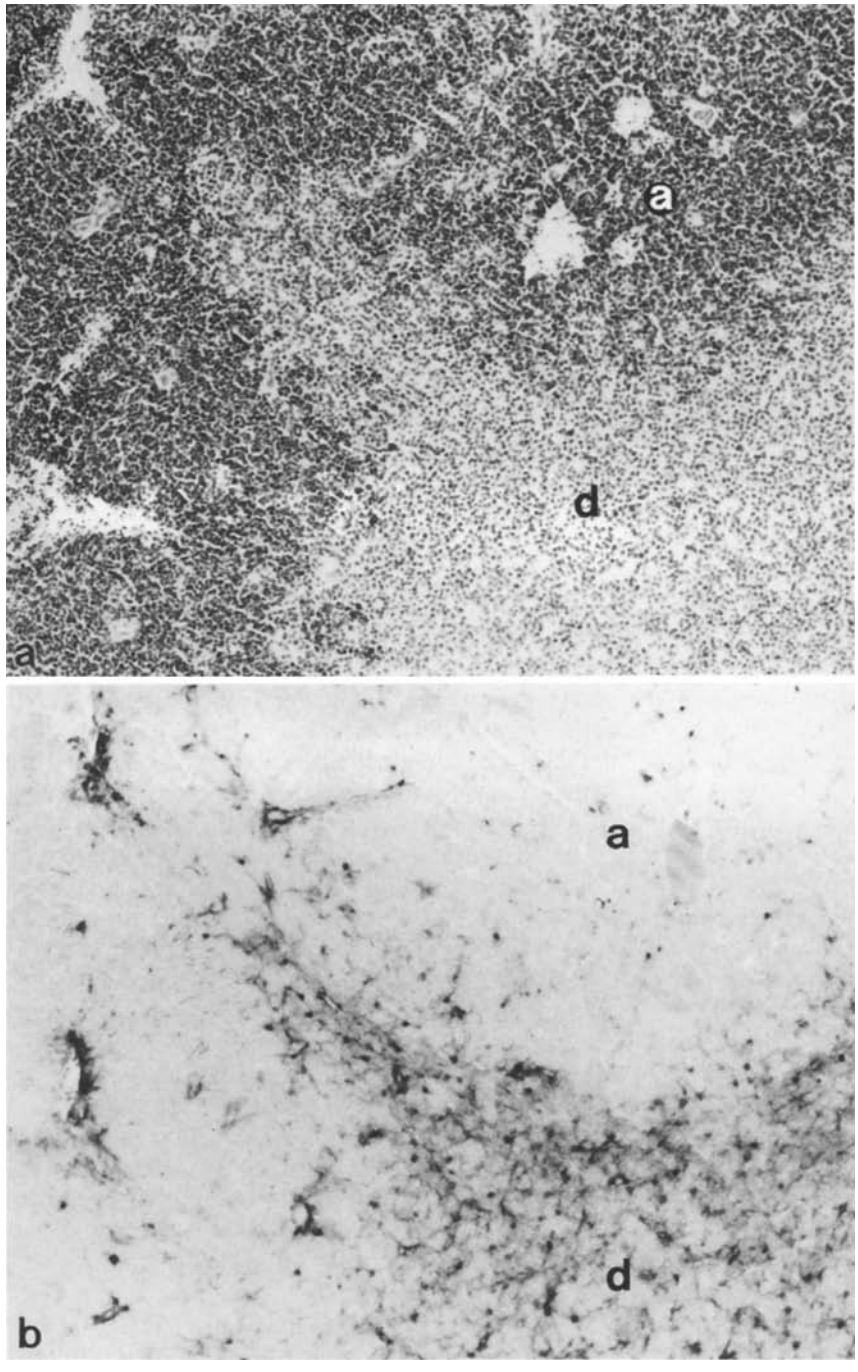
Some distinct differences exist between various authors observations on the presence and distribution of glial markers as well as neuronal markers in retina and retinoblastomas. In this connection it must be kept in mind that differences between both GFAP and S-100 antibodies have been reported (Jessen et al. 1984; Takahashi et al. 1984; Loeffel et al. 1985).

The normal human retina contains two types of glia, the Müller cell and the astroglia of the nerve fiber layer. The astroglia is by all characterized immunohistochemically by containing GFAP (Lane and Klintworth 1983; Terenghi et al. 1984; Loeffel et al. 1985). Some investigators have found S-100 in the astroglial cells while these cells have been described as not containing this protein in other studies (Messmer et al. 1985; Terenghi et al. 1984; Molnar et al. 1984). The findings in Müller cells are even more complex. In a study on retina of normal human eyes no GFAP was found in the Müller cell but they presented carbonic anhydase type C, an enzyme also found in oligodendroglia (Kumpulainen et al. 1983). Moreover myelin associated glycoprotein has been demonstrated in Müller cells (Molnar et al. 1984). GFAP has, however, been found in the Müller cells in the present study as in previous investigations on intact retina in retinoblastoma eyes (Lane and Klintworth 1983; Terenghi et al. 1984; Messmer et al. 1985). These findings fit well with experimental observations. The normal rat retina thus shows no GFAP reactivity in Müller cells while lesions in the retina and even in the optic nerve induce significant GFAP-production (Bignami and Dahl 1979). In the present study Müller cells did not express S-100 reactivity but one earlier study (Terenghi et al. 1984) has demonstrated S-100 positivity in these cells.

The retina-near and perivascularly localized GFAP/S-100 positive cells seen in the present study appear to be identical with those described by Terenghi et al. (1984) and Messmer et al. (1985) and to the GFAP positive cells observed by Lane et al. (1983). These cells have been suggested to represent outgrowth from the retina and optic nerve into the tumour. The S-100 positivity of the retina-near and perivascular glial cells in the present study points to an astroglial and not a Müller cell nature of this cell population.

The exclusively S-100 positive cells in the tumours appear to be identical with the S-100 positive cell by Terenghi et al. (1984) and suggested by them to be tumour cells with glial differentiation. But while Terenghi et al. found these cells richly represented they were few in the presently investigated cases. This cell type was not at all recognized in the studies of Messmer et al. (1985) and Molnar et al. (1984).

Messmer et al. (1985) studied 51 retinoblastomas. In one tumour entirely composed of differentiated cells and in two showing focal differentiation, they found that glial markers were present in cells even if the cells were not adjacent to vessels. These three tumours, however, were apparently not representative of all tumours with signs of differentiation. Terenghi et al. (1984) looked for glial elements in differentiated areas but did not find them. In the present study 13 tumours contained differentiated areas defined as Flexner rosette formation or presence of light areas, and all of these, except two of those with rosettes, presented glial



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elements. This may represent growth of retinal glia in the tumour. Thus it might be deduced that the more mature retinoblastoma cells induce proliferation of non neoplastic glial cells. In contrast, the idea that glial cells induce maturation of the tumour is much less likely, since many undifferentiated areas containing glia exist in the vicinity of the retina. Alternatively, it might be suggested that the glia in the differentiated areas represents a maturation of the tumour in a glial direction. The bipotential nature of primitive retinoblastomas has been suggested on results from tissue culture investigations (Kyritsis et al. 1984). The finding of S-100 positive cells in undifferentiated parts of the tumours supports this point of view (Terenghi et al. 1984 and present study). In other immature central

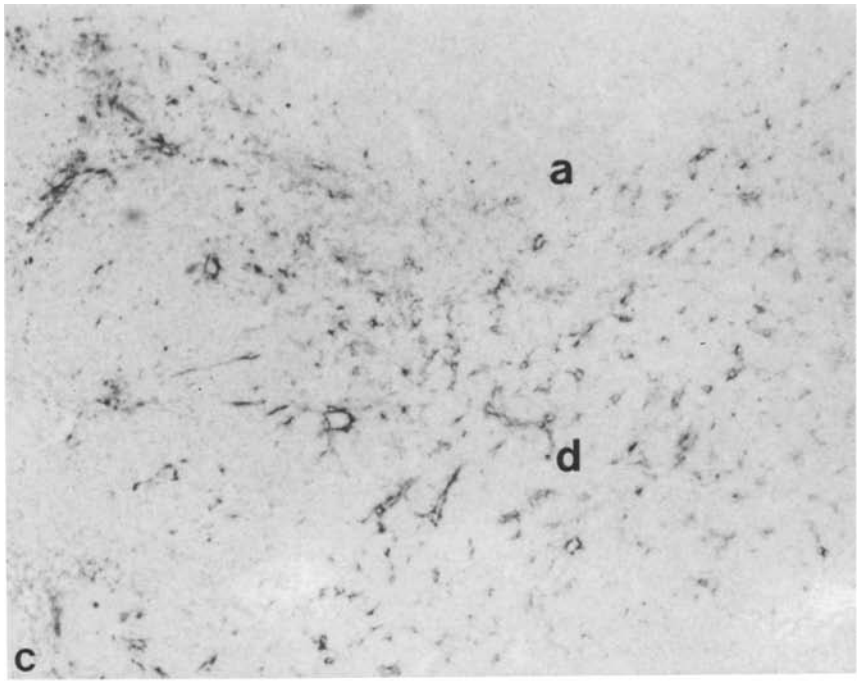

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Fig. 2. Part of retinoblastoma showing anaplastic (a) and differentiated (d) areas. a. Haematoxylin eosin, b. S-100 and c. GFAP. The immunoreactivity in b. and c. is found corresponding to the differentiated area defined in a. ×101

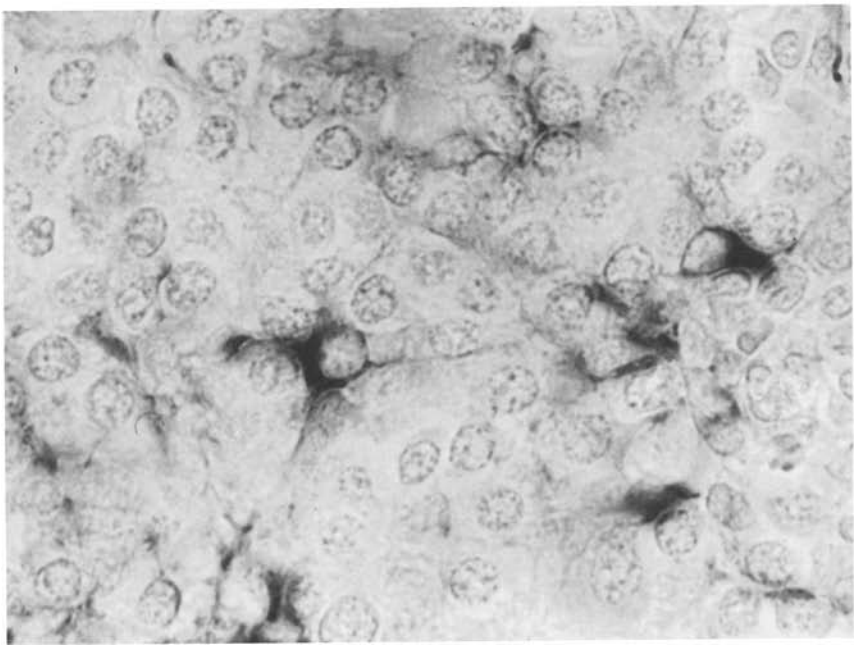

Fig. 3. Light area, same section as Fig. 2 c. GFAP positive cells are seen with a nuclear morphology similar to the unstained cells. ×1010

and peripheral neuronal tumours signs of glial/Schwann cell differentiation have been found (Deck et al. 1978; Mannoji et al. 1981; Roessmann et al. 1983; Nakajima 1982; Bonnin and Rubinstein 1984). Likewise in the neural tumours containing mature neuronal elements, glial-cells/Schwann cells are present, (Nakajima et al. 1982; Bonnin and Rubinstein 1984; Schrøder and Johannsen 1986). Thus the existens of glial elements in the differentiated parts of retinoblastomas would represent a parallel to these earlier observations.

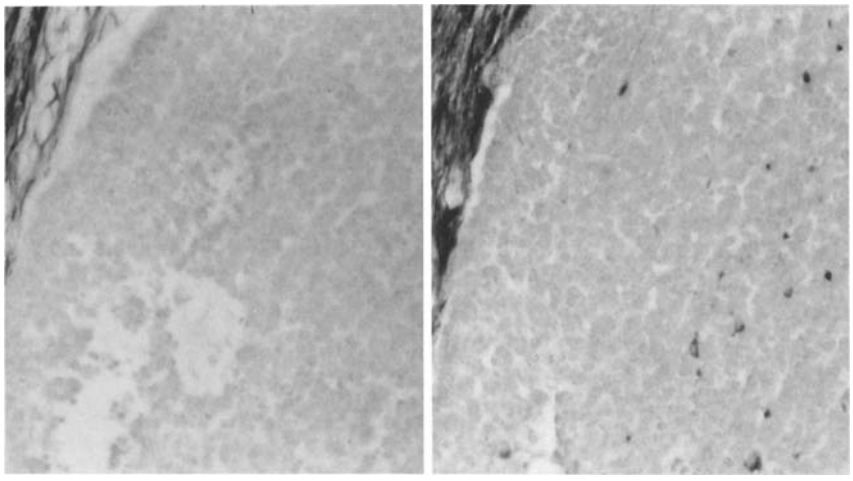

Fig. 4. Anaplastic part of a tumour adjacent to the retina. The glia cells of the ganglion cell layer are both GFAP positive (a) and S-100 positive (b) while immunoreactive cells in the tumour only are seen in (6). ×194

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