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Gamma-enolase activity in choroidal melanoma

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Abstract. Gamma-enolase is a glycolytic enzyme that is found in high concentration in neural and neuroendocrine tissues. The enzyme has been considered clinically useful as a tumor marker in the evaluation of small-cell undifferentiated malignancies. A preliminary report suggested that gamma-enolase activity may be used in the assessment of the malignant potential of choroidal melanoma. To test this hypothesis and document the distribution of gamma-enolase in the eye, we studied the immunohistochemical activity of gamma-enolase in six eyes from persons with choroidal melanoma. Although no correlation could be found with gamma-enolase activity and tumor size or tumor cell type, the distribution of gamma-enolase in normal retina and optic nerve was described. The areas of greatest immunohistochemical reactivity were the perikaryon of ganglion cells, inner and outer plexiform layers, and the nerve axons of the nerve fiber layer and optic nerve. Gamma-enolase may be a potentially useful marker of retinal and optic nerve glycolysis, and of neuronal function.

Introduction

Enolases are glycolytic enzymes that catalyze the conversion between 2-phosphoglycerate and phosphoenolpyruvate in the glycolytic pathway. Although the gamma-isoenzyme of enolase was initially considered to be present exclusively in neural tissue (so-called neuron-specific enolase), it has been subsequently identified in a variety of other tissues (Schmechel 1985; Schmechel et al. 1978). The highest concentrations are found, however, in neurons and neuroendocrine cells. The use of gamma-enolase as a tumor marker has been proposed by several investigators and may be most helpful in the differential diagnosis of small, undifferentiated, round cell tumors (Schmechel et al. 1978; Tsokos et al. 1984; Terenghi et al. 1984). Dhillon et al. (1982) have found it useful in the identification of certain cutaneous melanomas. The presence of gamma-enolase in choroidal melanomas has been established and quantitated by Royds et al. (1983) who studied tissue homogenates from seven tumors. Although the authors realized that their study contained too few cases to be statistically analyzed, they proposed that the estimation of gamma-enolase activity may be useful in the assessment of the malignant potential of choroidal melanoma. In order to confirm their findings and explore the potential value of using currently available hospital laboratory techniques to detect gamma-enolase, we correlated the immunohistochemical activity of gamma-enolase in six choroidal melanomas with tumor volume and cytologic classification.

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Materials and methods

Gamma-enolase activity was measured using a standard avidin-biotin peroxidase technique described elsewhere (Hsu et al. 1981), and a commercially available polyclonal rabbit antibovine gamma-enolase, the specificity of which was tested by adsorption with purified gamma enolase (Dakopatts). Negative controls used rabbit serum in place of rabbit antibovine gamma-enolase. Methods to assess specificity of staining have been described elsewhere (Tsokos et al. 1984). Human cerebral cortex and oat-cell tumor of the lung were used as positive controls. Retina and optic nerve, which stain intensely positive for gamma-enolase, served as a positive internal control and as an indicator of the uniformity of immunohistochemical reaction throughout each microscopic section. Histologic sections were prepared from human tissue that was fixed in formalin and embedded in paraffin. The following stains were obtained: hematoxylin and eosin, luxol fast blue for myelin, and Bodian for nerve axons. Tumor size was calculated after enucleation using average tumor cord length and maximum tumor height. Tumors were catagorized pathologically according to a modified Callender classification as being either nevus, spindle cell melanoma, mixed cell type melanoma, or epithelioid melanoma. Semi-quantitative assessment of the immunoperoxidase staining activity was based on a scale ranging from 1 to 5, with 5 representing maximum reactivity. After initially inspecting the immunoperoxidase stained sections, it was apparent that retina and optic nerve would serve as a reliable internal standard equal to maximum staining intensity.

Results

Four of six tumors stained positive, but in a patchy, uneven manner. This staining pattern was considered real because adjacent retina and optic nerve stained positive and in a uniform fashion in each case (Fig. 1). Maximum tumor staining was less than half the intensity of either retina or optic nerve. Tumor size, cell type, and semiquantitative assessment of immunoperoxidase staining activity are listed in Table 1. There was no correlation between positive stain-

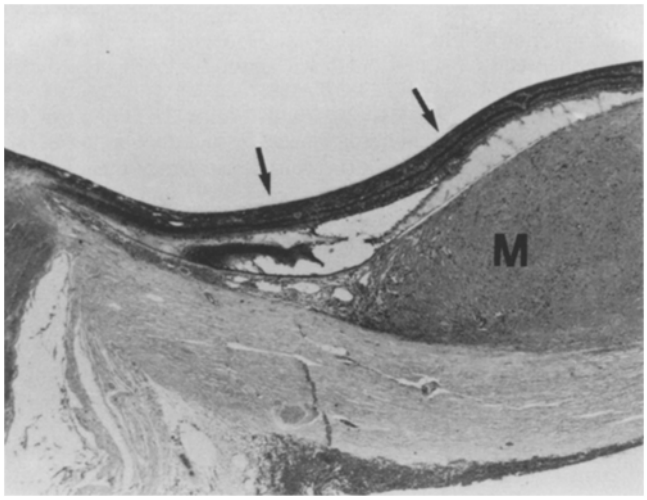

Fig. 1. The retina (*arrows*) stains strongly positive for gamma-enolase, while the choroidal melanoma (*M*) reacts very weakly. The tumor was composed of spindle cells (case 2) and the intensity of staining was graded as 1+. Some of the darker staining areas in the tumor represent melanin, which at this magnification cannot be distinguished from the immunohistochemical stain (immunoperoxidase, ×4,5)

Table 1. Choroidal melanoma and gamma-enolase activity

Case	Cell type	Tumor volume (mm^3)	Gamma-enolase: intensity of stain[a]
1	Mixed	1,440	1+
2	Spindle cell	605	1+
3	Mixed	200	0
4	Spindle	720	2+
5	Epithelioid	200	0
6	Mixed	122	1+

[a] Scale range: 1 to 5+. Retina and optic nerve were used as internal standards equal to 5+, or maximum staining intensity

ing and either tumor size or cell type. There was no correlation noted between positive staining within individual tumors and cell type in melanomas of mixed cell population.

Staining for gamma-enolase was observed throughout the neurosensory retina except for the outer segments of photoreceptors. Axons in the nerve fiber layer, cytoplasm of ganglion cells, and the cytoplasmic network of the inner and outer plexiform layers stained more intensely than the inner and outer nuclear layers (Fig. 2). This difference may simply reflect the greater amount of cytoplasm in the non-nuclear layers. The non-pigmented epithelium of the pars plana and pars plicata also stained positive. A faint reaction was also detected in the retinal pigment epithelium in cells with few melanin granules.

Nerve axons of the optic nerve stained strongly positive (Fig. 3). Focal areas of non-staining in some areas correlated with axon degeneration when compared with Bodian stains for nerve fibers.

Discussion

The variable activity of gamma-enolase in chorodial melanomas, measured by standard immunohistochemical techniques, makes interpretation difficult and clinical application limited. Although gamma-enolase concentrations determined by radioimmunoassay from melanomas biopsied following enucleation did roughly correlate with cytologic grading in the study by Royds et al. (1983), several aspects

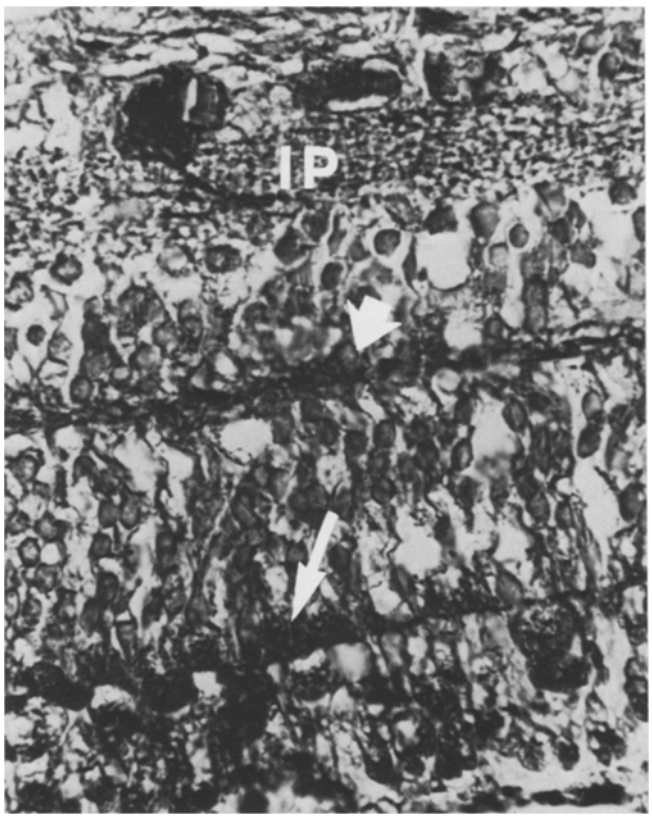

Fig. 2. The entire retina stains strongly positive for gamma-enolase. Particularly heavy reactivity is present in the inner plexiform layer (*IP*), in the central portion of the outer plexiform layer near the plane of photoreceptor synapses (*short, thick arrow*), and the outer nuclear layer near the external limiting membrane (*long, thin arrow*) (immunoperoxidase, ×288)

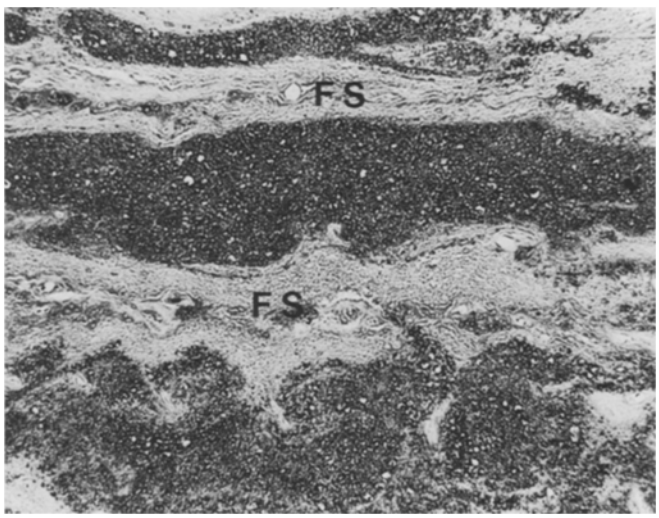

Fig. 3. The neural component of the optic nerve stains strongly positive for gamma-enolase. The fibrous septae (*FS*) do not stain and appear light grey (immunoperoxidase, ×22,5)

about tissue enolase levels should be reviewed. Actual measured concentrations of gamma enolase vary considerably, depending on the type of tissue: from 250 ng/mg of protein in lymphocytes to greater than 20,000 ng/mg of protein in certain parts of the brain (Kato et al. 1983; Marangos et al. 1979). While neural and neuroendocrine tissues contain substantially higher concentrations of the enzyme than do other tissues, gamma-enolase is distributed widely throughout certain organs in the body in low concentrations less than 600 ng/mg protein (Schmechel 1985). Gamma-enolase levels in two spindle-cell melanomas studied by Royds and associates averaged 615 ng/mg of protein, which was almost three times the concentration measured in any of their remaining five mixed cell melanomas. Since the retina and subretinal fluid are potential contaminants in tumor biopsies, one should be cautious not to attribute too much significance to variations in gamma-enolase measurements occurring in tissue with low concentration.

The functional significance of the expression of the gamma-enolase gene is not understood. In the nervous system, expression is closely associated with neural differentiation, and enzyme levels have been correlated with levels of synaptic activity in neurons (Schmechel et al. 1980b). Gamma-enolase participates in the formation of a high-energy phosphate bond before the second and final step in glycolysis (Schmechel 1985). The gamma-subunit of enolase is enormously enriched in the brain and represents approximately 2% of the brain's total soluble protein (Hullin et al. 1980). Gamma-enolase may be a potential marker of glycolysis and neural function and, in that context, be of interest to ocular physiologists and clinical investigators.

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