

Differential Expression of the Retinoblastoma Gene Family Members in Choroidal Melanoma: Prognostic Significance¹

Mina Massaro-Giordano,² Gianluca Baldi,
Antonio De Luca, Alfonso Baldi,³ and
Antonio Giordano

Scheie Eye Institute, University of Pennsylvania, Philadelphia, Pennsylvania 19104 [M. M.-G.]; Istituto di Clinica Oculistica, Seconda Università degli Studi di Napoli, Naples 80131, Italy [G. B.]; and Departments of Pathology Anatomy and Cell Biology, Sbarro Institute for Cancer Research and Molecular Medicine, Jefferson Medical College, Philadelphia, Pennsylvania 19107 [A. D. L., A. B., A. G.]

ABSTRACT

We evaluated 55 samples of choroidal melanoma managed by enucleation. Knowing that the immunohistochemical expression of the retinoblastoma gene family members Rb/p105, p107, and pRb2/p130 was inversely correlated with the degree of malignancy in at least some histological types, we investigated the expression of these three proteins in choroidal melanoma. We focused on the relationship between patient survival and the immunohistochemical detection of the retinoblastoma proteins. No correlation with clinical outcome was found for Rb/p105 and p107. However, we found pRb2/p130 to be an independent prognostic factor correlating positively or directly with patient survival times and indirectly or inversely with the degree of malignancy. Demonstration of the prognostic value of the immunohistochemical expression of pRb2/p130 is of significance, even if additional studies are required to confirm these data and to compare the prognostic value of pRb2/p130 immunodetection to that of other recently proposed markers, such as p53.

INTRODUCTION

Choroidal melanoma is the most frequent intraocular malignancy in adults (1). Although the Callender classification and its modification (2, 3) are used to study the prognosis of patients with uveal melanoma after enucleation, an accurate prediction of survival in patients with choroidal melanoma is difficult (4–6). The etiology of the disease is unclear, and little research has been carried out to identify and analyze the molecular pathways involved in the development and prognosis of chori-

dal melanoma. We evaluated the expression of the retinoblastoma gene, one of the most well known tumor suppressor genes, and its relatives as possible prognostic factors for this malignancy.

The retinoblastoma gene family is composed of three members: (a) the product of the retinoblastoma gene (pRb), which is one of the most well-studied tumor suppressor genes; and (b) two related proteins, pRb2/p130 and p107, which have been shown to be structurally and functionally similar to pRb (7–9). The three retinoblastoma family members show growth suppressive properties, although the growth arrest mediated by each of the three pocket proteins is not identical (10, 11). This suggests that although the different members of the retinoblastoma gene family may complement each other, they are not fully functionally redundant. In a recent study of 71 lung cancers, using immunohistochemical techniques, we showed that these proteins display distinctive expression patterns when compared and contrasted to different parameters (12). Additional studies on pRb2/p130 expression in lung cancer (13) and in endometrial carcinomas (14) point out a tight inverse correlation between tumor malignancy and pRb2/p130 expression, thus suggesting an involvement of this protein in the development and progression of these diseases. With this information as background, we studied the immunohistochemical expression of the pRb2/p130 gene as well as the other two members of the retinoblastoma gene family in a large number of samples of choroidal melanoma.

The distinct levels of expression of the three proteins in relation to patient survival, as well as the clinical significance of a lack of or a decrease in their expression in the course of choroidal melanomas, is discussed.

MATERIALS AND METHODS

Specimen Profile. We evaluated 55 samples of choroidal melanomas treated by enucleation. The cases were selected from the Registry of Surgical Pathology of the Second University of Naples. To optimize our statistical analysis, we selected all of the cases obtained from patients who had not undergone either chemotherapy or radiotherapy before surgical enucleation; in addition, none of the selected melanomas showed extrascleral extension and/or metastatic spread at the time of the enucleation. There were 31 male and 24 female patients (mean age, 56 years).

The histological diagnoses and classifications were performed using the Modified Callender Classification (3), which contains three categories: (a) spindle cell melanomas; (b) mixed cell melanomas; and (c) epithelioid cell melanomas. All of the enucleated eyes had been formalin-fixed and sectioned along the longitudinal diameter, passing through the tumor and the optic nerve. The specimens were then processed through paraffin wax.

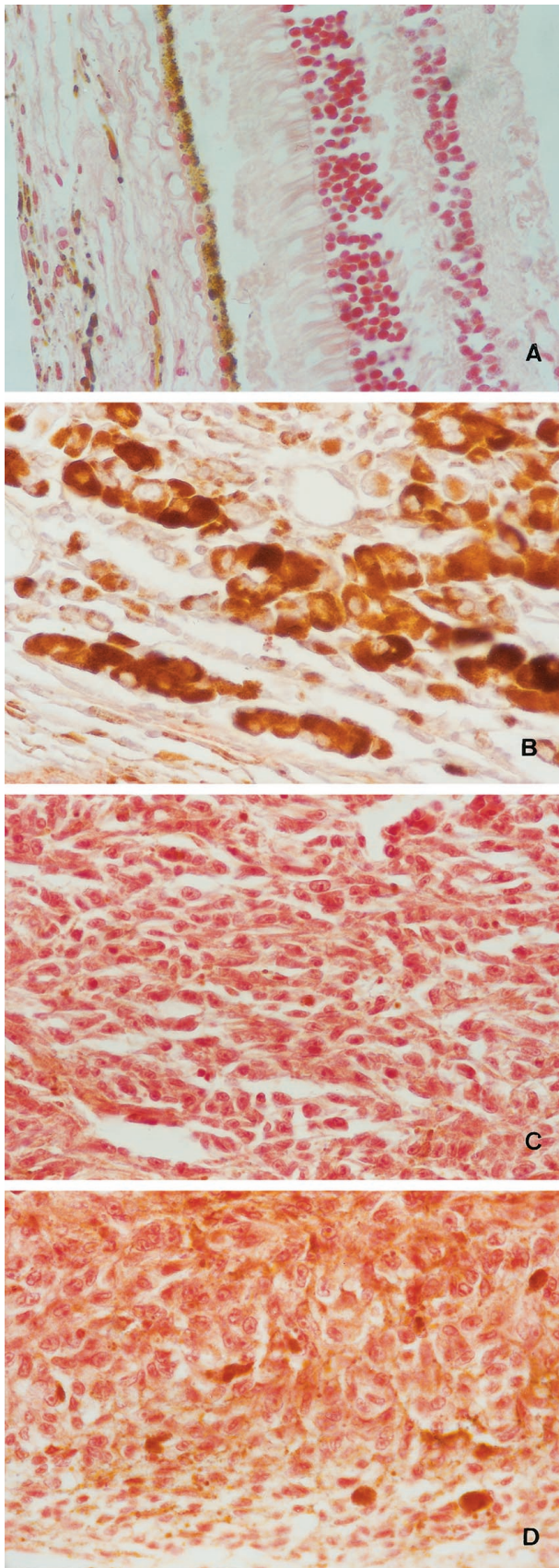
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² To whom requests for reprints should be addressed, at Scheie Eye Institute, 51 North 39th Street, Philadelphia, PA 19104. Phone: (215) 662-8100.

³ Present address: Campus BioMedico University, Rome, Italy.



Immunohistochemistry. Immunostaining for the retinoblastoma gene family members was performed using polyclonal antibodies ADL1 for pRb2/p130 and ADL2 for p107 and the monoclonal antibody XZ77 for Rb/p105 (12–14). The suitability for immunohistochemistry and the specificity of these antibodies have been described previously (12, 13). The APAAP⁴ conventional method was used as the final chromogene, and hematoxylin was used as the nuclear counterstain. The results were evaluated independently by three observers (G. B., A. B. and A. G.) and scored as follows based on the percentage of positive cells: (a) score 1, 0–30% positive cells; (b) score 2, 30–60% positive cells; and (c) score 3, >60% positive cells. The level of concordance, which was expressed as the percentage of agreement among the three observers, was 94.5% (52 of 55 specimens). In the remaining three specimens, the score was obtained from the judgements of the two observers who were in agreement.

Statistical Analysis. The Kaplan-Meier method (15) was used to estimate disease-free survival curves according to the immunohistochemical expression of retinoblastoma gene family members. The event used as an end point was death. The statistical significance of the difference between groups was assessed by the Mantel-Cox and Breslow tests. Multivariate Cox proportional hazards models stratified by histotype were used to assess pRb2/p130 expression in relation to survival. Additional models that controlled for DNA ploidy, PCNA expression, and tumor size were also used.

RESULTS

In normal eye samples, all members of the retinoblastoma gene family are expressed at high levels in all layers of the retina and choroid, with no significant differences between them (Fig. 1A). Considering the high degree of malignancy of melanoma, we looked carefully at the expression pattern of the retinoblastoma gene family members with well-described growth-suppressive properties (Fig. 1, B–D). pRb was expressed at a low to undetectable level in all of the examined specimens, except one in which a medium pRb expression level was detected. Unexpectedly, we found that p107 and pRb2/p130 were always expressed at different levels in these specimens. In summary, p107 showed a low expression level in 13 specimens (23.5%), a medium expression level in 22 specimens (40%), and a high expression level in 20 specimens (36.5%). pRb2/p130 also exhibited expression levels ranging from low (15 specimens; 27.2%) to medium (36 specimens; 65.5%) to high (4 cases; 7.3%). These data are summarized in Table 1.

⁴ The abbreviations used are: APAAP, alkaline phosphatase anti-alkaline phosphatase; PCNA, proliferating cell nuclear antigen.

Fig. 1 A, expression pattern of pRb2/p130 in normal retina and choroid. Choroid is on the left of the figure (APAAP, $\times 500$). B, negative expression of pRb/p105 in an epithelioid melanoma (APAAP, $\times 500$). C, high expression level of p107 in a spindle cell melanoma (APAAP, $\times 500$). D, high expression level of pRb2/p130 in a mixed cell melanoma (APAAP, $\times 500$).

Table 1 Expression of retinoblastoma gene family members in choroidal melanoma

Score ^a	pRb/p105	p107	pRb2/p130
1	54 (98%)	13 (23.5%)	15 (27.2%)
2	1 (2%)	22 (40%)	36 (65.5%)
3	0 (0%)	20 (36.5%)	4 (7.3%)

^a Expression score: 1, 1–30% positive cells; 2, 30–60% positive cells; 3, more than 60% positive cells. Numbers in parentheses represent the percentage of specimens achieving that particular score.

Statistical analysis was performed on the data to verify the relationship between the immunohistochemical detection of the retinoblastoma gene members and patient survival at 5 years after enucleation to evaluate the value of these factors in the prognosis of choroidal melanomas. The expression of neither pRb/p105 nor p107 was correlated with patient clinical outcomes. However, pRb2/p130 expression did correlate statistically with overall patient survival. Kaplan-Meier survival percentage curves based on a comparison between the survival (expressed in days) and the level of expression of pRb2/p130 was examined. The statistical significance of these data was measured by the Mantel-Cox test ($P = 0.0063$) and the Breslow test ($P = 0.0060$; Fig. 2).

In the multivariate Cox proportional hazards model stratified by histotype, pRb2/p130 was found to be significantly related to survival ($P = 0.02$). Additional models that controlled for DNA ploidy, PCNA expression, and tumor size did not provide a better fit to the data, and the significance of the effect of pRb2/p130 was reduced to a borderline significance in these models. Table 2 displays the final multivariate model for Rb2/p130.

DISCUSSION

The clinical course of patients with choroidal melanoma is capricious. Recent reappraisal of the widely used Callender system (3) has improved histological correlation with malignancy, but its value as a prognostic factor remains controversial. To obtain more accurate and reproducible prognostic information, other features are needed. In a previous study, we investigated the DNA content, PCNA level, and AgNOR count in the same cohort of patients, showing that each had a clinical and a significant influence on the survival of patients. However, each of these prognostic factors was strongly related to one or both of the classic prognostic factors: (a) tumor cell size; and (b) type (16). One of the problems in the management of choroidal melanomas is the lack of understanding of the biological factors that govern its behavior. Therefore, a careful analysis of the molecular elements responsible for the neoplastic phenotype in this kind of tumor is necessary to clarify the pathogenesis and the progression of choroidal melanomas. The analysis of the expression of the tumor suppressors belonging to the retinoblastoma gene family represents a first step toward this goal.

The role of members of the retinoblastoma gene family as tumor suppressors is well known (7). However, few studies have been reported dealing with altered patterns of Rb/p105, p107, and pRb2/p130 in some neoplasms. Altered pRb2/p130 expression in primary lung cancer has been associated with a high

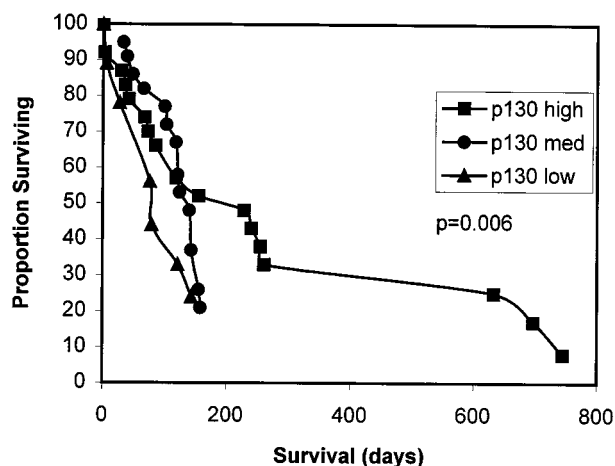


Fig. 2 Kaplan-Meier survival percentage curves showing that a higher expression of pRb2/p130 correlates with a better prognosis for patients with choroidal melanoma.

Table 2 Multivariate Cox proportional hazards models stratified by histotype

Variable	Risk ratio	Lower	Upper	P
pRb2/p130				
1	Baseline			
2	0.525	0.218	1.267	0.02
3	0.327	0.126	0.847	

proliferative index and with advanced tumor stages (12, 13). Studies of endometrial carcinomas confirm a relationship between altered expression of pRb2/p130 and the clinical course of the neoplasm (14). A recent study shows that the retinoblastoma members could be directly involved in the pathogenesis of human mesotheliomas (17).

Furthermore, genetic alterations disrupting the nuclear localization of Rb2 in human cell lines and primary tumors have recently been documented (18). Such mutations may reduce growth inhibition, probably due to insufficient levels of the protein in the nucleus. Furthermore, pRb2/p130 has been shown to have antiproliferative and tumor-suppressive potentials both *in vivo* and *in vitro* using a tetracycline-dependent overexpression system. A JCV-transformed tumor cell line injected into nude mice formed lesions that regressed in the presence of induced pRb2/p130 (19). Similar results were obtained in a study investigating the effects of pRb2/p130 expression in *c-erbB-2*-overexpressing SKOV-3 tumor cells (20).

Drawing on this background, the analysis of the expression of the retinoblastoma gene family members in choroidal melanomas was considered relevant. No statistically significant correlation was found between the expression of Rb/p105 and p107 and the clinical course of choroidal melanoma, at least in our group of specimens. The level of expression of Rb/p105 in these tumors was always very low, and the biological significance of this phenomenon remains unclear. However, when we statistically evaluated patient outcome and correlated it with pRb2/p130 expression, we found pRb2/p130 to be an independent

prognostic factor correlating with the overall survival time of the patients. Moreover, we compared the expression of Rb2/p130 with that of other well-established indicators (DNA content, PCNA level, and tumor size), showing that pRb2/p130 expression was the most significantly related to survival.

These results, as noted previously, are in agreement with previous work on lung and endometrial cancers (12–14). In the absence of a widely accepted prognostic marker for choroidal melanoma, demonstration of the prognostic value of a simple assay, such as the immunohistochemical analysis of pRb2/p130 status on routinely formalin-fixed, paraffin-embedded specimens, acquires a high value. However, additional studies are required for more accurate modeling of these data and to compare the prognostic value of pRb2/p130 immunodetection to other recently proposed markers, such as p53 (21).

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