Significance of DNA Abnormalities in Primary Malignant Melanoma and Nevi, a Retrospective Flow Cytometric Study

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ABSTRACT

DNA ploidy of melanocytic skin tumors from 87 patients (53 primary melanomas, 34 nevi) was determined by flow cytometry from routinely prepared paraffin blocks. Ploidy data correlated strongly with conventional morphological parameters. Only 1 of 34 nevi, but 13 of 53 melanomas were aneuploid. Among the melanomas, none of 21 levels I–III melanomas was aneuploid, but 13 of 32 levels IV and V melanomas were aneuploid. There was also a significant correlation between increasing Breslow thickness and the presence of DNA aneuploidy. For 33 melanoma patients with over 2 yr of follow-up (average, 7.1 yr), only 4 of 23 diploid tumors have recurred, but 9 of 10 aneuploid tumors have recurred. We conclude that the biological potential of melanocytic skin tumors is strongly linked to DNA aneuploidy. Since this parameter can be conveniently determined from paraffin blocks, determination of ploidy abnormalities in these tumors may be clinically useful.

INTRODUCTION

The prognosis for patients with Stage I cutaneous malignant melanoma is currently projected from various morphological criteria, none of which has ideal predictive usefulness. Parameters reported to have prognostic significance include lesion thickness, level of invasion, mitotic index, presence of ulceration or regression, microscopic satellite lesions, host inflammatory cell response, growth pattern, and blood vessel invasion (1–10). Lesion thickness has been identified as the single most important prognostic factor in Stage I cutaneous malignant melanoma (11). The prognostic value of various combinations of morphological parameters has also been assessed, but it is unclear which combination of factors is most useful. It is clear, however, that morphological parameters are not always adequate to predict the biological behavior of melanoma, particularly for lesions of intermediate thickness (12).

Abnormal DNA content (DNA aneuploidy), as detected by FCM, has emerged as a significant diagnostic and prognostic parameter in several solid tumors (13–15). Application of flow cytometry to melanocytic skin tumors might provide useful prognostic information in patients with uncertain risk of recurrence, provided the specimen preparation could be made simple and convenient. We sought to determine whether ploidy could be determined from routine paraffin-embedded melanoma specimens by FCM and whether ploidy data correlated with established morphological parameters and clinical course.

MATERIALS AND METHODS

Patient Selection. The study material consisted of a single specimen from each of 57 patients with primary cutaneous malignant melanoma and 38 patients with melanocytic nevi. All patients with Stage I primary cutaneous malignant melanoma seen at Rush-Presbyterian-St. Luke’s Hospital between 1969 and 1985 were included in this study if the paraffin block from the primary lesion was available for FCM. All tissue had been fixed in buffered formalin. Melanocytic nevi were selected randomly from the pathology files.

Histopathological study of hematoxylin and eosin stained sections of each case was carried out by one of us (S. K.) without knowledge of the FCM data. All melanomas were morphologically evaluated as to their Clark’s level (16), Breslow’s thickness (17), and presence of ulceration and regression.

Follow-up data were obtained through patient follow-up and review of hospital and office charts.

Sample Preparation. Blocks to be processed for FCM were chosen to be representative of the tumor and to contain a volume of tumor tissue adequate for processing. Only one block was processed for each tumor. Preparation of the blocks for FCM was accomplished with a modification of the method of Hedley et al. (18). In brief, thick sections (30 μm) from the formalin-fixed tissue blocks were dewaxed, rehydrated, and mechanically minced with a scissors. The tissue slurry was then digested with 0.5% pepsin (Sigma, St. Louis, MO), in 0.9% NaCl at pH 1.5 for 30 min at 37°C to produce a suspension of bare nuclei. The suspension was washed, then filtered through a 41-μm nylon mesh to remove aggregates, and the concentration was adjusted to 2 × 10^6 nuclei/ml. The nuclei were then incubated for 10 min with RNase A, 0.1 mg/ml (Sigma), then stained for DNA with propidium iodide (Sigma) at 0.13 mg/ml for 15 min, and analyzed by FCM within 1 h of staining.

FCM Analysis. Nuclei were adjusted to a concentration of 5 × 10^6/ml in dimethyl sulfoxide-citrate buffer for FCM analysis as described previously (19). The relative DNA content (red fluorescence) of 10^5 nuclei was analyzed in a Coulter Epics V (Hialeah, FL) flow cytometer equipped with a coherent (Worcester, MA) argon ion laser operating at 200 mW at 488 nm. All tumor samples contained diploid inflammatory and stromal cells which served as an internal diploid standard as previously described (19). The high voltage to the photomultiplier tube was adjusted so that the G1/G0 peak of the diploid cell was located in channel 50 of 256 total channels. Nondiploid populations were defined by the presence of discrete G1/G0 populations differing from diploid by at least 10%. The DNA index for nondiploid populations was calculated as the quotient of the channel of the nondiploid peak divided by the channel of the diploid peak.

Statistical Methods. For comparison of data the χ² test was used. Significance was assessed at P < 0.05.

RESULTS

Thirty-eight nevi and 57 melanomas were examined by light microscopy. Adequate cell counts (>10^6) after tissue preparation were present in all but one specimen. Samples with adequate cell counts were analyzed by FCM. Seven specimens produced uninterpretable DNA histograms, defined as having no peak with a coefficient of variation less than 10%. The remaining samples, 34 nevi and 53 melanomas, form the basis of this report. Representative examples of DNA histograms of nevi and melanomas are shown in Fig. 1.

Thirty-four nevi were analyzed. An aneuploid cell population (DNA index, 1.3) was found in only one specimen, a compound nevus which even in retrospect showed no atypia or other...
FCM OF MELANOMAS

3 as level V lesions. None of the levels I—III melanomas contained aneuploid cell populations. Ten of the 29 level IV and all 3 of the level V lesions were aneuploid. The correlation between level and the presence of aneuploid cell populations was significant (P < 0.001). The presence of aneuploid cell populations also correlated with Breslow thickness (Table 1). The “thin” lesions, those measuring less than 0.76 mm, were a homogenous group. All 12 cases in this group contained only diploid cell populations. As the lesion thickness increased, the percentage of cases containing aneuploid cell populations also increased. In lesions measuring greater than 3.0 mm, 5 of 6 cases (83%) contained aneuploid cell populations. There was a significant correlation between increasing Breslow thickness and the presence of aneuploid cell populations (P < 0.005).

Ulceration was noted in 12 of the 53 melanomas. Microscopic ulceration measuring >3 mm was identified in 5 of 40 diploid samples and in 7 of 13 samples containing aneuploid DNA patterns. There was a significant correlation between ulceration and the presence of aneuploid cell populations (P < 0.005). Regression was seen in 8 of the 53 melanomas. All of these cases contained only diploid cell populations.

Thirty-three of the patients with melanoma had follow-up of greater than 2 yr with an average follow-up time of 7.1 yr (range, 2–18 yr). In this group of 33 patients, 10 patients had lesions with aneuploid populations. Nine of those 10 patients have recurred. Only 4 recurrences have been seen among the 23 patients with diploid DNA histograms. The correlation between the presence of aneuploid cell populations and recurrence was statistically significant (P < 0.0005). This significant association was upheld when the study group was evaluated by level (Table 2) (P < 0.001) or by lesion thickness (Table 3) (P < 0.01). Looking specifically at the 21 patients with intermediate thickness lesions (0.76–3.0 mm), a significant correlation between recurrence and the presence of aneuploid cell populations was again identified (P < 0.01). Recurrences were seen in 6 of 7 patients with aneuploid populations and in 4 of 14 patients with diploid DNA distributions.

DISCUSSION

This study demonstrates that the cellular DNA content of cutaneous malignant melanoma can be analyzed with FCM distinguishing histological features (Fig. 2). Of the 53 melanomas evaluated, 13 contained DNA aneuploid cell populations. Multiple aneuploid populations were identified in two specimens, a level V melanoma measuring greater than 4 mm in depth and a level IV lesion measuring 2.8 mm. The frequency of aneuploid DNA patterns was significantly greater in primary malignant melanoma than in melanocytic nevi (P < 0.025).

Conventional histological parameters for evaluation of melanoma, including level, lesion thickness, ulceration, and presence of regression were correlated with FCM results. There were 21 melanomas classified as levels I–III, 29 as level IV, and
Table 1 Correlation between lesion thickness and ploidy

<table>
<thead>
<tr>
<th>Lesion thickness (mm)</th>
<th>Total no. of patients</th>
<th>Diploid no.</th>
<th>Aneuploid no.</th>
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<td>17</td>
<td>4</td>
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<tr>
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<td>7</td>
<td>5</td>
<td>2</td>
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<tr>
<td>2.26–3.0</td>
<td>7</td>
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<tr>
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Table 2 Correlation between level of invasion, ploidy, and recurrence

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<th>Level</th>
<th>Total no. of patients</th>
<th>State of ploidy</th>
<th>No. of occurrences</th>
<th>Recurrences</th>
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<td>Diploid</td>
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<td></td>
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<td></td>
<td>Aneuploid</td>
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Table 3 Correlation between lesion thickness, ploidy, and recurrence

<table>
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<tr>
<th>Thickness (mm)</th>
<th>Total no. of patients</th>
<th>State of ploidy</th>
<th>No. of occurrences</th>
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<td>Aneuploid</td>
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using paraffin-embedded material. The laboratory methods used in this study, a modification of the technique described by Hedley et al. (18), led to interpretable DNA histograms in 92% of the cases analyzed. This was true in spite of the fact that the tissue samples were often quite small. A good correlation between the DNA histograms produced using this technique and those obtained with fresh tissue from the same tumor have been seen in Hedley’s laboratory (18) as in our own (19); furthermore, we have compared the paraffin method with conventional cytogenetic analysis for detection of aneuploidy in bladder biopsies and found high (87%) agreement (19). This method provides a relatively simple, reproducible, and objective means of evaluating DNA ploidy characteristics of melanoma. It allows for retrospective analysis of pathologic material. This may allow a fairly rapid definition of the prognostic relevance of aneuploidy in malignant melanoma.

Although DNA aneuploidy has been found to be diagnostic of malignancy in several organ systems (13, 20) we identified an abnormal DNA histogram in one of 34 benign nevi. Complete processing and evaluation of this aneuploid sample was repeated with identical results. Similarly, Sondegaard found 4 of 16 nevi to be aneuploid as determined by flow cytometric DNA analysis (21). He noted that “histologically all nevi were undoubtedly benign” as was the case with the aneuploid nevus in our series (21). Brüchner et al. (22) analyzed the DNA content of 422 benign melanocytic skin lesions. DNA aneuploidy using FCM analysis was identified in 4 of 46 congenital nevi and in 0 of 376 acquired nevi. Stenzinger and his colleagues have suggested that DNA aneuploidy is an indicator of a premalignant condition in congenital nevi (23). Although our data and that of other investigators indicate that DNA aneuploidy is unusual in unequivocally benign melanocytic skin lesions, it appears that abnormal DNA content should not be used as a specific criterion of malignancy in these lesions.

The aneuploidy rate among malignant melanomas in this study was 25%. This is significantly less than the percentage of aneuploid melanomas reported by other series which studied fresh rather than paraffin-embedded tissue. Much of the discrepancy appears to be attributable to differences in our patient population compared to others. Only stage I cutaneous malignant melanomas, including many thin lesions, were evaluated in this study. We were able to study this unique but highly relevant population because we used archival, paraffin-embedded material unlike most other studies, where difficulty in obtaining adequate fresh tissue apparently limited the analysis to lesions of greater depth and/or stage which are more likely to be aneuploid. For example, Frankfurt et al., (24) reported abnormal DNA histograms in 48 of 61 cases of melanoma; however, only 5 of the 61 samples were taken from the primary site, the others being metastases. Brüchner et al., (22) reported the results of flow cytometric DNA analysis in 721 melanomas. Of 230 primary tumors, abnormal DNA histograms were identified in 125 (54.3%) whereas 466 of the 491 metastatic tumors (94.9%) contained aneuploid populations. The stage of each patient was not reported. Only 41% of lesions less than or equal to 1.50 mm thick were aneuploid. Sondegaard et al. (21) found 26 of 35 primary malignant melanomas to be aneuploid by flow cytometry. Here too, the discrepancy in frequency of aneuploidy is most likely due to a significantly greater number of thin and low level lesions in our series. Sixty-two % of our patients’ melanomas measured less than or equal to 1.50 mm thick were aneuploid. Sondegaard et al. (21) found only 20% of the primary lesions were of this depth. In summary, the difference in rate of aneuploidy between our series and others may be significant but does not appear large if differences in the patients’ stage and lesions studied are considered. Whether study of paraffin-embedded material intrinsically yields a somewhat lower frequency of aneuploidy than does analysis of fresh tissue could only be conclusively determined by parallel determinations using both methods; unfortunately, this would be difficult for small lesions.

The presence of aneuploid DNA content correlated strongly with conventional prognostic features of melanoma. Lesion thickness is the single most important histological parameter for the assessment of prognosis in cutaneous malignant melanoma. We found a significant correlation between DNA aneuploidy and lesion thickness as well as with the level of invasion. Sondegaard et al. (21) reported a correlation between the presence of aneuploid DNA populations and histological features suggestive of a poor prognosis, such as high mitotic activity, nuclear pleomorphism, large nucleoli, and lesions greater than 2.25 mm in depth; however, he did not find a significant...
correlation between level of invasion and ploidy. The number of primary lesions evaluated in his study was small (35 cases). This may, at least in part, explain this difference.

Not only did we identify a correlation between conventional morphological parameters and ploidy, but we found a significant correlation between aneuploidy and the likelihood of recurrence. Among the 33 patients with 2 years or more of follow-up, aneuploid populations were identified in 10 cases. Nine of those 10 patients have recurred. In the 23 patients with DNA histograms showing only diploid populations, 4 patients have developed recurrent disease. This is consistent with the data of Brüchner et al. (22) which showed a 3-fold increase in the 2-yr recurrence rate in patients with primary lesions with abnormal ploidy characteristics as compared to patients with tumors with normal DNA histograms.

Morphological parameters frequently do not reflect biological behavior in patients with intermediate thickness lesions (12). Evaluation of the clinical course in these patients on the basis of thickness and ploidy suggests that the latter may add important prognostic information, since DNA aneuploidy was significantly associated with recurrence in this group.

Although the patient population we have studied so far is relatively small, the data suggest that there is a need to define the role of FCM DNA analysis in the determination of prognosis in melanoma patients. This is of particular interest in patients with intermediate thickness lesions and uncertain risk of recurrence. To this end we have undertaken a large retrospective study to assess the usefulness of ploidy data for prognostication and to compare its value with the many already recognized clinical and histopathological parameters used to predict clinical course in patients with melanoma.

REFERENCES


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