

Immuno-morphometric study of choroid melanoma angiogenesis

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Abstract

Introduction: The lack of local epidemiologic data, the progress of current diagnosis and treatment methods motivated us to evaluate our patients from the perspective of tumor angiogenesis. The metastatic potential of the choroidal melanoma is well known, and the angiogenesis is an essential promoter of it. That is why we proposed a study to examine the tumor angiogenesis with immuno-histochemical staining and digitalized image processing

Material and method: We present 21 cases of choroid malignant melanoma, 14 investigated, treated and followed at the Ophthalmology of the University Emergency Hospital Târgu Mures, and 7 at the "Dr. Constantin Papilian" Military Emergency Hospital Cluj Napoca between 01.01.2005 – 31.12.2009. For all the patients ophthalmologic examination, imagistic examination – ultrasound, CT/MRI, and radical treatment was done, followed by histopathologic examination and imunohistochemic staining. The histological staining used was Hematoxilyn-eozin (HE). Immunologic staining were for CD31, CD105, Collagen IV and Smooth Muscle Actin (SMA). The stained sections were scanned by a digital scanner (Zeiss Mirax Scan) at the Pathology Department. The digitalized data was visualized with the aid of Pannoramic Viewer 1.15 RTM, 3DHistech program, and area selection was done. For every section 4 central and 4 peripheral areas were selected, "hot spot" area at 40x magnification (1760x1050 pixel, representing an area of 660x430 μm) These areas were digitally processed with the use of ImageJ 1.43m (Wayne Rasband, National Institute of Health, USA) software.

Results: Maximal tumor diameter was 20 mm, mean value 15,11 mm. Maximal tumor volume was 6400 mm³, mean volume 2573 mm³. The morphometric

study based on comparing mean immune-positive surfaces (MIS) for CD31 positivity present significant differences between central and peripheral areas, in the favor of peripheral areas. The CD105 reaction had statistically significant difference of MIS values in favor of central areas. The $p=0,0054$ means that newly formatted tumor vessels are more frequent in central areas. The CD31-MIS and CD105-MIS values in central areas showed significant difference $p<0,001$. In the central areas CD105-MIS values dominate, so the tumor endothelial cells are in greater proportion. In the peripheral areas the difference of CD31 and CD105 MIS are less evident without a statistical difference ($p=0,656$). Though the examination of SMA staining was more obvious peripheral, the morphometric analysis revealed $p=0,393$ with no statistical significance. The morphometric study on Collagen IV-MIS resulted statistically stronger reaction in peripheral areas. The five year survival rate of the examined patients (death by all causes) was 24,37%, much under the literature data.

Conclusions: The visual acuity of the affected eye showed no correlation between cellular type, or tumor pigmentation. Regards of the accuracy of different imagistic examination methods, results obtained by ultrasound examination are similar with those of MRI, and much alike to the direct histological measurements. 16 from the 21 melanoma cases were associated with clinical complications at the moment of diagnose, visual acuity was affected, glaucoma and/or retinal detachment was present. 7 of 21 were blind, with no light perception. Only 4 patients presented at the first examination with maintained but decreased visual acuity. There is a statistically significant correlation between tumor dimensions (maximal tumor diameter) an intraocular pressure values. The lesion of the vascular wall is evident, meaning the modification of structure as well as the proportion of the elements. The difference between the peripheral and central area is evident, well documented imunohistochemic and morphometric. The differences are statistically significant.

Key words: choroid melanoma, angiogenesis, immune-morphometry