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RELATION OF CYTOGENETIC ABNORMALITIES AND CLINICAL OUTCOME IN METASTATIC MELANOMA

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Abstract The value of chromosomal analysis is well established in human hematologic neoplasms. In contrast, the relation between chromosomal abnormalities and clinical outcome in solid tumors in humans has received little study. We undertook this study to determine whether chromosomal abnormalities could provide information on the survival of patients with malignant melanoma.

Chromosome-banding analysis was performed on tumor-biopsy samples from 62 patients with metastatic melanoma, and recurring cytogenetic abnormalities were correlated with survival. Patients with structural abnormalities of chromosome 7 or 11 had significantly shorter survival than patients without these abnormalities. We conclude that cytogenetic analysis may provide useful prognostic information about patients with metastatic melanoma. (*N Engl J Med* 1990; 322:1508-11.)

CYTOGENETIC analysis plays an integral part in the diagnostic workup of a number of hematologic neoplasms. In addition to the convincing association of specific chromosomal rearrangements with distinguishing hematologic features, chromosomal abnormalities have been conclusively demonstrated to be of prognostic value in patients with leukemias and lymphomas.¹⁻³ Furthermore, molecular examination of specific chromosomal abnormalities has pinpointed the site of genes important in the process of growth regulation (cellular oncogenes).^{4,5}

Despite the fact that solid tumors are far more common and contribute more to morbidity and mortality than hematologic neoplasms, much less is known about the specificity and importance of the chromosomal abnormalities in solid tumors (e.g., carcinomas, sarcomas, and melanomas). This has resulted primarily from technical difficulties in analyzing the karyotypes of solid tumors because of their low mitotic index, their complex karyotypes, and the often suboptimal morphology of the chromosomes. Recently, there have been important advances in identifying consistent cytogenetic abnormalities in solid tumors,^{6,7} and the correlations between cytogenetic and morphologic abnormalities appear to be as

specific for these tumors as for hematologic neoplasms.

The present study was undertaken to determine the distribution of clonal chromosomal abnormalities and to correlate abnormal karyotypes with clinical information in patients with metastatic melanoma. Our analysis has revealed that selected cytogenetic abnormalities are predictive of clinical outcome.

METHODS

Cells for cytogenetic analysis were obtained during clinically indicated biopsies in accord with protocols approved by the University of Arizona Human Subjects Committee. When specimens were received, the clinical information about each case was reviewed to ascertain whether the case met the eligibility criteria for inclusion in our clinical data base. For cases that met the eligibility criteria, more complete clinical information was sought from the medical records. The common three-stage melanoma staging system was applied, in which Stage I indicates localized melanoma, Stage II regional metastases, and Stage III distant metastases.⁸ Because the data base is updated regularly, we periodically "close out" the data base and concentrate on the analysis of the resulting subset of patients until the next periodic close out. When the melanoma data base contained material on 62 patients, we created a file for our initial analysis. In this report we present the results of our analysis of these 62 patients.

Follow-up data were available on all 62 patients. The study included 30 female and 32 male patients whose mean age was 45.9 years (range, 15.8 to 76.0). On the basis of clinical staging, 21 patients had Stage II and 41 had Stage III disease. Twenty-two patients in this study had received chemotherapy before the biopsy sample was obtained for cytogenetic analysis. The site of the biopsy was as follows: lymph node (32 patients), skin (23), viscera (3), brain (2), lung (1), and bone (1).

The harvesting and banding techniques used for the chromosomes have been described previously.⁹ Briefly, tumors were mechanically disaggregated and placed in specialized growth medium M15¹⁰ for 48 to 96 hours. In the final two hours of culture, demecolcine (Colcemid; 0.05 μ g per milliliter) was added, the cells were exposed to a hypotonic solution (0.075 M potassium chloride) for 30 minutes at 37°C and fixed in methanol-glacial acetic acid (3:1), and air-dried slides were prepared. Metaphase cells were G-banded with a modified Wright-staining protocol that has been described elsewhere.⁹ Chromosomal abnormalities were classified according to the International System of Human Cytogenetic Nomenclature.¹¹

Several studies have reported the nonrandom nature of chromosomal abnormalities in melanoma, particularly those involving chromosomes 1, 6, 7, and 11.^{7,12-17} The incidence and distribution of alterations involving chromosomes 1, 6, 7, and 11 among the patients in our study are shown in Figure 1. Structural abnormalities that occurred 10 or more times were examined for their influence on survival. Among our patients with melanoma, the most frequent sites of abnormalities were found on chromosomes 1 (31 times), 6 (15 times), 2 (14 times), 7 and 9 (11 times each), and 11, 21, and 3 (10 times each).

Survival distributions were estimated according to the method of Kaplan and Meier,¹⁸ and the significance of differences between survival distributions was tested with the log-rank statistic.²⁰ The differences in percentages for discrete variables were tested with the chi-square statistic.²¹

RESULTS

Of the 62 patients, 46 (74 percent) had sufficient metaphases for study, and 43 of these (93 percent) had abnormal karyotypes. Of the 46 patients whose cells demonstrated mitoses, 36 had totally abnormal mi-

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Figure 1. Idiograms of Human Chromosomes 1, 6, 7, and 11 Depicting the Regions Most Frequently Altered in Human Malignant Melanoma.

The dots adjacent to each chromosome represent the loci of structural rearrangements in the 43 patients with abnormal mitoses. Some patients had more than one rearrangement of a given chromosome. No attempt was made to determine whether the cytogenetic changes were uniform in patients who had multiple lesions; however, our previous studies¹⁸ have indicated that the majority of clonal changes are retained over time and between various biopsy sites. The heavy brackets indicate the most frequently altered band regions. The loci of cellular oncogenes mapped to chromosomes 1, 6, 7, and 11 are also indicated. TCR β denotes T-cell receptor β .

toses, 7 had a mixture of normal and abnormal mitoses, and 3 had completely normal mitoses. There was no correlation between the presence or absence of normal mitoses and the death rate ($P = 0.89$). In addition, there was no correlation between the death rate and the modal chromosome number (range, 35 to 110 chromosomes per cell) among patients in the study ($P = 0.70$).

Because of the nature of our data, different numbers of patients were analyzed depending on the survival distribution examined. Of the 62 patients, information on structural abnormalities of particular chromosomes was available for 31. All 31 of these patients were included in analyses relating structural abnormalities to the time from diagnosis to death. Of these 31, 22 had Stage III disease, so that analyses of the interval between Stage III and death included only 22 patients.

We also examined three issues related to the representativeness of our patient population and the effect of considering particular subsets of patients in our analyses. First, we used the patient-registry data base of the Arizona Cancer Center to compare all the patients with melanoma who were seen at the center with those whose biopsy samples were analyzed in the cytogenetics laboratory. We found no significant differences in any of the demographic or clinical characteristics considered for these two groups, including survival.

Second, we examined the results for patients whose tissue samples were analyzed cytogenetically to determine whether these results differed from those obtained from patients for whom cytogenetic analysis could not be done. Of the samples received by the

laboratory, a proportion could not be evaluated because no growth or no metaphases were seen after the chromosomes were harvested. We conducted a pilot study in this group of patients with melanoma, comparing their clinical and demographic features with those of patients for whom cytogenetic analysis was successful. None of the clinical or demographic features examined differed significantly between the two groups.

Finally, since some of our analyses included only the patients for whom structural information was available, we compared this group with the group of patients for whom this information was not available. Of the 62 patients included in our clinical data base, 31 had complete information available on structural abnormalities and 31 did not. We therefore compared these two groups of patients to ascertain whether they differed significantly in any way. None of the demographic or clinical features, including survival, differed significantly between these two groups. Taken together, these three analyses suggest that the patients in our study group were representative of those seen at our cancer center.

Figure 2 shows that some of the karyotypic analyses were performed several years after the diagnosis of the patient's disease. In the ideal study design, the biopsy for cytogenetic analysis would have been obtained at the time of diagnosis, with patients followed until death. In our study, the actual study design was to obtain biopsy samples from patients with metastatic disease at some point after the diagnosis. The small size of most primary lesions often requires their use in entirety for diagnosis. We found, however, that the time from the diagnosis to the biopsy in our patient population was not significantly related to any chromosomal abnormality ($P > 0.26$).

We did not find any correlation between specific

Figure 2. Interval between the Diagnosis of Malignant Melanoma and the Biopsy for Cytogenetic Analysis.

The interval between the diagnosis and the biopsy was not significantly related to any chromosomal abnormality ($P > 0.26$). The inset depicts the ideal and actual study design for this investigation (see text).

chromosomal changes and the size (Clark's test) or thickness (Breslow) of the diagnostic biopsy samples. Also, we observed no significant difference in the frequency of chromosomal changes between patients who received chemotherapy before their biopsy for cytogenetic analysis and those who were not treated.

Specific chromosomal abnormalities were then examined for their association with clinical outcome. Estimated survival distributions according to karyotype are presented in Figures 3 and 4. Patients with a structural abnormality of chromosome 7 or 11 had a significantly shorter survival ($P < 0.001$ and $P < 0.02$, respectively) than patients without such chromosomal abnormalities (Fig. 3). This was true when survival was analyzed as the interval from cytogenetic study to death (Stage III to death; Fig. 3) and as the interval from diagnosis to death (data not shown). We then examined whether survival among patients with an abnormality of either chromosome 7 or 11 differed from that among patients who did not have an abnormality of either chromosome. A significantly shorter survival was observed among patients with alterations in either chromosome 7 or 11, whether they were evaluated from Stage III to death ($P < 0.003$) or from diagnosis to death ($P < 0.005$) (Fig. 4). The other karyotypic abnormalities examined (including those

Figure 3. Survival Curves among Patients Who Had Stage III Malignant Melanoma with and without Abnormalities in Chromosome 7 or 11.

Diagonal lines intersecting survival curves denote patients for whom data were censored.

Figure 4. Survival Curves among Patients Who Had Malignant Melanoma with or without Abnormalities in Chromosome 7 or 11.

Survival was studied from the time Stage III disease was noted to death (top panel) and from the time of the diagnosis of malignant melanoma to death (bottom panel). Diagonal lines intersecting survival curves denote patients for whom data were censored.

of chromosomes 1, 2, 3, 6, 9, and 21) did not correlate significantly with shorter survival.

Finally, although the numbers involved in our study at this point are insufficient to support a definitive multivariate analysis, we did perform analyses of the relations of structural abnormalities of chromosomes 7 and 11 to survival from biopsy, diagnosis, and the occurrence of Stage III disease until death; the variables included age, sex, nodal involvement, the presence of visceral metastases, and Stage III disease in a proportional-hazards model with an underlying Weibull survival distribution.²⁰ The results of univariate analysis (with regard to the relation of these structural abnormalities) remained significant when subjected to multivariate analyses.

DISCUSSION

Chromosomal analysis of malignant cells from patients with hematologic neoplasms has provided prognostic information independent of other clinical or laboratory features of disease.¹⁻³ However, the relation between chromosomal abnormalities and the prognosis for solid tumors (including malignant melanoma) in humans has received little study. This report on karyotypic abnormalities in patients with metastatic melanoma examines the relation between banded

chromosomal analysis and clinical outcome of disease for a human solid tumor.

One feature that might complicate this analysis is the influence on survival of treatment after the biopsy. Whereas treatment improves survival for some forms of metastatic cancer, no treatment for melanoma has yet been reported to alter survival in patients with metastatic disease.²² Accordingly, we do not believe that the treatment received by the patients in this study after biopsy would be likely to have had significant confounding effects on the prognostic factors examined, including chromosomal abnormalities.

The karyotypic abnormalities in this study that portended shorter survival involved structural rearrangements of chromosomes 7 and 11. Although the abnormalities involving these chromosomes were diverse, the region of each chromosome involved was consistent. For example, the rearrangements of chromosome 7 almost exclusively involved the short arm (25 of 27 rearrangements) (Fig. 1). Similarly, there was a clustering of chromosomal breakpoints along chromosome 11, although they were divided almost equally between the short arm (bands p11 to p14) and the long arm (bands q23 to q25) (Fig. 1). Differences in survival were not evaluated statistically for individual rearrangements of chromosomes or specific band regions because of the limited number of observations.

As shown in Figure 1, several cellular oncogenes map to chromosomes 7 and 11, most notably *c-erbB*, *H-ras*, and *ets-1*. The influence of these oncogenes on the negative prognostic effect of alterations in chromosome 7 or 11 is unclear. Koprowski and colleagues, however, have described the frequent alteration of chromosome 7, and specifically the elevated expression of *c-erbB*, in early stages of malignant melanoma and dysplastic nevi.²³ It will be of interest to determine whether changes involving chromosome 7 or 11 are common to primary melanoma and its precursor lesions.

In summary, we have shown that chromosomal abnormalities are predictive of clinical outcome in some patients with metastatic melanoma. Because of the large number of comparisons necessitated by the study design and the number of individual chromosomes examined, our results will require corroboration. Nonetheless, inasmuch as cytogenetic analysis has provided useful information about hematologic neoplasms, it seems likely that the analysis of solid

tumors, such as melanoma, will prove to be of similar value.

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