Fine Mapping of Chromosome 3 in Uveal Melanoma: Identification of a Minimal Region of Deletion on Chromosomal Arm 3p25.1-p25.2

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ABSTRACT

To identify minimal common areas of allelic loss on chromosome 3, we have mapped both arms of the chromosome in 21 uveal melanomas that did not show monosomy 3 in our previous allelotype study. DNA was isolated from microdissected paraffin sections and amplified by PCR. In an initial screening, 14 microsatellite markers on chromosome arm 3p and 13 on chromosome arm 3q were used. Loss of heterozygosity for at least one marker was found in 9 of 21 tumors (43%) on 3p and 8 of 21 tumors (38%) on 3q. The initial analysis defined two common regions of allelic loss on 3p, a 7.3-Mb region between markers D3S1263 and D3S3510 spanning 3p25.3-24.3 and a larger region between markers D3S3578 and D3S1284. The two common regions of allelic loss were further mapped with an additional 14 microsatellite markers. A 1.4-Mb minimal region of allelic loss was identified between microsatellite markers D3S3610 and D3S1554 on 3p25.1-3p25.2. A total of 10 tumors had allelic loss in this region; 2 of these tumors had corresponding putative homozygous deletions. These homozygous deletions may further narrow the region of interest to 0.1 Mb. This 1.4-Mb minimum region of deletion includes several genes that might be involved in the carcinogenesis of uveal melanoma as well as other important tumor types.

INTRODUCTION

Carcinogenesis occurs as a stepwise accumulation of genetic alterations in genes that control cell growth, cell death, and DNA repair (1). One of the critical events that lead to carcinogenesis is the inactivation of one or more TSGs.5 Allelic loss, manifested as LOH of microsatellite markers flanking the TSG, is recognized as hallmark of sporadic tumors, with inactivation of the second allele by gene deletion, point mutations or promoter hypermethylation (1).

Prior uveal melanoma is a relatively rare tumor, but it is associated with a high visual morbidity and can pose a serious threat to life (2). About 30% of patients will develop distant metastases within 10 years of successful management of the local tumor (3). The most common genetic abnormality in uveal melanoma is the loss of one copy of chromosome 3. Cytogenetic and comparative genomic hybridization studies have demonstrated monosomy 3 in ~50% of cases (4–8). Complete or partial deletions of chromosome 3 have been detected by microsatellite marker analysis (9, 10). In our allelotype of 50 uveal melanomas, ~60% of tumors had LOH of all markers on chromosome 3 consistent with monosomy or isodisomy 3 (11).

Genetic abnormalities of chromosome 3 and, in particular 3p, are also critical to the development of several other tumor types, including small cell lung carcinoma, breast cancer, ovarian cancer, adrenocarcinoma of the cervix, renal cell carcinoma, and head and neck tumors (12). Several regions on chromosome 3p are frequently rearranged in tumors and are therefore believed to contain TSGs. Two of these regions are located on the centromeric portion of the short arm (12). The 3p12-13 region contains the gene ROBO1/DUTT1 and the 3p14.2 region spans the FHIT gene. Three other hot spots for rearrangements were reported on 3p21. The first is located on 3p21.1-p21.2 and includes the gene BAP1 that binds the RING finger domain of the BRCA1 gene. The second is the lung cancer TSG region (LUCA) that includes the RASSF1 gene frequently hypermethylated in cancer. The third is located on 3p21.3 and contains the DLCl gene found deleted in a number of small cell lung cancer cell lines and primary tumors. The telomeric region 3p25-26, containing the VHL gene, has also been reported to be frequently rearranged (12).

Although monosomy or isodisomy 3 are very common in uveal melanoma, only a few partial deletions of this chromosome have been described (13, 14). To gain more insight into the patterns of chromosome 3 deletion and possible genes involved in the carcinogenesis of uveal melanoma, we analyzed 21 tumors from our allelotype cohort that did not show monosomy 3, with a total of 41 microsatellite markers (11). We identified a 1.4-Mb shared MRD on chromosome 3p25.1-p25.2 between markers D3S3610 and D3S1554 that contains 9 National Center for Biotechnology Information reference sequences and a number of predicted genes.

MATERIALS AND METHODS

Specimens and DNA Extraction. We evaluated 21 cases of posterior uveal melanoma treated by enucleation from 1993 to 1998 and archived as paraffin blocks by the Eye Pathology Laboratory of the Wilmer Ophthalmological Institute. DNA was extracted from paraffin-embedded tissue as described previously (15). Briefly, unstained paraffin sections of each block were manually dissected into tumor and normal tissue samples under a microscope to ensure that tumor samples contained at least 80% cancer cells (15). The normal ocular tissue was used as the source of normal control DNA for each patient. Normal and tumor tissue was digested with Sdx/proteinase K for 24 h at 48°C, and samples were extracted with phenol/chloroform. Pigments, which coisolated with the DNA after the extraction, tended to inhibit the PCR. Therefore, DNA samples were additionally purified using GeneClean II according to the manufacturer’s instructions (Bio101; Vista, CA).

Fine Deletion Mapping. Twenty-one uveal melanomas from the original allelotype cohort (15 tumors with retention of all tested markers on 3p, 4 tumors with LOH of 3p or 3q, and 2 tumors noninformative for markers on 3p or 3q) were tested (15). DNA from normal and tumor tissues were analyzed by LOH by amplification of STS microsatellite markers using PCR and the conditions described below.

For an initial screening, 27 primer pairs (Research Genetics, Huntsville, AL) were analyzed: 14 on chromosome arm 3p (D3S1270, D3S1597, D3S1263, D3S1265, D3S1286, D3S3510, D3S1293, D3S1283, D3S1007, D3S1538, D3S1578, D3S1234, D3S1210, D3S1284) and 13 on chromosome arm 3q (D3S2318, D3S1251, D3S1603, D3S1546, D3S1310, D3S1292, D3S1238, D3S1764, D3S1268, D3S3715, D3S1580, D3S2748, D3S1311). The microsatellite markers were chosen to span ~5-Mb intervals on chromosome 3p and ~8-Mb intervals on chromosome 3q. The relative positions of the markers were determined using the Human Genome Working Draft Sequence Browser November 2002 (Ref. 16; Fig. 1).5 Markers D3S1597, D3S1284, D3S3610, D3S3510 were also evaluated.

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5 The abbreviations used are: TSG, tumor suppressor gene; LOH, loss of heterozygosity; MRD, minimal region of deletion; STS, sequence-tagged site.
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Fig. 1. Chromosome 3 mapping. Twenty-one uveal melanomas were tested with 27 microsatellite markers (14 for chromosomal arm 3p and 13 for chromosomal arm 3q). Retention of both alleles; loss of heterozygosity; NI, noninformative; ND, not determined; the first row indicates the STS microsatellite marker. Two common regions of allelic loss (CRL) were identified on 3p, D3S1263-D3S3510 (CRL1) and D3S1578-D3S1284 (CRL2). Positions of the markers on the physical map were determined using the Human Genome Working Draft Sequence Browser (16).

D3S1292 and D3S1268 had been previously used in the allelotype (11). The common regions of loss on 3p were further mapped with additional 14 microsatellite markers (D3S1259, D3S3701, D3S656, D3S3610, D3S100, D3S2385, D3S1585, D3S1554, D3S1479, D3S1360, D3S1076, D3S1295, D3S3635, D3S3507). In total, each tumor was tested with 41 markers. The MRD identified on chromosome 3p25.1-p25.2 was defined using only tumors with at least two consecutive losses.

Using T4 kinase (Life Technologies, Inc., Gaithersburg, MD), 500 ng of one primer from each pair was end labeled with [32P]ATP (20 mCi; Amersham, Piscataway, NJ) in a total volume of 20 µl containing 20 ng of genomic DNA, 25 ng of end-labeled primer, 60 ng of each unlabeled primer, and 2 units of TaqDNA polymerase (New England Biolabs, Beverly, MA). PCR reactions were carried out in a total volume of 10 µl containing 20 ng of genomic DNA, 25 ng of end-labeled primer, 60 ng of each unlabeled primer, and 2 units of TaqDNA polymerase (New England Biolabs, Beverly, MA). PCR conditions were as follows: 35 cycles (95°C for 1 min, 54°C–58°C depending on primer pairs for 1 min, 72°C for 1 min) followed by a final 4-min extension at 72°C. About 1 min, 72°C–C.

RESULTS

After the initial screening with 27 STS microsatellite markers, LOH for at least one marker was found on 3p in 9 of the 21 uveal melanomas (43%) and on 3q in 8 of the 21 tumors (38%; Fig. 1). A 7.3-Mb common region of LOH (CRL1) on 3p25.3–24.3 between markers D3S1263 and D3S3510 was identified (Fig. 1). Six of the 9 uveal melanomas with loss on 3p displayed LOH in at least one marker in this region (UM55, UM28, UM6, UM35, UM10, and UM50). Two of these six tumors (UM10 and UM50) showed loss of all informative markers on 3p with the exception of marker D3S1252, included in the 7.3-Mb minimal common region of LOH. This apparent retention of a marker within a region of LOH is consistent with a possible homozygous deletion in these tumors (see “Discussion”; Ref. 17). The remaining 3 tumors with 3p LOH (UM46, UM9, and UM31) showed losses in a 19.7-Mb region (CRL2) between markers D3S1578 and D3S1284 on chromosome 3p14–p12 (Fig. 1).

The chromosomal region 3p25.3–p24.3 between markers D3S1263 and D3S3510 was further mapped with 10 additional microsatellite chosen at ~0.5-Mb intervals (Fig. 2A). Four additional markers were used to map the 3p14–12 region (Fig. 2B). With the additional markers, 4 more tumors (UM22, UM13, UM14, and UM26) were found to have LOH in the common region of loss on 3p25.3–24.3, and 2 more tumors (UM14 and UM57) were found to have LOH in the common region of loss on 3p14–11. In total, the 3p chromosomal arm was mapped with 28 STS microsatellite markers, and 15 of the 21 uveal melanomas (71%) harbored LOH of at least one marker.

A 1.4-Mb shared MRD between microsatellite markers D3S3610 and D3S1554 on 3p15.1-12.5 was identified (Figs. 2A and 3). The centromeric boundary was defined by tumors UM28 and UM6, and
tumors had retention of only 1 microsatellite marker D3S1252 (UM10). Within this MRD, 2 tumors had retention of both alleles for marker D3S1256, LOH of the top allele for marker D3S3610; retention of marker D3S1554. Tumor UM10 from top to bottom: retention of both alleles for marker D3S656; LOH of the bottom allele for marker D3S1585. Tumor UM28 from top to bottom: retention of both alleles for marker D3S656; LOH of the bottom allele for marker D3S1252; retention of both alleles for marker D3S1286.

Fig. 3. LOH analysis of posterior uveal melanoma. Autoradiographs depicting microsatellite analysis of representative tumors UM6, UM10, and UM28 are shown. Markers are labeled above each normal (N) and corresponding tumor (T) lane. Arrows indicate relative loss of alleles in tumor samples. Tumor UM6 from top to bottom: retention of both alleles for marker D3S656; LOH of the top allele for marker D3S3610; retention of marker D3S1554. Tumor UM10 from top to bottom: LOH of the bottom allele for marker D3S656; apparent retention of both alleles caused by amplification of residual normal DNA for marker D3S1252; LOH of the bottom allele for marker D3S1585. Tumor UM28 from top to bottom: retention of both alleles for marker D3S656; LOH of the bottom allele for marker D3S1252; retention of both alleles for marker D3S1286.

DISCUSSION

Monosomy 3 is the most common genetic abnormality in uveal melanoma, but only a few reports describe interstitial deletion or translocation of this chromosome. Homozygous deletion in the THRBI locus was described in 3 of 19 uveal melanomas, but this result was not confirmed in subsequent studies (9, 18). Translocations of chromosomal regions 3p14 and 3q23 were found in primary culture but did not help to pinpoint the region(s) involved in chromosome 3 carcinogenesis (13, 14).

Chromosomal arm 3p abnormalities are frequently found in tumors. At least six major regions of chromosomal rearrangement have been described, and they are often multiple and discontinuous (12). We defined a common region of loss on 3p14-12, which maps to the centromeric region of chromosome 3 and includes two of these frequently rearranged regions, 3p12-13, and the 3p14.2. The latter contains the putative TSG FHIT gene that spans the FRA3B fragile site. However, occurrence of intragenic mutations in FHIT are very rare, thus some researches have argued that FHIT abnormalities may only represent alterations in the fragile site. One tumor, UM31, showed loss of the pericentromeric region of the chromosome suggesting the presence of a complex rearrangement that cannot be further characterized by microsatellite analysis.

Frequent allelic losses on 3p25-26, corresponding to our MRD, have been reported in lung, renal, breast, and other solid tumors, and in vitro studies indicate that this region is able to suppress growth of tumor cells (19). The VHL gene, located on 3p25.3, is associated with a familial cancer syndrome characterized by kidney cancer and multiple benign tumors, but it is telomeric to our 3p25.1-p25.2 region (20).

In a previous study of uveal melanoma, Tschentscher et al. (10) defined by comparative genomic hybridization and microsatellite analysis a 2.2-Mb MRD on 3p25-26 between markers D3S2450-D3S3691. On the human draft sequence, their markers map to 3p25.3-3p26.1 (6.7-8.9 on the physical map), a region slightly telomeric to our 3p25.1-p25.2 MRD (10). This conflict may simply be explained by the presence of two TSGs closely spaced and both important in the tumorigenesis of uveal melanoma. Even if we conservatively define our telomeric boundary by D3S656 and 3 tumors (UM6, UM28, UM35), our MRD does not overlap that of Tschentscher et al. (10). If the 6 tumors with partial deletion of 3p in the study by Tschentscher et al. (10) are considered, 5 of them show allelic losses in areas that include our MRD.

Two uveal melanomas (UM10 and UM50) showed loss of all informative markers on chromosomal arm 3p except for one marker, D3S1252, included in the 1.4-Mb MRD (Figs. 2A and 3). This pattern of apparent retention of at least one marker, flanked by loss of at least two other markers, is the previously described criteria for a homozygous deletion as determined by microsatellite analysis (17). This apparent retention, shown to represent homozygous deletion of this marker by correlation with Southern blot and FISH analyses, is caused by amplification of a small amount of residual normal DNA, which remains after careful microdissection of uveal melanomas (17). The homozygous deletion of the region corresponding to marker D3S1252 further limits the region of interest to 100 Kb (between markers D3S2385 and D3S1585) and suggests that a TSG may exist in the region between markers D3S2385 and D3S1585.

Analysis of our 1.4-Mb MRD with the University of California–Santa Cruz at Genome Browser (16) indicates the presence of 9 National Center for Biotechnology Information reference sequences. Of those, five have known function. The xeroderma pigmentosum complementation group C (XPC) gene is involved in the DNA excision repair pathway and is associated with the Xeroderma pigmentosum syndrome in which both cutaneous and uveal melanomas have been described previously (21, 22). The WNT7A gene belongs to the wingless-type mouse mammary tumor virus integration site (WNT) family and seems to be involved in embryo development (23). The expression of WNT7A was recently found to be reduced in lung cancer (24). The other three genes encode an extracellular matrix protein FBLN2 (25), a novel member of the histone deacetylase family HDAC11 (26), and a protein LSM3 that binds the U6 snRNA, an essential element of spliceosome in humans (27). Each of these genes represents a good candidate for mutational screening in uveal melanoma. In addition to these genes, four other poorly characterized RefSeq proteins map in the MRD, and the presence of other genes in the D3S2385-D3S1554 region is suggested by UniGene Cluster analysis and gene prediction computer programs.

Allelic losses in the 3p25-26 region that do not overlap the VHL gene have been described in several tumor types (12). In uveal
melanoma, we have identified a 1.4-Mb MRD on 3p25.1-p25.2, the smallest reported MRD at this site. Deletions described in studies of breast cancer and oral cancer overlap our MRD (28, 29), and it is quite possible that a TSG on 3p25.1-p25.2 may be involved in other important tumor types in addition to uveal melanoma. Follow-up mutational analysis of candidate genes in the region will be needed to identify this putative TSG.

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