Expression of PCNA and Ki-67 in posterior uveal melanomas in adults

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Abstract

The aim of the study was to evaluate the expression of cell proliferation markers (PCNA and Ki-67) in posterior uveal melanomas in adults. Thirty-six enucleated eyes (without prior treatment) were included in histopatological study. A series of 15 cases of spindle cell type melanomas, 12 epithelioid and 9 of a mixed type were assessed, using the immunohistochemical method with monoclonal PCNA and Ki-67 antibodies. PCNA expression was observed in 75% and Ki-67 in 13.8 % of all the examined tumours. The mean score of PCNA and Ki-67 index were the highest in tumours, which contained epithelioid cells and in large, more advanced (pT3, pT4) uveal melanomas. The results showed that the evaluation of expression of PCNA and Ki-67 may provide an additional information about the progression of tumor process.

Key words: PCNA, Ki-67, uveal melanoma.

Introduction

Different kinds of proteins play an important role in the control of the cell cycle. Some of them take place in cytological transformation and tumour progression. PCNA and Ki-67 are two of the nuclear markers, used to demonstrate the proliferative phase of the cell cycle. Proliferating cell nuclear antigen (PCNA) is a co-factor of DNA polymerase δ, which is necessary for DNA replication. The expression of PCNA is the highest in G1/S cell cycle phase. PCNA labelling was performed in several series of brain neoplasm [5, 7]. Ki-67 is an antigen, expressed in all the phases of the cell cycle, excluding the G0 phase

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Material and method

Thirty-six patients: 20 females (the mean age 59.5 years, the age range 36-82 years) and 16 males (the mean age 68.4, the age range 58-78) were operated for posterior uveal melanoma (melanoma malignum choroideae) between 1994 and 2003 at the Department of Ophthalmology of the Medical University in Białystok. All the eyes were enucleated because of posterior uveal melanoma without prior treatment (they were disqualified for brachytherapy). They were included into histopatological study. The obtained material was fixed in formalin, routinely processed and paraffin embedded. The cytological material was stained with H-E. Those specimens were histopathologically (Callendar's classification) [2] and TNM (TNM Classification of Malignant Tumours) classified. The histological types of tumours, according to Callendar's classification, included 15 (41.7%) spindle cell type tumours, 12 (33.3%) that contained epithelioid cells and 9 (25%) mixed tumours. All the tumours were evaluated, using pathological tumour grading pT (range pT1 -pT4). In different histological types of melanomas, the proportions of tumours in the same stage were similar. Seventy-five (75) percent of uveal melanomas belonged to pT3 and pT4 grade. Table 1 shows the association between the histopatological type of the uveal melanomas and the grades (pT feature). Cell proliferation was determined by immunoreactivity for the proliferation marker with monoclonal antibodies Ki-67 (clone MIB-1; M 7240, DAKO) and PCNA (clone PC 10; M 0879, DAKO), using a LSAB KIT with (DAKO) DAB as a chromogen to visualize the antigen/antibody complex. The protein expression was defined as positive (+) if more than 10% of the cells showed

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Table 1. Association of histological cell type of melanomas to their grades.

Histological cell type	Number of tumours	pT1	pT2	pT3	pT4
spindle cell	15	0	4	8	3
epithelioid cell	12	1	2	2	7
mixed	9	1	1	2	5

Table 2. PCNA and Ki-67 expressions in histological cell type of uveal melanomas

Histological cell type of melanoma	Number of cases (n)	PCNA (+) (%)	Ki-67 (+) (%)
spindle cell	15	10 (66.7%)	2 (13.3%)
epithelioid cell	12	10 (83.3%)	2 (16.7%)
mixed	9	7 (77.8%)	1 (11.1%)

(+) - positive reaction for PCNA and Ki67 protein above 10% of the cells

Table 3. The proliferation index of PCNA (IP PCNA) and Ki-67 (IP Ki-67) according histological cell type of melanomas.

IP PCNA			
Histological cell type of melanoma	**Number of cases (n)**	**IP PCNA ±. SD**	**Range values**
spindle cell	15	13.2 ±7.4*	4.2-28.6
epithelioid cell	12	24.9 ±13.8*	6.8-50.1
mixed	9	16.1±9.0*	4.5-38.1
IP Ki-67			
Histological cell type of melanoma	**Number of cases (n)**	**IP Ki-67 ±. SD**	**Range value**
spindle cell	15	1.7±1.1*	0.5-14.3
epithelioid cell	12	3.1±1.9*	0.8 -27.6
mixed	9	1.9±2.1*	0.2-12.6

SD: standard deviation, *p<0.05

positive reaction for PCNA and Ki-67; and as negative (-) if less than 10% of cells had negative immmunostaining. The percentage of positive nuclei was expressed as a proliferating index (IP) (the number of positive-staining cells for antigen K-67 and PCNA was divided by the total cell count and expressed as a percentage) For the evaluation of IP, tumour areas with a high density of labelling were chosen. For each tumour, a total of five fields (total cell number > 2000) were counted. The MicroImage InCD UDF Packed Writing Software for Window Olympus was used for those calculations. The significance of differences in scores among all the groups was assessed, using Student's 't' test. Values at $p<0.05$ were considered significant.

Results

PCNA immunopositive nuclei (more than 10% cells, showing positive reaction for PCNA protein) were observed in 27, out of 36 cases. The highest percentage of those (83.3%) was found in the tumours that contained epithelioid cells. The expression in the spindle cell group was the lowest and was observed in 66.7% of cases. Positive immunostaining for Ki-67 was found in 5, out of 36 uveal melanomas: 2 cases in spindle cell and 2 epithelioid type tumours, and 1 in the mixed group (Table 2). PCNA and Ki-67 overexpression were found in a large number of big tumours (pT3, pT4).

The score of IP PCNA ranged from 4.2 to 50.1. The highest value of PCNA -24.9 was found in the epithelioid cell melanoma group. In the tumours, containing spindle cells, this index was more than 1.5 times lower (13.2) than that in the previous group. In the last group (mixed cells) the mean score of IP PCNA was insignificantly higher than that in the spindle cell type melanomas. The score of IP Ki-67 ranged from 0.2 to 27.6. The highest and the lowest values of IP Ki-67 were observed in similar proportions and in the same histological cell type tumours, like IP PCNA. In all the cases, the mean PCNA IP value was higher than the mean Ki-67 IP (Table 3). In more advanced tumours (pT3, pT4), IP PCNA and IP Ki-67 values were much higher than those in melanomas of grades pT1 and pT2.

Discussion

Different subjective methods, such as mitotic index and necrosis, which are used in determining the grade of tumour activity, are complemented by immunohistochemical analysis.. Karlsson et al. [6] have reported a high rate for Ki-67 and PCNA staining in 79 uveal melanomas. The activity of these nuclear markers was correlated with histopathological type and tumour size (large and epithelioid cell type tumours). Chowers et al. [3] have found a high incidence of Ki-67 positive cells in epithelioid cell melanomas too. The high levels of Ki-67 positivity were also

associated with shorter survival times. Liang et al. [8] have determined the score of Ki-67 index as 0.75 ±1.02. The IP Ki-67 was significantly higher in large tumours and in uveal melanomas that contained epithelioid cells. In our study, IP Ki-67 ranged from 0.6 to 27.6. The highest IP Ki-67 were found in epithelioid cell type. Our results of Ki-67 were higher than the ones reported in the literature because our five immunopositive melanomas were large (pT4). In the present study, PCNA immunostaining was more prominent than Ki-67 in all the cell types of uveal melanoma. According to Kayaseluck'a et al.[7], this feature may result from a long half-life of PCNA. Strong PCNA nuclear immunopositivity was observed in a large number of cells in 36 tumours. PCNA expression was the highest in epithelioid melanomas .This is consistent with other reports [4, 6]. In uveal melanomas, which contained epithelioid cells, local tumour recurrence (after local resection) was more often observed [1]. Some of the researchers concur with the opinion that Ki-67 is a more specific antibody to detect the proliferation index [7]. It is necessary to combine subjective methods, such as histology of tumours, with more objective methods, such as immunohistochemical analysis [4]. Evaluation of proliferating cell protein expressions (PCNA and Ki-67) in uveal melanoma cells may provide an additional information about the progression of tumour process.

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