

Stereological study on malignant melanoma of the choroid and ciliary body

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Abstract

This study is aimed to find a correlation between the cell type and the vascularization of the malignant melanoma of the choroid and ciliary body, using stereological methods. HE stained slices were made of specimens from 56 patients. The microscopic tumor types, established according to Callender's classification, modified were: malignant melanoma spindle classic, malignant melanoma mixed classic, malignant melanoma spindle pleomorphic and malignant melanoma mixed pleomorphic. The stereologic measurements were made on 16 of the 56 cases, using the Prodit 5.2 program. We observed that in spindle cell type the vascularization of the tumor occupies a smaller volume than in the mixed type. The tumor blood vessels occupied a larger volume in the pleomorphic spindle type and pleomorphic mixed type. Thus, we believe that malignant melanoma of the choroid and ciliary body containing spindle pleomorphic cells may have a worse prognostic.

Keywords: malignant melanoma - choroid - stereologic method

Introduction

Malignant melanoma of the choroid and ciliary body represent the most frequent encountered malignant intraocular tumor in white patients. They arise from the choroidal melanocytes, localised in the choroidal stroma. These cells produce and contain melanin pigment, and their origin is the neural crest.

The aim of this study is to find a correlation between the cell type and the relative volume occupied by blood vessels in the malignant melanoma of the choroid and ciliary body, using stereological methods. The cell type and the vascular pattern are two of the most important prognostic parameters in malignant melanoma.

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Materials and methods

We examined 56 cases of uveal malignant melanomas enucleated in 1992-1998 from the Register of Ophthalmic Pathology, Department of Pathology, Debrecen, Hungary.

After enucleation, the eye globes containing the tumors were embedded in paraffin and sectioned at 6 μm . Sections were HE stained (after bleaching by potassium permanganate method, for a better visualisation of the cell type and tumor blood vessels).

The microscopic tumor type was established according to Callender's classification modified by the pathologists from AFIP (Armed Forces Institute of Pathology, Washington D.C., USA) (1).

The stereological studies were made on 16 of the 56 cases (8 from the spindle type and 8 from the mixed type) using a video camera equipped Nikon microscope, connected to a PC Pentium. Images were digitized using Prodit 5.2 software. This interactive program allowed us to make many quantitative measurements including the stereological studies.

The method combined test-points with test-lines inside the standard surfaces, which allowed the estimation of the volumes occupied by the compounds of the tumor: tumor cells, tumor vessels and tumor stroma. For this reason, the observer chose video images from the centre of the tumors and superposed the standard grid. The grid was adapted by the observer in order to correspond to the parameters of the studied tissue. The

steps of the measurement are: identification of the tumor structures (tumor cells, tumor stroma, tumor vessels); definition of the geometrical Weibel grid superposed on the microscopic image; definition of specific characteristics of the tumor; counting the total number of points; automatic counting of the stereologic report; statistic evaluation of the parameters; graphic representation; interpretation of the stereological results.

In each case we examined with the 40x objective a test surface of 0.0877 mm^2 , which corresponds to 480 points on the Weibel grid test; the distance between two points is $d = 14.53 \mu\text{m}$.

Results

One of the 56 cases was of necrotic type and it was eliminated because it was not appropriate for the stereologic studies. The remaining 55 cases were: 27 of spindle type and 28 of mixed type. In some tumors of spindle or mixed type, we observed pleomorphic spindle cells, having indistinct borders, eosinophil cytoplasm, two or more nuclei, with irregular shapes and eosinophilic nucleolus. According to the presence of characteristic cell types, we classified tumors in: malignant melanoma spindle classic (MMSC) (Fig.1), malignant melanoma mixed classic (MMMC), malignant melanoma spindle pleomorphic (MMSP), malignant melanoma mixed pleomorphic (MMMP). (Fig. 2).

We examined by stereological method 16 cases.

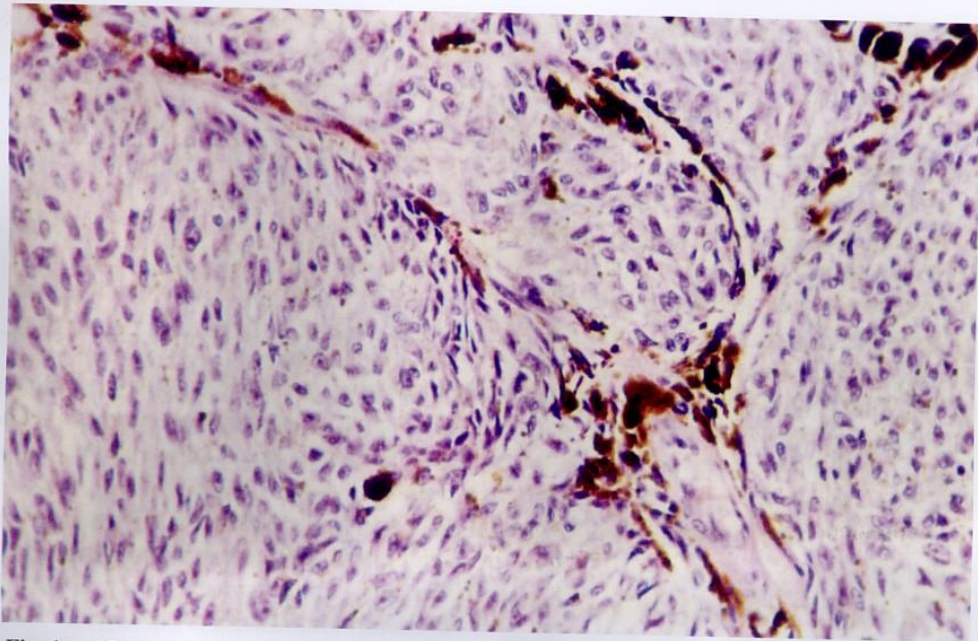


Fig. 1 Malignant melanoma classic spindle type

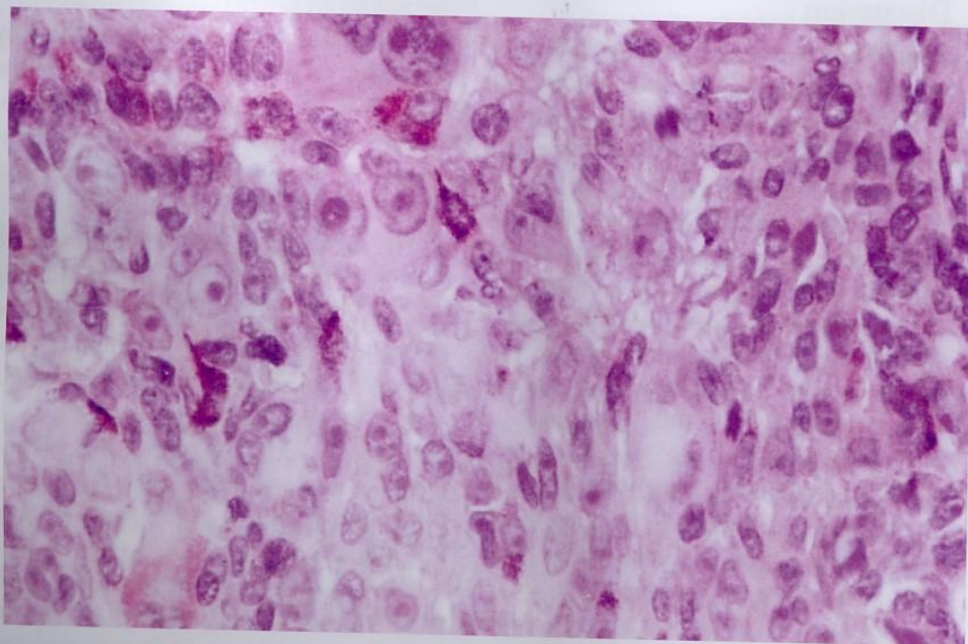


Fig. 2 Malignant melanoma mixed type, with pleomorphic spindle cells

Table 1 Mean of the volume occupied by malignant cells, stroma and lumen vessels in the four microscopic types of malignant melanoma

	MMSC	MMSP	MMMC	MMMP
volume % malignant cells	67,1	64,6	72,3	66,0
volume % tumor stroma	31,0	32,3	21,7	24,8
volume % lumen vessels	1,9	2,9	6,0	9,2

The results of the stereological measurements are shown in Table 1.

The volume occupied by the tumor vessels is smaller in MMSC than in MMMC and on the other hand is smaller in both MMSP and MMMP than in MMSC and MMMC.

Discussion

The first microscopic classification of the uveal malignant melanoma was made in 1931 by Callender. He used histologic and cytologic criteria for his classification (2). He described 6 microscopic types: spindle A type, spindle B type, mixed type, fascicular type, epithelioid type and necrotic type. He and his colleagues demonstrated the prognostic value of this classification: mixed, epithelioid and necrotic types have a worse prognostic than spindle and fascicular type (3).

Spindle A tumor type has cells with a spindle shape with indistinct borders, small nucleus, indistinct nucleolus and rare mitotic figures. Spindle A cells grow in a compact cohesive manner.

Spindle B tumor type has cells with a similar morphology but larger, with a

larger plumper nucleus with a prominent nucleolus, coarse chromatin network, and frequent mitotic figures. They grow cohesively.

Cells of the epithelioid type tumor are larger and polygonal with pleomorphic nucleus, abundant cytoplasm, distinct borders, and distinct nucleolus. These cells lack cohesion and show many mitotic figures.

The mixed type tumor described by Callender contains spindle A, B and epithelioid cells in a variable proportion.

The fascicular type tumor contains spindle A, B cells and epithelioid cells, but with a different disposition.

The pathologists from AFIP simplified Callender's classification by eliminating the fascicular type, which was based on the histologic pattern, by combining the spindle A and B types (4), and by eliminating the epithelioid type which is in fact part of the mixed type. Later, pathologists recognized many other cell types: intermediate cells (5,6), vacuolated cells (7), epithelioid intermediate cells (8,9).

Recently, the authors of this study described a new cell type found in the malignant melanoma of the choroid and

ciliary body; the spindle pleomorphic cells (7). According to the presence of these cells, we classified the spindle and mixed types, respectively, in: classic (without spindle pleomorphic cells) and pleomorphic (with spindle pleomorphic cells).

In the last decades many pathologists and ophthalmologists studied this tumor in order to find new and important prognostic parameters. One of this parameter is the tumor vascularisation of the malignant melanoma. Folberg *et al* described for the first time 9 types of tumor blood vessels in malignant melanoma of the choroid and ciliary body and emphasized the prognostic value of these types (10,11):

- normal type: normal choroidal vessels are present;
- silent type: no normal or tumor blood vessels are present in a field viewed with a 10x objective;
- straight type: vessels without dichotomic branching which are not cross-linked to each other;
- parallel type: straight vessels, lacking dichotomous branching or cross-linking are aligned parallel to each other;
- parallel with cross-linking: parallel vessels are cross-linked to each other, similar to the switching tracks in a rail yard;
- arc type: incomplete loops;
- arc type with branching: arch with dichotomic branching;
- loop type: vessels completely closed;
- network type: composed of at least three back-to-back closed loops.

Folberg demonstrated that normal, silent, straight, parallel, arcs, arcs with

branching correlate with a good prognosis while parallel with cross-linking, loops and networks correlate with a bad prognostic and are significantly associated with the ability of ciliary body and choroidal melanomas to generate metastases (12).

Folberg *et al* established a relationship between cell type and certain vascular patterns: straight vessels develop in zones with spindle melanoma cells and loops, networks and parallel with cross-linking in zones with epithelioid cells (11). The authors of this study did not find a similar correlation between the cell type and the vascular pattern (13). Especially spindle or mixt type tumors, containing pleomorphic spindle cells have loops and networks (data not shown).

In this study, we tried to correlate the microscopical pattern and the volumes occupied by the tumor cells with the volumes occupied by the tumor blood vessels. We observed that in spindle cell type the vascularisation of the tumor occupies a smaller volume than in mixed type. The tumor blood vessels occupied a larger volume in the pleomorphic spindle type and pleomorphic mixt type than in classic spindle type and classic mixed type.

Thus, we believe that malignant melanoma of the choroid and ciliary body containing spindle pleomorphic cells may have a worse prognosis. The presence of spindle pleomorphic cells in these tumors may determine a bad prognostic, even for malignant melanomas spindle type. At the same time, the stereological method demonstrated the value

of the cytomorphometrical studies in the appreciation of the malignant potential of these tumors. The appreciation of the vascularisation in malignant melanoma may offer important data for the tumor prognostic and tumor progression. Because the microscopic classification of these tumors is actually very difficult for the ophthalmic pathologists and involves subjective judgement, it will be necessary in the future to develop new and more objective techniques to appreciate the important prognostic parameters in malignant melanoma of the choroid and ciliary body.

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