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Advances in Brief

Loss of Heterozygosity for 10q22–10qter in Malignant Melanoma Progression[1]

Rudolf A. Herbst,[2] Jürgen Weiss, Alexander Ehnis, Webster K. Cavenee, and Karen C. Arden

Ludwig Institute for Cancer Research [R. A. H., W. K. C., K. C. A.], Center for Molecular Genetics [W. K. C.], and Department of Medicine [W. K. C., K. C. A.], University of California at San Diego, La Jolla, California 92093-0660, and the Hautklinik Mannheim, Fakultät für Klinische Medizin Mannheim der Ruprecht-Karls-Universität Heidelberg, Mannheim, D 68135, Germany [R. A. H., J. W., A. E.]

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 stages of melanoma as well as 7 cases in which samples from at least 2 different 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 United States between 1980 and 1990. Roughly 10% of all malignant melanoma cases appear to be familial and linkage analysis of large, multigeneration families has mapped at least two predisposing genes to chromosomal regions 1p36 and 9p21 (for review see Ref. 2). As with other cancers, linkage analyses are restricted to familial disease; thus the detection of cytogenetic rearrangements and other molecular changes such as LOH[3] have proved to be another powerful tool in determining the chromosomal location of oncogenes and tumor suppressor genes in sporadic tumors. In malignant melanoma such losses have been demonstrated at the sites of the two known tumor suppressors, TP53 and NF1 (2), as well as for other chromosome regions including 1p, 6q, 9p, 11q, 11p, and 16q (2, 3). Karyotypic changes of the long arm of chromosome 10 have been reported in early melanocytic lesions (4) and LOH for markers on 10q have been reported in malignant melanomas of undefined clinical stage (5, 6). The region 10q22–10qter has been shown to be involved in several types of tumors such as non-Hodgkin's lymphoma, renal cell carcinoma, prostate carcinoma, as well as neural crest-associated tumors such as advanced meningioma and glioblastoma (reviewed in Ref. 6). In the latter there are indications that LOH for 10q involves at least two distinct regions (7) and occurs during tumor progression (8). In a recent study of 18 cases of metastatic melanoma LOH for chromosome 10 was found in 50% (7 of 14) of informative cases; in 5 of the 7 cases one entire chromosome 10 was lost and in the remaining 2 cases the complete long arm of chromosome 10 was lost (6). These reports of rearrangements of chromosome 10 in early melanocytic lesions as well as in metastatic disease prompted us to ask at which stage 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 unselected 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): *CHLC.GGAA2F11* [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)]; *CHLC.ATC3.2044* [a 3-nt repeat (10)]; *D10S168* [a 2-nt repeat (11–13)]; *D10S187* [a 2-nt repeat (14)]; *D10S610* [a 4-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 [γ-^{32}P]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 ^{35}S-labeled sequencing products of M13 mp18 DNA (United States Biochemical). To ensure reproducibility, at least two independant 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

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[2] To whom requests for reprints should be addressed, at the Ludwig Institute for Cancer Research, San Diego Branch, 9500 Gilman Drive, La Jolla, CA 92093-0660.

[3] The abbreviations used are: LOH, loss of heterozygosity; nt, nucleotide(s).

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

LOH analysis of tumors from 19 different patients (1–19). Columns from left to right: No., patient identification. Tumor: LN, distant lymph node metastasis; RM, regional metastasis; SM, organ metastasis to skin; BO, intestinal metastasis. Year obtained: year of sample collection. Type: clinical melanoma subtype: SSM, superficial spreading melanoma; NM, nodular melanoma; ALM, acral lentiginous melanoma; LMM, lentigo maligna melanoma; NC, subtype not classified. Initial diagnosis: year of initial diagnosis. Initial stage: staging at initial diagnosis was according to the revised AJCC/UICC pTNM staging system (1988). Loci are shown in the most likely order from 10qcen to 10qter from left to right. ●, LOH; ○, heterozygous tumor; ∅, additional allele detected in tumor; –, noninformative (constitutionally homozygous) locus; ND, not done.

						Loci	⟨ 10 qcen								10qter ⟩
No.	Tumor	Year obtained	Type	Initial diagnosis	Initial stage	GGAA	110	108	88	ATC	168	187	610	221	169
1	PT	1990	SSM	1990	IB	○	–	○	○	○	–	○	○	○	–
2	PT	1993	NM	1993	IB	○	–	–	–	○	–	○	○	–	–
3	PT	1986	SSM	1986	IIA	○	–	–	–	–	–	○	ND	○	ND
4	PT	1993	NM	1993	IIA	–	–	–	–	○	–	○	○	○	○
5	PT	1988	SSM	1988	IIA	○	○	○	–	●	●	–	–	–	○
6	PT	1986	SSM	1986	IIA	●	–	–	●	●	○	○	ND	○	○
7	PT	1993	NM	1993	IIA	–	–	–	●	●	●	●	–	●	–
8	PT	1993	NM	1993	IIB	●	–	○	○	–	–	–	–	●	–
9	LN	1989	SSM	1988	IA	○	–	–	–	–	–	○	–	○	○
10	LN	1993	SSM	1989	IB	–	–	–	–	○	–	○	○	○	○
11	RM	1991	NC	1990	IIA	○	–	○	–	○	–	–	–	○	○
12	RM	1993	SSM	1985	IIB	●	–	–	●	–	●	–	●	●	–
13	SM	1993	ALM	1990	IIB	○	–	○	–	–	○	–	–	○	–
14	SM	1993	ALM	1989	IA	●	–	–	–	–	●	○	–	–	○
15	SM	1991	SSM	1989	IIA	–	○	–	–	–	–	–	●	∅	–
16	LN	1993	LMM	1992	IIB	○	–	–	–	–	○	○	○	∅	–
17	SM	1992	ALM	1991	IIB	●	–	–	○	–	–	–	●	○	–
18	LN	1992	ALM	1992	III	–	●	○	–	●	●	●	–	–	●
19	BO	1992	NC	1989	NC	●	●	●	–	–	–	●	●	●	–

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 (patient 16 in Table 1 and Fig. 1); this gain was detectable in two skin metastases obtained from different sites and two months apart. A similar gain of one allele was also observed in another tumor at the *D10S221* locus in combination with LOH at the *D10S610* locus (patient 15 in Table 1 and Fig. 1).

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 consecutive 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

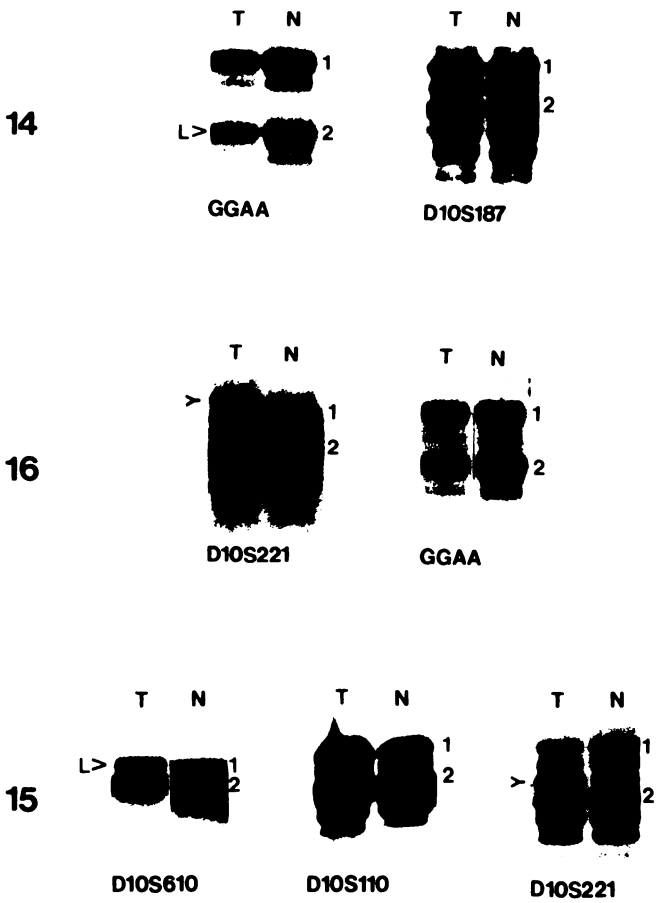

Fig. 1. LOH and gain of additional alleles in tumors from three patients. Patient 14 (designation corresponds to Table 1): LOH (*L*>) of the shorter size allele 2 at locus GGAA in tumor (*T*) compared to normal (*N*); locus *D10S187* shows retained constitutional heterozygosity. Patient 16: >-, an additional 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 nt longer than allele 2 at locus *D10S221* in tumor.

Table 2 *LOH for chromosome 10q22–q24 in progressive malignant melanoma*

LOH analysis of tumor samples obtained sequentially from seven different patients (I–VII) at different, progressive points in disease (a–d). Columns from left to right: No., patient identification. Tumor: LN, distant lymph node metastasis; BO, intestinal metastasis; BR, brain metastasis; RM, regional metastasis; LU, lung metastasis. Year obtained: year of sample collection. Type: clinical melanoma subtype: ALM, acral lentiginous melanoma; SSM, superficial spreading melanoma; NM, nodular melanoma. Initial diagnosis: year of initial diagnosis. Initial stage: staging at initial diagnosis was according to the revised AJCC/UICC pathologic-tumor-nodes-metastasis staging system (1988). Loci are shown in the most likely order from 10qcen to 10qter from left to right. ●, LOH; ○, heterozygous tumor; –, noninformative (constitutionally homozygous) locus; ND, not done.

						Loci	⟨ 10 qcen								10qter ⟩
No.	Tumor	Year obtained	Type	Initial diagnosis	Initial stage	GGAA	110	108	88	ATC	168	187	610	221	169
Ia	LN	1986	ALM	1986	IB	○	○	○	–	○	○	○	–	–	–
Ib	LN	1987				○	○	○	–	○	○	○	–	–	–
Ic	LN	1988				○	○	○	–	○	○	○	–	–	–
IIa	PT	1985	SSM	1985	IIA	●	●	–	–	○	○	–	ND	–	○
IIb	LN	1986				●	●	–	–	●	○	–	ND	–	○
IIIa	LN	1986	NM	1985	IIA	○	–	○	–	○	–	–	ND	–	○
IIIb	LN	1988				●	–	●	–	○	–	–	●	–	○
IVa	PT	1985	NM	1985	IIA	○	–	○	–	○	–	○	–	–	○
IVb	LN	1990				●	–	●	–	●	–	●	–	–	●
Va	LN	1986	SSM	1985	IIA	○	○	–	○	–	–	○	○	○	○
Vb	LN	1989				○	○	–	○	–	–	○	○	○	○
Vc	BO	1990				●	●	–	●	–	–	●	●	●	●
Vd	BR	1990				●	●	–	●	–	–	●	●	●	●
VIa	PT	1985	NM	1985	IIB	●	–	–	–	–	–	–	ND	–	–
VIb	RM	1986				●	–	–	–	–	–	–	ND	–	–
VIc	LN	1986				●	–	–	–	–	–	–	○	–	–
VIIa	RM	1986	NM	1986	III	ND	ND	–	○	ND	○	ND	ND	ND	ND
VIIb	LN	1986				ND	ND	–	ND	ND	●	ND	ND	ND	ND
VIIc	LU	1987				○	ND	–	●	–	●	ND	ND	ND	ND

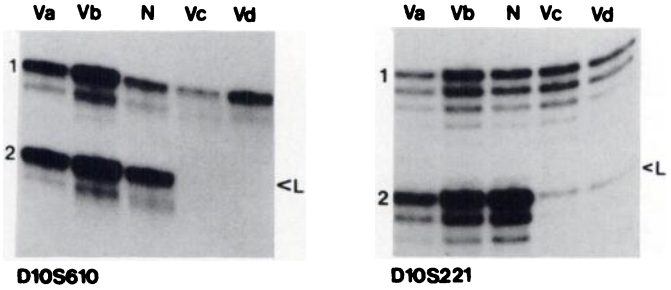

Fig. 2. LOH (*<L*) of the shorter size allele 2 at loci *D10S610* and *D10S221* in two later stage tumors of patient V (designation corresponds to Table 2) that represent an intestinal metastasis (*Vc*) and a brain metastasis (*Vd*) compared to normal tissue (*N*) and two lymph node metastases obtained one (*Vb*) and three (*Va*) years earlier.

stages of disease and/or tumors of a higher grade at initial diagnosis suggest that LOH for chromosome 10 is an event occurring later in tumor progression comparable to the situation in brain tumors (8). This hypothesis also gained support by the demonstration of late occurrence of LOH in a series of sequential tumor samples from individual patients which represented progressive disease. The data also indicate that in most cases only limited portions of chromosome 10 undergo LOH. We were, however, unable to define a more narrow region of common loss perhaps because, in order to be able to utilize a large number of polymerase chain reaction-based polymorphic loci, we had to use markers isolated by 3 different groups (10, 13, 14). The relative positions of markers from a single source have been determined by linkage analysis, but the relative positions of markers from different sources is difficult to establish unequivocally and, although we present the most likely order of the loci used in this study, it may not be entirely accurate. The pattern of losses suggests, however, that there is more than one region involved, similar to the findings in brain tumors. Studies including markers that are better placed within a common map and that include a larger number of cases and stages of progressive disease may clarify this issue. This information could eventually prove to be useful for prognostic purposes.

This is also the first report to our knowledge documenting observation of additional alleles in malignant melanoma. This may be a 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.

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