Prognostic significance of PCNA grade in malignant melanoma

Proliferating cell nuclear antigen (PCNA) grade (a semi-quantitative assessment of the entire tumor profile) and estimation whether PCNA grade is low (0-50% positive cells) or high (51-100% positive cells) has been demonstrated to be of prognostic significance in gastric carcinoma. Sixteen vertical growth phase melanomas with 8 or more years of clinical follow-up were reacted with antibody to PCNA. PCNA grade was independently evaluated by both authors without knowledge of clinical outcome. PCNA grade was subsequently correlated with clinical outcome and other prognostic indicators. Agreement in assigning PCNA grade was noted between the two observers in 13/16 cases. However, PCNA grade failed to accurately predict patient survival or death. Additionally, PCNA grade was not significantly correlated with other recognized prognostic attributes. While interpretation of the prognostic significance of PCNA grade based upon this small series of cases should be conservative, a semi-quantitative estimate of PCNA-positive cells appears to have little utility in predicting the clinical outcome in malignant melanoma.

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Proliferating cell nuclear antigen (PCNA) is a 36 kD protein involved in DNA synthesis and repair. The molecule is phylogenetically highly conserved in terms of both structure and function (for review, see Ref. 1). Tight linkage to cell proliferation has led to the investigation of its role in the evaluation of tumors for prognosis. Indeed, the proliferative index assessed via PCNA immunohistochemistry appears to have a prognostic value in hemangiopericytomas (2), gastric carcinomas (3), gastrointestinal lymphomas (4), colorectal cancer (5), and soft tissue sarcomas (6). Takahashi et al. (7) noted a progressive increase in percent of PCNA positive cells in melanomas with increasing tumor thickness, but did not correlate PCNA staining with clinical outcome. Surprisingly, an apparent survival advantage for thick melanomas having high levels of PCNA expression for a given mitotic index has been noted (8). Both Takahashi et al. (7) and Evans et al. (8) estimated PCNA positivity by examining between 3 and 10 selected high-

power fields ($\times 400$), and made no attempt to quantify positivity over the entire tumor profile. Such a quantization protocol always contains the danger of selection bias and thus could lead to an over- or under-estimation of the average PCNA positivity. While it is possible to determine the PCNA index (PCNA-positive cells/1000 tumor cells) for the entire cross-sectional profile of a tumor, this becomes too time-consuming for most lesions. Thus, small, and possibly unrepresentative, areas must be selected for evaluation. Jain et al. (3) have adopted a different approach to the assessment of the PCNA-positive fraction of tumor cells in gastric carcinoma. They scan the entire tumor profile at low power ($\times 100$) and estimate whether 0-50% of tumor cells are positive (low PCNA grade) or 51-100% of tumor cells are positive (high PCNA grade). These authors observe that a high PCNA grade correlates with poor prognosis in gastric carcinoma. Using a similar approach, it was the objective of the study presented here to deter-

Fig. 1. Low PCNA grade. Less than 50% of the nuclei in the tumor profile mark with antibody to PCNA. A. $\times 40$. B. $\times 400$.

mine if PCNA grade is predictive of prognosis in malignant melanoma.

Material and methods

Sixteen vertical growth phase malignant melanomas having 8 or more yr of clinical follow-up were examined. PCNA immunohistochemistry using monoclonal antibody 19A2 (Coulter Laboratories, Hialeah, FL) and a biotin-streptavidin detection system (Biogenex Laboratories, San Ramon, CA) with aminoethylcarbazole as chromagen was performed as previously described (9). Tissue sections were lightly counterstained with hematoxylin. Prognostic factors recognized by the tumor progression model of Clark et al. (10) (tumor thickness, mitotic figures/mm², presence and extent of tumor-infiltrating lymphocytes, histologic regres-

sion, sex, and anatomic site) were also assessed. For each case, the entire vertical growth phase was examined at X100 and the fraction of PCNA-positive nuclei was estimated to fall into one of two classes: 0–50% PCNA-positive (low PCNA grade) or 51–100% PCNA-positive (high PCNA grade) (3). Any level of nuclear positivity was scored as positive. PCNA grade was determined independently by each observer without knowledge of clinical outcome. In cases of observer disagreement, each case was reviewed and consensus was obtained.

Results

Examples of tumors falling into low (Fig. 1A and B) and high (Fig. 2A and B) PCNA grades are demonstrated. Kappa statistical analysis shows a high level of agreement between the two inde-

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Results

Examples of tumors falling into low (Fig. 1A and B) and high (Fig. 2A and B) PCNA grades are demonstrated. Kappa statistical analysis shows a high level of agreement between the two inde-

Fig. 2. High PCNA grade. Greater than 50% of the nuclei in the tumor profile mark with antibody to PCNA. A. $\times 40$. B. $\times 400$.

pendent observers (Table 1) in assigning each tumor to either a high or a low PCNA grade. However, PCNA grade failed to correlate with clinical outcome (Table 2) or with attributes recognized to have prognostic significance (Table 3).

Discussion

PCNA grade can be independently assigned by two observers with a good level of agreement. However, in this small case series, the PCNA grade of a malignant melanoma did not correlate with patient survival or with any of the attributes recognized to have prognostic value in the model of Clark et al. (10). One might expect PCNA grade to better correlate with mitotic figure count. Our observation is in disagreement with Evans et al. (8) who note a strong correlation between percent of

tumor cells which are PCNA-positive and the number of mitotic figures/mm² of tumor profile. However, the method we used for mitotic figure counting (10) requires selection of the most mitotically-active portion of the vertical growth phase and does not provide the average mitotic frequency for the entire vertical growth phase. A recent report (11) described a trend toward greater PCNA positivity in malignant melanomas having either a greater depth of penetration or an advanced stage of progression. However, the PCNA scoring method used was different from that described above, most notably in that only a portion of the tumor profile was evaluated. These investigators did not correlate PCNA positivity with clinical outcome. Both Takahashi et al. (7) and Evans et al. (8) identified a significantly lower percentage of PCNA-positive cells in the melanomas they examined than did the

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Table 1. Reproducibility of scoring between two independent observers
Reproducibility of Scoring PCNA Grade

Code #	PCNA Grade	
	Observer 1 (JTW)	Observer 2 (DRD)
1	High	High
2	High	Low
3	High	High
4	Low	Low
5	High	Low
6	High	High
7	High	Low
8	Low	Low
9	High	High
10	Low	Low
11	Low	Low
12	High	High
13	Low	Low
14	Low	Low
15	Low	Low
16	Low	Low

Scoring concordance 13/16 (81%).

Kappa statistic = 0.625 (p-value = 0.0069).

present study. This may reflect, at least in part, different methodology employed in the immunohistochemical reaction (9).

Several investigators have urged caution in the interpretation of PCNA immunohistochemical staining patterns (see Ref. 1 for review). PCNA immunohistochemistry has been demonstrated to grossly overestimate the growth fraction of xenograft tumors grown from the LoVo cell line in nude mice when compared with the fraction of labelled mitoses method (11). The authors conclude that this could be most simply explained by a relatively short cell-cycle time and a long half-life for PCNA. Francis et al. (12) caution that PCNA immunohistochemistry overestimates growth fraction of cultured coronary arteries compared with autoradiography of [³H]thymidine pulse-labelled specimens. Darnton et al. (13) have noted that in two systems (low and high grade human lymphomas, normal and hyperproliferative rat gastric mucosa), double staining for PCNA and AgNORs (also considered a marker for cell proliferation when present in increased numbers) showed that many cells negative for PCNA contained increased numbers of AgNORs. Lack of correlation between PCNA staining of human colon carcinoma cells and bromodeoxyuridine staining, S-phase fraction, and survival has been demonstrated by de Goeij (14). Finally, del Giglio et al. (15) found that high levels of PCNA expression in adult patients with acute myelogenous leukemia did not correlate with the percentage of cells in S+G2+M measured by flow cytometry. Rather, higher levels of PCNA

expression correlated with decreased responsiveness to chemotherapy (possibly reflecting a hyperactive DNA repair mechanism in these cells). Support for the latter observation can also be found in the study by Takahashi and coworkers (7). They note that increased PCNA staining was observed in keratinocytes of the suprabasal layer, but not in the basal layer of normal skin exposed to sunlight, although it is the basal layer that is proliferatively active. This suggests that some of the PCNA reactivity observed may be due to DNA excision repair which is known to occur in cells irradiated with UV light (16, 17).

Therefore, PCNA immunostaining in melanomas may reflect a cohort of several "PCNA populations", PCNA expressed as a function of cell replication, PCNA redistributed as a function of DNA excision repair, and PCNA over/underexpressed due to alteration in the PCNA gene or deregulated PCNA transcription or translation. Deregulated PCNA transcription or translation was also suggested in cases where increased immunohistochemically detectable PCNA was observed in histopathologically normal breast lobules adjacent to breast tumors, as well as in pancreatic exocrine parenchyma adjacent to endocrine and exocrine tumors of the pancreas (18). In these cases, it was postulated that some of the tumors are actively secreting growth factors that are stabilizing the PCNA mRNA and thus inducing PCNA protein accumulation without actually inducing DNA synthesis.

We cannot be certain that the concept of PCNA grading will have no prognostic utility in other cutaneous neoplasms or that evaluation of a larger case series of melanomas will not reveal prognostic utility. However, this study and those cited above suggest that caution be applied to the interpretation of data from PCNA immunohistochemical studies. We join McCormick and Hall (19) in our concern that the marking of a cell nucleus by antibody to PCNA may be subject to interpretations other than simply that the cell is replicating.

Acknowledgments

We would like to thank Regula Candrian for providing excellent immunohistochemical stains.

Table 2. Correlation between PCNA grade and patient survival or death
Survival versus PCNA Grade

	Low	High
Alive	4	4
Dead	4	4

Fisher's exact test (p-value = 1.000).

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Table 3. Correlation between PCNA grade and other factors recognized to have prognostic significance in malignant melanoma

Thickness (mm) versus PCNA Grade

0.50	Low
0.60	High
0.70	Low
0.70	High
0.89	Low
1.00	High
1.00	Low
1.00	Low
1.20	High
2.40	High
2.90	Low
3.25	Low
3.30	Low
5.00	High
10.30	High
18.50	High

Spearman correlation test (p-value = 0.3886).

Sex versus PCNA Grade

	Low	High
Male	1	4
Female	7	4

Fisher's exact test (p-value = 0.282).

Tumor Infiltrating Lymphocytes versus PCNA Grade

	Low	High
Brisk	0	0
Non-brisk	4	5
Absent	4	3

Fisher's exact test (p-value = 1.000).

Regression versus PCNA Grade

	Low	High
Absent	7	6
Present	1	2

Fisher's exact test (p-value = 1.000).

Location versus PCNA Grade

	Low	High
Axial	7	4
Extremity	1	4

Fisher's exact test (p-value = 0.282).

Mitotic Rate versus PCNA Grade

	Low	High
0	3	1
0.1-6	2	3
> 6	3	4

Fisher's exact test (p-value = 0.674).

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