Loss of Heterozygosity for 10q22–10qter in Malignant Melanoma Progression


Abstract

Karyotypic and molecular data indicate that genetic events involving the chromosome region 10q22–10qter may be related to tumorigenesis in malignant melanoma. To test this we analyzed 10 polymorphic microsatellite repeats in the region 10q22–qter, using a polymerase chain reaction-based assay for loss of heterozygosity and DNA isolated from normal and tumor tissue from 26 individuals with malignant melanoma. The samples included 19 paired normal and malignant tissues representing various points in time during tumor progression were available. Our findings indicate that loss of heterozygosity of 10q22–10qter is a frequent event, that the observed loss of heterozygosity does not result from whole chromosome loss, and that it is associated with tumor progression. Finally, the appearance of new alleles in two of the tumors may indicate the involvement of DNA replication errors in melanoma analogous to such events in other tumor types.

Introduction

The process of tumorigenesis and tumor progression appears to result from the temporal accumulation of multiple genetic lesions (1). This paradigm has been tested in several neoplastic diseases, and, in the case of cutaneous malignant melanoma, has been driven by a dramatic global increase in the incidence with a doubling in the case of cutaneous malignant melanoma, has been driven by a dramatic global increase in the incidence with a doubling in the state of disease LOH for 10q22–qter occurred in nonfamilial malignant melanoma. We analyzed LOH for this region of human chromosome 10 using pairs of DNA samples isolated from both normal and tumor tissue obtained from each patient and, unlike previous studies, included samples from different stages of disease. We also investigated allelic loss in tumor samples obtained from the same individuals which represented sequential stages of disease.

Materials and Methods

Patients and Tissues. Tumor samples were obtained from unscreened patients who underwent surgery for primary or metastatic melanoma in Mannheim between 1989 and 1993; normal skin or peripheral blood lymphocytes served as controls. For the sequential analysis of different stages in the same patient we also utilized formalin-fixed paraffin-embedded tissue obtained between 1985 and 1990.

DNA Analysis. DNA was extracted from tissue samples, peripheral blood lymphocytes, and paraffin-embedded tissue according to standard procedures (9). Polymerase chain reaction-based LOH analyses were performed using 100 ng of DNA and primers for amplification of polymorphic microsatellite repeats at the following 10 loci on chromosome 10q22–10qter (in the most likely order from 10cen–10qter, see discussion): CHLCA.GGAZA.F1 (a 4-nt repeat (10)); D10S110 (a 2-nt repeat (11–13)); D10S108 (a 2-nt repeat (11–13)); D10S88 (a 2-nt repeat (11–13)); CHLCA.TC3.2044 (a 3-nt repeat (10)); D10S168 (a 2-nt repeat (11–13)); D10S187 (a 2-nt repeat (14)); D10S610 (a 2-nt repeat (10)); D10S221 (a 2-nt repeat (14)); D10S169 (a 2-nt repeat (11–13)). Conditions for denaturation, annealing, and elongation were largely as described in the original publications with prolongation of steps for paraffin-extracted material as proposed previously (9). One of each set of primers was labeled using [32P]ATP (Amersham) and products were resolved by electrophoresis on 6–8% formamide/polyacrylamide gels. After transfer of the gels onto a paper support, autoradiography was performed for 2–96 h and allele size was determined by comparison to comigrated 35S-labeled sequencing products of M13 mp18 DNA (United States Biochemical). To ensure reproducibility, at least two independent reactions were performed and evaluated per sample.

LOH Analysis. Evaluation of LOH was performed by comparing intensities of the two alleles in informative cases; only cases in which simple visual inspection was sufficient to easily discriminate between LOH or retention of constitutional heterozygosity were included here. In some cases (where the alleles were clear and well separated) these conclusions were verified by scanning laser densitometry where LOH was imputed by a signal loss of greater than 50%.

Results

We first examined paired normal/tumor tissues from 19 patients described in Table 1: 8 of 19 were primary melanomas; the remaining 11 were from later stages of disease such as regional or distant lymph node metastases or organ metastases. Four of the eight (50%) primary tumors showed LOH at one or more loci; three of these four retained
constitutional heterozygosity for at least two other informative loci on 10q. The primary melanomas that showed LOH were classified according to the revised AJCC/UICC pathologic tumor-nodes-metastasis staging system (1988) (15) as being stage IIA or greater at the time of initial diagnosis. Of the 11 samples representing later stage disease, 4 tumors retained heterozygosity at all informative loci; 2 were distant lymph node metastases for which the primary melanoma had been graded as stage IA and IB, respectively, while the other 2 samples were derived from a regional lymph node metastasis (primary tumor; stage IIA) or a distant lymph node metastasis (primary tumor; stage IIB). Of the 7 (64%) later stage tumors that displayed changes, 2 showed LOH at all informative loci, while 4 retained heterozygosity for at least 1 informative locus. The remaining tumor did not show LOH but a new allele not present in the normal tissue was detected at the D10S221 locus in combination with LOH at the DJOS61O locus. The remaining tumor did not show LOH at all informative loci in three lymph node metastases resected in three consecutive years. The primary tumor was classified as stage IB at the time of initial diagnosis.

In an additional seven patients, we investigated LOH in two or more tumor samples excised from the individuals at different stages of disease (Table 2). Six of the seven (86%) showed LOH, whereas only one of them (patient 1 in Table 2) retained heterozygosity at all informative loci in three lymph node metastases resected in three successive years. The primary tumor was classified as stage IB at the time of initial diagnosis. Two cases showed LOH in the earliest sample examined (patients II and VI in Table 2; in both cases the primary tumor, staged IIA and IIB) whereas 4 of 6 displayed LOH only in tumor samples obtained 2-5 years after initial diagnosis. In 2 of 6 cases LOH was present at all informative markers (patients IV and V in Table 2; for patient V see Fig. 2), whereas 4 of 6 retained heterozygosity for at least 1 informative marker. Due to limited availability of DNA only 5 loci and not all stages could be examined in patient VII.

Discussion

Previous reports of chromosomal rearrangements and molecular changes (4-6, 16) led us to focus our attention on the chromosomal region 10q22-10qter in nonfamilial malignant melanoma. Parts of this region appear by linkage analysis to be excluded from harboring a gene predisposing to familial melanoma (12). This and our finding of a higher frequency of LOH in that region in tumor samples of later

Table 1 LOH for chromosome 10q22-q24 in malignant melanoma of different stages

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Fig. 1. LOH and gain of additional alleles in tumors from three patients. Patient 14 (designation corresponds to Table 1): LOH (•) of the shorter size allele 2 at locus GGAA in tumor (T) compared to normal (N); locus D10S187 shows retained constitutional heterozygosity. Patient 16: -•-, an additional allele found in the tumor at locus D10S221 that is 2 nt longer than allele 1; locus GGAA shows retained heterozygosity. Patient 15: LOH of the longer allele 1 at locus D10S610 in tumor; retained heterozygosity at locus D10S110; gain of an additional allele 2 at longer than allele 2 at locus D10S221 in tumor.
This hypothesis also gained support by the demonstration of late stage tumors of patient V (designation corresponds to Table 2) that represent an intestinal occurrence of LOH in a series of sequential tumor samples from suggesting that LOH for chromosome 10 is an event occurring later in metastasis (Vc) and a brain metastasis (Vd) compared to normal tissue (N) and two lymph we present the most likely order of the loci used in this study, it may also indicate that in most cases only limited portions of chromosome region of common loss perhaps because, in order to be able to utilize manifestation of repetitive DNA-instability as has been demonstrated recently for several other malignancies (17–22). It has been proposed that such instability may be another mechanism of inactivation of tumor suppressor genes. The role that this mechanism plays in the etiology of melanoma remains to be determined.

Acknowledgments

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References


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