Advances in Brief

Prognostic Significance of Periodic Acid-Schiff-positive Patterns in Primary Cutaneous Melanoma

Michael A. Warso, Andrew J. Maniotis, Xue Chen, Dibyen Majumdar, Minu K. Patel, Anne Shilkaitis, Tapas K. Das Gupta, and Robert Folberg


Abstract

The patterns of periodic acid-Schiff (PAS) staining of extracellular matrix in histological sections of certain melanomas may be predictive of outcome. Recent in vitro and molecular genetic data suggest that the appearance of these patterns in both uveal and cutaneous melanoma is a function of aggressive tumor cells. We studied 96 patients with primary cutaneous melanomas treated at the University of Illinois at Chicago who were monitored for disease-free survival. Survival probabilities were determined by Kaplan-Meier estimates, and prognostic factors were evaluated by multivariate analysis. By univariate analysis, there was a significant decrease in disease-free survival among patients whose tumors contained parallel with cross-linking or network patterns (PXN; \( P = 0.0070 \)). Stepwise regression with Cox models that included the combinations of the PAS-positive patterns, tumor thickness, female gender, ulceration, and age yielded a model with thickness and the PAS-positive parallel with cross-linking or networks. Despite the relatively small sample size in this study, the detection of the PAS-positive parallel with cross-linking or networking in cutaneous melanoma was associated with a decrease in disease-free outcome. Additional studies of the prognostic significance of these patterns is warranted on larger data sets.

Introduction

A strong statistical association was established between the presence of PAS\(^3\)-positive patterns and outcome in primary uveal melanoma (1, 2). Specifically, the histological presence of closed loops of PAS-positive material encircling small clusters of tumor cells, networks (defined as at least three back-to-back loops), and cross-linking parallel PAS-positive linear structures was strongly linked to death from metastatic uveal melanoma. The identification of these patterns is reproducible (2–4), and the prognostic significance of these patterns has been confirmed by multiple independent groups (3, 5–8).

Recently, Maniotis \textit{et al.} (9) showed that aggressive primary and metastatic uveal melanoma cells reconstituted PAS-positive patterns \textit{in vitro} without the presence of endothelial cells, fibroblasts, or soluble growth factors; in contrast, less aggressive melanoma cell lines did not generate these patterns \textit{in vitro}. The generation of these patterns was, therefore, linked to the aggressive tumor cell phenotype, and recently it was suggested that these patterns may reflect a profile of deregulated gene expression (10). Microinjection experiments demonstrated that portions of the \textit{in vitro} tumor cell-generated patterns were capable of conducting dye (9). Mueller \textit{et al.} [Ref. 11; and subsequently others (12, 13)] suggested that the PAS-positive patterns seen in tissue sections contribute to a functional microcirculation in these tumors. This interpretation was based on the histological data showing anastomoses between these patterns and blood vessels (14, 15) and a correspondence between the \textit{in vivo} confocal angiograms and corresponding histological observations of these tumors. The \textit{in vitro} reconstitution of matrix patterns from the tumor cells was termed “vasculogenic mimicry” (16), because, like vasculogenesis, the channels were generated \textit{de novo}; however, unlike vasculogenesis, the pattern channels were apparently generated by tumor cells rather than endothelial cells.

Some have questioned whether the PAS-positive patterns described by Folberg \textit{et al.} (2) contribute to a functional microcirculation, however, there is a consensus even among those who are critical of the concept of vasculogenic mimicry (17) that the prognostic significance of PAS-positive patterns described by Folberg \textit{et al.} (2) is valid. Maniotis \textit{et al.} (9) suggested that metastatic cutaneous melanoma formed these patterns \textit{in vitro}; histologically, looping PAS-positive patterns have been demonstrated in both primary (15) and metastatic cutaneous melanoma anecdotally (9, 18). Although one group has studied the prognostic significance of these patterns in conjunctival melanoma (19), there are no studies, to date, on the prognostic significance, if any, of these patterns in cutaneous melanoma. This study was designed to explore the prognostic significance of these patterns in cutaneous melanoma.
significance of the PAS-positive patterning in primary cutaneous melanoma on DFS and to investigate the significance of patterning relative to other established prognostic features such as thickness and ulceration.

Materials and Methods

Patient Material. The Department of Surgical Oncology at the University of Illinois maintains a database of all melanoma patients. This database contains all of the clinical and pathological records, methods of treatment, and outcomes. The original slides or blocks of the primary tumor, whenever possible, are stored in our melanoma tissue and sera bank. From this database, the patients for whom unstained slides of the primary melanoma were available are included in this study. The institutional review board of the University of Illinois at Chicago has reviewed the maintenance of the databases and tissue and sera bank.

Light Microscopy. Slides for evaluation of paraffin sections were cut at a thickness of 5 μm. These were stained with PAS without a hematoxylin counterstain (1). The PAS-positive patterns were highlighted under a green filter, and investigators (A. J. M., X. C., and R. F.), who were masked to outcome, examined them for the presence or absence of the previously described PAS-positive patterns (2): parallel patterns without and with cross-linking, arcs without and with branching, loops, and networks (Fig. 1, A–C).

Data Analysis. All statistical evaluations were performed using SPSS or SAS software. The presence or absence of each of the above-described patterns was noted for each case and tested against the outcome measure of DFS, defined as free of all evidence of melanoma from the time of diagnosis to follow-up. The primary outcome measure was DFS, which was investigated both by Kaplan-Meier survival curves and by Cox modeling. Kaplan-Meier survival curves were plotted for the data set according to the presence or absence of PAS-positive patterns appearing individually or in combination. Relationships between patterns and other known prognostic features, such as tumor thickness and ulceration, were tested with the $t$, $\chi^2$, and Fisher’s exact tests. The relative importance of PAS-positive patterns and conventional melanoma characteristics was determined by multivariate analysis with Cox’s proportional hazards model (20) using stepwise selection. In constructing Cox models, we permitted all conventional prognostic cutaneous melanoma characteristics to enter the models: tumor thickness, ulceration, sex, and age (21), together with each of the patterns individually and in combination. For all tests, the level of significance was set at $P = 0.05$.

Results

The investigators identified one histological pattern that was not encountered in uveal melanoma: the presence of circu-
lar packets of melanoma cells in the dermis that were surrounded, at least in part, by dermis (Fig. 1D). Because of their spheroidal shape, these dermal packets may be confused with looping patterns that form networks that are prognostically very significant in uveal melanoma. By contrast, in the looping pattern, PAS-positive matrix subdivides the cellular compartment of the tumor such that tumor cells are present on both sides of matrix partition (Fig. 1, compare D with C). It is of interest that uveal melanomas seldom incorporate a significant quantity
of fibrous stroma unless there has been previous radiation treatment or necrosis.

Biopsy samples from 96 patients (56 men and 40 women) were studied for DFS. Forty-four patients experienced recurrence. A comparison of baseline characteristics between patients who did and did not experience recurrence is summarized in Table 1. Kaplan-Meier survival statistics (22) were tested for each pattern individually and in combination. The only pattern that indicated a significant separation in disease-free outcome was networks. Among the combination of patterns tested, the presence of either the network or the parallel with cross-linking substantially improved the statistical separation (Table 2). The Kaplan-Meier curve for this combination (PXN) is shown in Fig. 2 and indicates that the group of patients whose tumors contained these PAS-positive patterns had significantly lower DFS probabilities than the group of patients whose tumors did not contain these patterns (Fig. 2; log rank $\chi^2 = 7.2607; P = 0.0070$).

Tumors that contained parallel with cross-linking or networks tended to be thicker (mean thickness for: parallel with cross-linking or networks absent, 1.96; parallel with cross-linking or networks present, 2.77; $t = 3.11, P = 0.0025$) and ulcerated (17.5% ulceration for parallel with cross-linking or networks absent, and 41.8% ulcerated for parallel with cross-linking or networks present; $\chi^2 = 6.34, df = 1, P = 0.0118$). In developing Cox models, each of the PAS-positive patterns was a component together with the tumor characteristics of thickness, sex, ulceration, and age. In this small data set, when PAS-positive patterns were excluded from entering the model and only "conventional" tumor characteristics were allowed entry (thickness, sex, ulceration, and age), the stepwise model contained only thickness. We tested the entry of each PAS-positive pattern in isolation and in combination, and the only pattern that appeared in a stepwise Cox regression model was the combination of networks and parallel with cross-linking (PXN). In fact, when PAS-positive parallel with cross-linking or

### Table 1 Comparison of patient and tumor characteristics between patients who did and did not experience recurrence

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Recurrence (n = 44)</th>
<th>No recurrence (n = 52)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean = 55 (95% CI = 50.65, 59.35) Median: 57.5 (range, 26–76)</td>
<td>Mean = 51.88 (95% CI = 46.84, 56.93) Median: 52 (range, 18–81)</td>
<td>0.3586</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 26 (59.1%) Female 18 (40.9%)</td>
<td>30 (57.7%) 22 (42.3%)</td>
<td>0.8898</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>mean = 2.55 (95% CI = 2.23, 2.87)</td>
<td>mean = 2.33 (95% CI = 1.87, 2.80)</td>
<td>0.4439</td>
</tr>
<tr>
<td>Ulceration</td>
<td>Absent 27 (61.4%) Present 16 (36.4%)</td>
<td>38 (73.1%) 14 (26.9%)</td>
<td>0.2830</td>
</tr>
<tr>
<td>PAS-positive patterns present</td>
<td>Straight 6 (13.6%)</td>
<td>11 (21.2%)</td>
<td>0.3364</td>
</tr>
<tr>
<td></td>
<td>Parallel without cross-linking 8 (18.2%)</td>
<td>12 (23.1%)</td>
<td>0.5562</td>
</tr>
<tr>
<td></td>
<td>Parallel with cross-linking 8 (18.2%)</td>
<td>9 (17.3%) 0.9110</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arcs without branching 32 (72.7%)</td>
<td>34 (65.4%) 0.4393</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arcs with branching 32 (72.7%)</td>
<td>33 (61.1%) 0.3333</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Loops 31 (70.5%)</td>
<td>30 (57.7%) 0.1955</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Networks 29 (65.9%)</td>
<td>25 (48.1%) 0.0793</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parallel with cross-linking or networks 31 (70.5%)</td>
<td>25 (48.1%) 0.0267</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dermal packets 24 (54.5%)</td>
<td>25 (48.1%) 0.5276</td>
<td></td>
</tr>
</tbody>
</table>

* CI, confidence interval.

Ulceration was missing for one observation in the data set.

### Table 2 Kaplan-Meier analysis for PAS-positive patterns

<table>
<thead>
<tr>
<th>PAS-positive patterns</th>
<th>Log-rank $\chi^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Straight</td>
<td>0.1948</td>
<td>0.6590</td>
</tr>
<tr>
<td>Parallel without cross-linking</td>
<td>0.8648</td>
<td>0.3524</td>
</tr>
<tr>
<td>Parallel with cross-linking</td>
<td>2.2256</td>
<td>0.1357</td>
</tr>
<tr>
<td>Arcs without branching</td>
<td>1.8124</td>
<td>0.1782</td>
</tr>
<tr>
<td>Arcs with branching</td>
<td>1.8956</td>
<td>0.1686</td>
</tr>
<tr>
<td>Loops</td>
<td>3.0098</td>
<td>0.0828</td>
</tr>
<tr>
<td>Networks</td>
<td>5.6335</td>
<td>0.0176</td>
</tr>
<tr>
<td>Parallel with cross-linking or networks</td>
<td>7.2607</td>
<td>0.0070</td>
</tr>
<tr>
<td>Dermal packets</td>
<td>0.0284</td>
<td>0.8663</td>
</tr>
</tbody>
</table>

![Fig. 2 Kaplan-Meier survival curve for DFS of patients with primary cutaneous melanoma: comparison of patients whose tumors contained versus lacked parallel with cross-linking or network PAS-positive patterns (PXN).](image-url)
networks were allowed to enter the model, the stepwise Cox regression model contained only thickness and parallel with cross-linking or networks (Table 3).

**Discussion**

It is generally assumed that in cutaneous melanoma the presence or absence of metastases to the regional lymph nodes is the single most significant prognostic indicator (23–25). Second to this, the thickness and presence or absence of histologic evidence of ulceration in the primary lesion(s) constitutes the most important prognostic markers (26). However, in a substantial subset of these patients, the regional lymph nodes are not involved. Therefore, presently thickness and ulceration are being considered as leading prognosticators in node-negative patients, and the American Joint Committee on Cancer has taken these microscopic features as the means of staging primary melanoma (21). With increasing evidence that these two parameters are not accurate predictors of the natural history of cutaneous melanoma as has been assumed (27, 28), a number of investigators have attempted to identify additional significant prognostic features.

The results obtained in this study are consistent with four previous observations. First, in a study of 234 patients treated for uveal melanoma, parallel with cross-linking and networks were independently associated with an adverse outcome (2). Second, when studying a smaller data set of patients with uveal melanoma, Anastassiou et al. (8) discovered a relationship between the presence of either the parallel with cross-linking pattern or networks and outcome. Third, in vitro observations have indicated that aggressive melanoma cells make PAS-positive patterns whereas nonaggressive cells do not (9). Aggressive melanoma cells make not only loops and networks, but also cross-linking parallel patterns (29), suggesting that each of these patterns in tissue sections reflects the presence of an aggressive tumor cell phenotype. Finally, the appearance of these architectural characteristics seems to reflect a distinctive gene expression (10).

In this relatively small sample of patients with primary cutaneous melanoma, the presence of parallel with cross-linking or networks carried prognostic significance: patients whose tumors contained either of these patterns had a worse outcome than patients whose tumors lacked these patterns. The stepwise Cox model suggests that both thickness and the presence of parallel with cross-linking or networks exert an independent adverse effect on DFS.

The patients were drawn from the research database of the Department of Surgical Oncology at the University of Illinois. They are patients for whom the clinical data are available and from whom representative sections of the primary tumor could be obtained. As such, they do not necessarily represent a cross-section of the melanoma population. Additional studies on the prognostic significance of these patterns in primary cutaneous melanoma are warranted.

**Table 3** Cox proportional hazards model analysis of prognostic factors in primary cutaneous melanoma for DFS

<table>
<thead>
<tr>
<th>Variable</th>
<th>DF</th>
<th>Parameter estimate</th>
<th>SE</th>
<th>Wald χ²</th>
<th>P</th>
<th>Hazard ratio</th>
<th>Lower 95% CL</th>
<th>Upper 95% CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>PXN a</td>
<td>1</td>
<td>0.73064</td>
<td>0.33966</td>
<td>4.6274</td>
<td>0.0315</td>
<td>2.076</td>
<td>1.067</td>
<td>4.040</td>
</tr>
<tr>
<td>Thickness</td>
<td>1</td>
<td>0.31362</td>
<td>0.12565</td>
<td>6.2296</td>
<td>0.0126</td>
<td>1.368</td>
<td>1.070</td>
<td>1.751</td>
</tr>
</tbody>
</table>

a −2logL, DF = 2, model χ² = 11.7669 (P = 0.0028); DF, degrees of freedom; SE, standard error; PXN, parallel with cross-linking, or networks.

**References**


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