Circulating DNA Microsatellites: Molecular Determinants of Response to Biochemotherapy in Patients With Metastatic Melanoma

Bret Taback, Steven J. O’Day, Peter D. Boasberg, Sherry Shu, Patricia Fournier, Robert Elashoff, He-Jing Wang, Dave S. B. Hoon

Although biochemotherapy appears to be a promising treatment for metastatic melanoma, its impact remains unpredictable. Microsatellite markers for loss of heterozygosity (LOH) appear to have prognostic significance when identified in primary tumors and serum and/or plasma from cancer patients. However, their association with response to systemic therapy has yet to be assessed. To determine whether microsatellite markers are associated with response to therapy, serum from 41 patients with metastatic melanoma, drawn before the initiation of biochemotherapy, was analyzed for LOH with nine microsatellite markers. During a median follow-up of 13 months, the overall response rate for these 41 patients was 56%, including 13 (32%) complete responses and 10 (24%) partial responses. LOH was detected in sera from 12 (29%) of the 41 patients. The response rate of these 12 patients was 17% (95% confidence interval [CI] = 5% to 45%), whereas that of the 29 patients without LOH was 72% (95% CI = 54% to 85%) (P = .001). All statistical tests were two-sided. The presence of LOH was statistically significant and independently associated with disease progression (multivariable analysis, P = .003). Circulating tumor DNA markers may be useful in assessing prognosis for advanced melanoma patients and their response to biochemotherapy. [J Natl Cancer Inst 2004;96:152–6]

Melanoma is characterized by increased genetic instability with advancing stage (1–3). One of the most frequent genetic alterations in melanoma is loss of heterozygosity (LOH) that occurs nonrandomly at certain chromosome loci, suggesting the involvement of putative tumor suppressor and regulatory genes (4–10). Acquisition of genetic alterations is an ongoing phenomenon that occurs throughout disease progression (11–13). Consequently, identification of molecular biomarkers associated with LOH may aid in treatment planning and provide more specific prognostic information.

Melanoma patients with systemic metastasis have an average median survival of 6–9 months (14–17). Such patients treated with biochemotherapy regimens have increased response rates compared with those for patients treated with chemotherapy and biologic response modifiers alone (18–22), which require inpatient hospitalization and are associated with considerable toxicity. We found (23) that use of a concurrent biochemotherapy regimen modified by administering interleukin 2 in a decrescendo fashion had a response rate equivalent to that of chemotherapy and biologic response modifiers alone but with less toxicity. However, the impact on overall survival was modest, and individual patient responses were unpredictable (24,25). Current staging criteria from the American Joint Committee on Cancer (AJCC) (26) do not predict treatment outcomes. To date, there are no established prognostic factors to assess or predict treatment response in patients with advanced-stage melanoma.

Circulating nucleic acids in the serum and/or plasma of cancer patients have allelic losses similar to those found in the primary tumor, suggesting the utility of such nucleic acids as potential markers for disease surveillance (27–32). The purpose of this study is to determine whether microsatellite markers for LOH detected in serum from patients with...
metastatic melanoma receiving a modified concurrent biochemotherapy regimen were associated with a patient’s response to treatment.

Blood (10 mL) was collected from 41 patients with American Joint Committee on Cancer (AJCC) stage IV melanoma at enrollment into our program of modified concurrent biochemotherapy between July 1995 and September 1997 at the John Wayne Cancer Institute after providing written informed consent (23). The collection, storage, and processing of serum and lymphocyte DNA were as previously described (33). Pretreatment evaluation, protocol criteria, biochemotherapy (consisting of dacarbazine, cisplatin, vinblastine, decrescendo interleukin 2, interferon alfa-2b, and tamoxifen) dosing and schedule, and patient monitoring and follow-up have been described previously (23).

Treatment response was evaluated only in patients completing at least two cycles of biochemotherapy, as described elsewhere (23). Patients were categorized as having a complete or partial response or stable or progressive disease, as defined previously (23). The duration of response was measured from the first day of treatment to the first evidence of progressive disease, last follow-up date, or death from any cause. Survival was measured from the first day of treatment to death or the date of the last follow-up.

LOH analysis was performed in a blinded fashion. The following nine microsatellite markers, on seven different chromosomes, that show frequent LOH in melanoma tumors were selected: D1S228 at 1p36, D3S1293 at 3p–3p24.2, D6S264 at 6q25–27, D9S157 and D9S161 at 9p21–23, D10S212 at 10q24–26, D11S2000 at 11q23, D14S51 and D14S62 at 14q31–32. Primer set acquisition, labeling, polymerase chain reaction amplification, and the electrophoresis and analysis of products are as previously described (33). LOH was scored when the intensity of the band for an allele from serum was more than 50% lower than that of the corresponding band from match-paired lymphocytes.

Chi-square (for categorical variables) and t (for numerical variables) tests were used to compare characteristics of patients who did and did not respond to biochemotherapy and characteristics of patients with and without LOH. A logistic regression model was developed to investigate whether the biochemotherapy response was associated with the following covariates: sex, age, Eastern Cooperative Oncology Group (ECOG) performance status, site of metastasis, number of metastatic sites, level of lactate dehydrogenase, prior therapy, and LOH status. A stepwise procedure was used to identify statistically significant covariates. The survival distribution was estimated with the Kaplan–Meier method. The log-rank test was used to compare the distributions of progression-free survival and overall survival between patients with and without LOH. The Cox proportional hazards model was used to study the association of LOH with survival, adjusting for the other clinical factors. The covariates listed above were included in the original model, and the stepwise procedure was also applied for final model selection. Residual plots (Cox–Snell, Martingale) were used for checking the assumption of the Cox proportional hazards model. Statistical analysis was performed with SAS software (SAS Institute, Cary, NC). All statistical tests were two-sided.

LOH was identified in serum from 12 (29%) of the 41 patients: eight (20%) patients for one marker, one (2%) for two markers, and three (7%) for three markers. LOH was not detected in serum samples for 29 (71%) patients or for 40 healthy donors. LOH was most common at microsatellite marker D9S157, occurring in four (12%) of 33 patients’ serum samples informative for the tested marker, followed by D3S1293 (11%), D11S2000 (5%), D14S62 (3%), D1S228 (3%), D9S161 (3%), and D10S212 (0%).

Patients were separated into two groups (23 responders and 18 nonresponders) by their clinical and radiographic responses to biochemotherapy. Responders had a complete or partial response, and nonresponders had stable or progressive disease (Table 1). The overall response rate was 56%, including complete responses in 13 (32%) of the 41 patients and partial responses in 10 (24%) of the 41 patients. Responders completed a mean of six cycles of biochemotherapy (range = 4–8 cycles); nonresponders, those patients demonstrating stable or progressive disease during treatment, were discontinued from the study after completing a mean of three cycles (range = 2–6 cycles). At the start of biochemotherapy, LOH for any one marker was identified in serum

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<th>Table 1. Patient demographics*</th>
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<td><strong>Factor</strong></td>
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*LOH = loss of heterozygosity; ECOG = Eastern Cooperative Oncology Group.
‡Two-sided chi-square test was used.
§Data are the mean ± standard deviation.
§Data are missing for two patients.
from only two (9%) of 23 responders: one with LOH for D3S1293 and the other with LOH for D6S264. Both patients had a complete response to biochemotherapy. In contrast, LOH was detected in sera from 10 (56%) of 18 nonresponders: six (33%) had LOH for one marker, one (6%) had LOH for two markers, and three (17%) had LOH for three markers. LOH was most frequently detected in informative nonresponders for D9S157 (27%), followed by D14S51 (25%), D3S1293 (17%), D6S264 (13%), D11S2000 (12%), D9S161 (6%), D14S62 (6%), and D15S228 (6%).

Univariate analysis detected a statistically significant association between nonresponders and the presence of LOH. The response rate of the 12 patients with LOH was 17% (95% CI = 5% to 45%), whereas that among the 29 patients without LOH was 72% (95% CI = 54% to 85%; \( P = .001, \chi^2 \) test). Failure to respond to biochemotherapy was associated with increased numbers of serum LOH markers (\( P = .002, \) Wilcoxon rank sum test). The only other factor associated with biochemotherapy response was ECOG performance status (\( P = .035, \chi^2 \) test). A logistic regression multivariable analysis was performed to investigate whether biochemotherapy response was associated with various covariates. The presence of LOH was the only factor statistically significantly associated with response to biochemotherapy (estimated odds ratio = 0.08, 95% CI = 0.01 to 0.43; \( P = .003 \)).

After a median follow-up of 13 months, 32 of the 33 patients whose disease progressed had expired; at time of publication, nine remaining patients were still alive, one with stable disease and eight with no clinical evidence of disease after additional biochemotherapy and/or surgery. The median time to progression for nonresponders was 2.3 months and that for responders was 10.3 months (\( P < .001, \) log-rank test), and median overall survival was 4.7 months for nonresponders and 41.6 months for responders (\( P < .001, \) log-rank test). Patients with LOH in serum had statistically significantly decreased median progression-free survival (3 months versus 9.5 months; \( P < .001 \)) and overall survival (6.3 months versus 15 months; \( P = .022 \)) compared with patients without LOH (Fig. 1). Univariate analysis also showed that the site of metastasis, level of lactate dehydrogenase, and history of prior therapy were associated with disease progression and/or recurrence and with overall survival from melanoma. Multivariable analysis showed that an LOH status (risk ratio [RR] = 3.87, 95% CI = 1.71 to 8.77; \( P < .001, \) Wald test) and a lactate dehydrogenase level of more than 190 U/L (RR = 3.76, 95% CI = 1.56 to 9.02; \( P < .003, \) Wald test) were the only statistically significant predictors of disease recurrence. The site of metastasis (soft tissue, lymph nodes, and lung versus other organs; RR = 0.32, 95% CI = 0.12 to 0.85) and a prior history of therapy (RR = 6.71, 95% CI = 1.67 to 27.00) were also statistically significantly associated with the overall survival of patients with advanced-stage melanoma treated with biochemotherapy.

Allelic loss on chromosome 9p occurs in lesions of all thicknesses and is one of the most common genetic aberrations identified in melanoma (5,10,34–36). We found that the most frequent serum microsatellite LOH marker in our study corresponded to this chromosomal location. We have previously hypothesized that microsatellite markers in melanoma patients’ plasma had prognostic significance (33); however, the patients analyzed in that study had different disease stages and treatments. Because higher concentrations of genomic DNA can be identified in serum than in plasma (37), LOH may be relatively underestimated, as seen in this study.

Analysis of primary and advanced-stage tumor tissues provides information relative to the time of the procedure and is not practical for serially assessing di-
ease. At present, additional methods are needed to further assess individual prognosis among patients within the same AJCC staging group and to identify potential responders to treatment protocols as early as possible. Molecular markers of metastatic disease obtained in a minimally invasive manner, such as through a blood test, would allow the disease to be followed as it progressed.

The presence of LOH in the serum of patients with advanced metastatic melanoma was associated with a poorer response to induction biochemotherapy and, independently, with patient outcome. To our knowledge, this is the first study to evaluate the association between circulating DNA tumor markers and a patient’s response to systemic therapy for a solid tumor. Genetic alterations found, such as circulating tumor microsatellites, serving as immediate determinants of disease progression and response to treatment will, no doubt, permit a more efficient use of our healthcare resources.

References

(37) Lee TH, Montalvo L, Chebrotow V, Busch MP. Quantitation of genomic DNA in plasma and serum samples: higher concentrations of genomic DNA found in serum than in plasma. Transfusion 2001;41:276–82.