Detection of Three Novel Translocations and Specific Common Chromosomal Break Sites in Malignant Melanoma by Spectral Karyotyping

Linda M. Sargent,[1]* Mark A. Nelson,[2] David T. Lowry,[1] Jamie R. Senft,[1] Amy M. Jefferson,[1] Maria E. Ariza,[2] and Steven H. Reynolds[1]

[1]Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, National Institute of Occupational Safety and Health, Morgantown, West Virginia
[2]Arizona Cancer Center, University of Arizona, Tucson, Arizona

Chromosomal aberrations in malignant melanoma cells have been reported using standard chromosome banding analysis and comparative genomic hybridization. To identify marker chromosomes and translocations that are difficult to characterize by standard banding analysis, 15 early passage malignant melanoma cell lines were examined using spectral karyotyping. All 15 tumor cell lines had lost all or part of 1p and 10q. Losses of material on chromosome arms 4p (12/15), 6q (12/15), 9p (15/15), 12p (13/15), 12q (13/15), 13q (11/15), and 19q (14/15) were the next most frequent events. Gain of chromosome arms 1q (11/15), 6p (13/15), and 20q11 (14/15) was also observed. Interestingly, we identified translocations der(12)t(12;20)(q15;q11), der(19)t(10;19)(q23;q13), and der(12)t(12;19)(q13;q13) in 4/15 tumors. Three recurring translocations involving four of the most frequent break points were detected. The identification of recurring translocations and unique chromosome break points in melanoma will aid in the identification of the genes that are important in the neoplastic process.

INTRODUCTION

The incidence of melanoma is increasing 5–7% per year and is notorious for metastasis early in the disease (Brozena et al., 1993). Melanoma occurs in young people and does not respond to current therapeutic intervention (Herlyn, 1993). The risk of melanoma is linked with occupational exposure to ultraviolet radiation, arsenic, ionizing radiation, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons (Everall and Dowd, 1978; Rushton and Alderson, 1981; Loomis et al., 1997). The regional distribution of melanoma in farm workers may be explained in part by exposure to arsenic (Philipp et al., 1983).

Although the number of melanoma cases is increasing in the population, the genetics of this cancer are not well described. The identification of recurrent break points and translocations in tumors is the first step in the isolation of genes whose regulation has been altered during the progression of neoplasia. Chromosome banding, fluorescence in situ hybridization (FISH), and comparative genomic hybridization (CGH) analysis have previously demonstrated numerous chromosome alterations in malignant melanoma. Karyotypic analysis of tumor metaphase preparations is hindered by a low mitotic index and complicated translocations as well as small rearrangements that are difficult to identify by traditional chromosome banding. Identification of translocations by banding analysis of good metaphase preparations has been shown to be less sensitive than spectral karotyping (Schrock et al., 1996; Kakazu et al., 1999). Although comparative genomic hybridization has made it possible to characterize the loss and gain of chromosomal material, this method cannot identify chromosomal translocations and has limited resolution.

The accuracy of the identification of karyotypic alterations has been greatly increased by the development of spectral karyotyping. Spectral karyotyping (SKY) is a novel imaging method that combines spectroscopy and imaging. This type of approach allows analysis of the full spectrum of light at all pixels of the image. With the spectral based approach, one can simultaneously identify 100% of the chromosomes in a metaphase cell. The loss, gain, or translocation of any chromosome in a metaphase cell can be characterized in one experiment. We have been able to confirm chromosome aberrations in malignant melanoma that had been identified by standard banding analysis (Thompson et

Supported by: NIH; Grant numbers: CA 70145, ES 06694; Melanoma Research Foundation.

*Correspondence to: Dr. Linda M. Sargent, 1095 Willowdale Road, MS-3014, Morgantown, WV 26505. E-mail: lqsl@cdc.gov

Received 16 November 2000; Accepted 23 January 2001

al., 1995). In addition, we have characterized marker chromosomes as well as three new translocations that were not detected in malignant melanoma by other methods.

MATERIALS AND METHODS

Cell Lines

Fifteen early passage cell lines prepared from malignant melanoma samples were obtained from the Arizona Cancer Center Tissue Culture Core. The cell lines were grown in Arizona M15 medium (Arizona Cancer Center, Tucson, AZ) supplemented with 5% fetal bovine serum. The cultures were grown tightly capped in a 37°C incubator. When the cells were 70% confluent, a range of 1 mg/ml to 100 mg/ml Colcemid (Gibco BRL, Rockville, MD) was added. Cell lines that were resistant to Colcemid (Gibco BRL) were given the higher dose. After a 10-h incubation, cells were harvested for chromosome banding, spectral karyotyping, and FISH analysis with 0.075 M KCl for 30–90 min and fixed using 3/1 acetic methanol/acid fixative.

Methods for Spectral Karyotyping

The experiments were carried out using commercial probes purchased from Applied Spectral Imaging (Carlsbad, CA). Spectral karyotyping (SKY) and inverted DAPI banding were carried out by standard methods (Schrock et al., 1996). At least 20 metaphase cells of good banding morphology and spectral hybridization quality were analyzed for each tumor. Chromosome abnormalities were included in the karyotype of a tumor if they occurred in at least 40.0% of the metaphase spreads. Translocations were confirmed with FISH using Vysis direct-labeled chromosome painting probes (Vysis Inc., Downers Grove, IL).

Statistics

Chromosome regions with elevated breakage were tested for statistical significance by the chi-square test. The *P*-values were corrected for multiple comparisons using the Bonferroni correction.

RESULTS

Molecular cytogenetic analysis of early passage malignant melanoma cell lines was performed using spectral karyotyping, FISH, and inverted DAPI banding. The statistically significant gains and losses of chromosomal material are summarized in Table 1. Nonrandom loss of the short arms of chromosomes 1 and 10 was observed in all of the tumor lines. The minimal regions of deletion were 1p36 to pter (14/15), 10q23 (15/15), and 19q13 (14/15). There was a significant, but less frequent loss of 2p13 to pter (12/15), 3p21 to pter (11/15), 13p11 to pter (12/15), and 14q14 to qter (9/15). Other common losses included 4p16 to pter (12/15), 6q22–24 (12/15), 9p21 to pter (14/15), 12p11 to pter (13/15), 12q12–15 (11/15), and 13q21 to qter (12/15). There was a gain of chromosomal material at 6p22 to pter (13/15) and 20q11 to qter (14/15). Statistically significant gain of 1q32 to 41 (11/15) was also observed. There was a significant, but lower frequency gain of 3p14 to pter (11/15), 7q31 to qter (10/15), 15q11 to qter (7/15), and 17q23 to qter (9/15). The recurring breakpoints of chromosomes 1, 4, 6, 9, 10, 12, 19, and 20 were the most frequent karyotypic changes in the melanoma cell lines.

TABLE 1. Chromosome Abnormalities Detected in Human Malignant Melanoma Cell Lines by SKY

	Ratio of tumors with aberration
Chromosomal deletions	
1p36 to pter	14/15
2p13 to qter	12/15
3p11–21 to pter	11/15
4p16 to pter	12/15
6q22–24	12/15
9p21 to pter	14/15
10q23 to qter	15/15
12q13 to qter	11/15
13q21 to qter	12/15
14q14 to qter	9/15
15q11 to qter	7/15
16q23 to qter	11/15
19q13 to qter	14/15
Chromosomal gains	
1q32–41	11/15
6p22 to qter	13/15
7q31 to qter	10/15
12p11 to qter	13/15
15q11 to qter	7/15
17q23 to qter	9/15
20q11 to qter	14/15
Translocations	
der(19)t(10;19)(q23;q13)	4/15
der(12)t(12;20)(q15;q11)	4/15
der(12)t(12;19)(q13;q13)	3/15
der(1)t(1;6)(p31;p22)	6/15
der(6)t(1;6)(q32;q21)	3/15

Common chromosome break sites that were observed by spectral karytoyping analysis and confirmation by fluorescence in situ hybridization. At least 20 karyotypes were analyzed for each of the 15 early passage cell lines. Chromosome changes that occurred in at least 40.0% of the cells from a cell line were included in the karyotype. The band regions are indicated next to the chromosome numbers that are listed in numerical order. The data are expressed as the ratio of the number of cell lines that had the specific band change to the total number of tumor cell lines that were analyzed. The table is divided into chromosomal deletions, gains, and translocations.

There were numerous aberrations of chromosome 10 that resulted in the loss of 10q23 to the end of the chromosome (Table 2). The translocation, der(19)t(10;19)(q23;q13), is illustrated in the composite spectral photograph of cell lines UACC 502, UACC 647, and A 375 (Fig. 1). The translocation was confirmed by fluorescence in situ hybridization. In addition to the loss of chromosome 10, there were numerous aberrations that resulted in the loss of chromosome band 19q13 (Table 1).

There were two translocations involving chromosomes 1 and 6. The translocation involving the short arm of 6, der(1)t(1;6)(p22;p22), was found in 6/15 of the cell lines, whereas a less common translocation of the long arm, der(6)t(1;6)(q21;q13), was seen in 3/15 of the cell lines (Tables 1, 2).

Two translocations involving chromosome 12 were observed in 50.0% of tumor cell lines; 4/15 of the tumor lines had the translocation, 4/15 der(12)t(12;20)(q15;q11) (Fig. 2), and 3/15, der(12)t(12;19)(q13;q13), (Fig. 3). A minor translocation involving the short arm of 12, der(12)t(12;20)(p15;q11), was also detected. There was a net gain of chromosome band 20q11ter and a loss of 12p11 to the top and 12q13 to the end. The identity of these translocations was confirmed by FISH.

The translocation between 12 and 19, der(12)t(12;19)(q13;q13), was present in 4/15 of the cells (Table 1, Fig. 3). There was a net loss of 12q13 to qter and of 19q13. The identity of the chromosomes involved in these translocations was determined by FISH.

The loss of 12p11 in 13/15 of and 16q23 in 11/15 of the tumor cell lines is a unique finding. Duplications of chromosome material on 20q were present in 14/15 of the tumor cell lines. Extra copies of chromosome 20 were in 4/15 and translocations involving chromosome 20 in 6/15 and isochromosomes of 20q in 5/15 of the tumor cell lines.

DISCUSSION

Karyotypic alterations in tumors identify chromosomal regions that are important in disease progression. The resulting changes in gene expression include downregulation of tumor suppressor genes, increased expression of genes that favor growth, and expression of fusion gene products that may exhibit altered activities. The consistent amplification of 20q11, 1q32, and 6p23 material would suggest the selection for genes that confer a growth advantage. The significant loss of 1p11–36, 3p11–21, 4p11–16, 6q22–24, 10q24, 12p11, 12q15–23, 13q21, and 19q13 would indicate that these regions contain candidate genes for growth suppression. The translocations of der(1)t(1;6)(p21;p22), der(6)t(1;6)(q21;q13) der(12)t(12;20)(q15;q11), der(19)t(10;19)(q23;q13), and der(12)t(12;19)(q13;q13) indicate the possibility of new fusion genes or altered gene regulation. We are currently mapping the break points to identify the critical genes that are altered.

The loss of human chromosome 1 has been reported frequently in malignant melanoma. Microcell fusion with the 1p36 region has been shown to suppress tumorigenicity (Dracopoli et al., 1989). Studies of familial melanoma suggest linkage to 1p (Goldstein et al., 1996). The minimal region of loss of 1p36 in malignant melanoma has been reported to involve the mutation, deletion, and decreased expression of the *PITSLRE* protein kinase (Nelson et al., 1999). The *CDC2L* locus encoding the *PITSLRE* protein kinases maps to 1p36.3. The *PITSLRE* protein kinase is a down-stream regulator of apoptosis signal transduction pathways, and alterations in *PITSLRE* can result in the loss or deregulation of apoptotic signaling (Ariza et al., 1999). Thus, a transformed cell may gain a growth advantage by the loss of a gene that regulates cell death. The spectral results indicated numerous translocations and deletions of the short arm of chromosome 1. The structural rearrangements between chromosomes 1 and 6 in malignant melanoma have been reported previously (Guan et al., 1998).

The alterations in chromosome 6 involved an increased copy of the short arm and deletion of the long arm. This is consistent with changes reported previously (Bastian et al., 1998; Su et al., 2000). The long arm of chromosome 6 has been shown to have genes that suppress the tumor phenotype (Su et al., 2000), indicating the possible presence of tumor suppressor genes.

The short arm of chromosome 9 was lost in 14 of the 15 tumor cell lines. The high incidence of this loss indicates that genetic aberrations of this chromosome are important in the development of the disease. This loss has been observed by chromosome banding analysis as well as comparative genomic hybridization (Thompson et al., 1995; Bastian et al., 1998). There is evidence that a mutation in the *CDKN2A* gene at 9p21 is correlated with familial melanoma (Hussussian et al., 1994). The loss of 9p21 material is an early event in the development of malignant melanoma (Bastian et al., 1998; Balazs et al., 1999) and has been reported in dysplastic nevi (Cowan et al., 1988). In late stage disease, there is often a homozygous deletion of

TABLE 2. Modal Karyotype of Melanoma Cell Lines

1. A375
59–83, XXY, der(1)t(1;6)(q21;p22), der(1)t(1;16)(p13;q22), der(2)t(2;20)(p16;q11), der(2)t(2;9)(p13–q32;q13), der(2)t(2;19)(p15–q32;q12), der(3)t(3;13)(p14;q22), −3, del(4)(q12), der(4) t(4;8)(p12;q11), −5; −6, der(6)t(6;Y)(q16;q11), der(6)t(6;22)(q11;q11), del(9)(p11), −10, del(10)(q23), i(11q), del(12)(q23), −12; −13, der(19)t(10;19)(q23;q13), dup(20)(q11) (modal number = 63)
2. UACC 502
70–79, XXY, del(1)(p32), −1, der(1)t(1;6)(1p22, 6p12), del(4)(p11), del(4)(q11), der(6)t(6;Y)(q24;q11), der(6)t(1;6)(q32;q21), +7, −8, der(9)t(9;13)(p11;q12), −10, der(11)t(11;13)(p15;q22), t(12;20;Y)(q15;q11;q11), t(12;19)(q13;q13), der(1)t(1;16)(q32; q13), der(16)t(1;4;16)(q13;q21;q32–42), der(17)t(15;17)(q22;q21), −18, der(19)t(10;19)(q23;q13); +20, −21 (modal number = 74)
3. UACC 647
64–79, XXY, der(1)t(1;5)(q31;q31), der(4)t(3;4)(p14;p11), −4, −5, der(5)t(5;7)(q21;q31), der(6)t(6;9)(p12;q12), der(6)t(6;16)(q23;q13), der(6)t(6;21)(p11;p11), der(7)t (7;16)(q21;p11), del(9)(q12), t(10;19)(q23;q13), i(13q), der(14)t(1;14)(q32; 1q11), der(15)t(5;15)(q31;p11), −16 (modal number = 72)
4. UACC 827
55–65, XX, i(1q), der(1)t(1;11;3)(q12;q14;q21), del(2)(p15), −2, del(3)(p21), −3, −4, del(6)(q13), der(6)t(6;22)(p21;p11); −8, der(9)t(9;13)(p21;q13), −9, −10, del(11)(q14), i(12p), del(13)(13q), der(12)t(12;13)(p11;q11), −14, del(14)(q24), der(14)t(1; 14)(q32–42; q31), der(15)t(9;15)(q31;q22), der(15)t(15;22)(p11;p11), −16, der(16)t(1;16)(q41;p13), −17, −19, der(19)t(10;19)(q23;q13), i(20q), −22 (modal number = 63)
5. UACC 1022
51–64, XX, −Y, del(1)(p21), −1, der(1)t(1;22)(p11–q32;q11), der(1)t(1;7;16)(p13–q32;q11–31;q23), der(2)t(2;19)(p11;q13), del(2)(p21), −2, −3, del(3)(p14), −4; der(4)t(4;6)(p16–q21;p12); del(4)(p15), −5, der(6)t(1;6)t(q32–42;q24), dup(7)(q11–31), +7, i(8q), der(9)t(3;9;15)(q11;p11;p14), del(9)(p13), i(10q), der(10)t(10;17)(q24;q23), del(11)(q23), +11, del(12)(p11), der(13)t(9;13)(p11;q11), der(14)t(14;22)(p11;q11), −16, del(16)(p12), −17, −18, del(19)(q13), i(20q), +20, −22, der(X)t(3;19; X)(q22–32;q13;q21) (modal number = 58)
6. UACC 1227
44–95, XY, der(1)t(1;6)(p21;p22), del(1)(p36), del(2)(p13), del(3)(p21), der(3)t(3;11)(q24;q21), del(4)(p15), der(6)t(1;6)(q22;q22), del(7)(q31), +7, der(7)t(7;12)(q11;p12), del(8)(q21), del(9)(p11), der(10)t(10;17)(q11;q22), del(10)(q24), del(11)(q22), −13, der(16)t(1;16)(q21;q32), del(16)(q22), del(19)(q13), add(20)(q11), i(20q), i(Yq) (modal number = 93 [2/20 cells had 44 chromosomes])
7. UACC 1460
49–79, XXX, der(1)t(1;17)(p11;p8), del(1)(p36), −2, del(2)(p13), del(3)(p21), −3, del(4)(p15), −4, −5, der(6)t(6;10)(p11;q23), +7, del(9)(p21), −10, del(10)(q23), +11, der(12)t(12;19)(q13;q13), der(13)t(13;20)(p11;q11), −14; −15, del(16)(q22), −16; −17, −18, der(19)t(19;22)(p11;q11), i(21q) (modal number = 74 [2/20 cells had 49 chromosomes])
8. UACC 375
63–136, XXYY, der(1)t(1;6)(p36;p11), −2, del(2)(p36), der(2)t(2;17)(q32;p12), del(3)(p21), der(4)t(4;22)(p11;q11), −5, −6, der(6)t(1;6)(q36–42;p21), der(8)t(7; 8)(q31; q23–24), del(9)(p21), der(9)t(9;21)(p11;q11), −10, del(10)(q24), del(11)(p14), del(12)(p11), der(13)t(11;13)(q22;q23), der(14)t(14;8)(p11;q23), der(14)t(14;15)(p11;q13), −16, der(17)t(3;17)(p21;q23), t(12; 19)(q13;q12), der(20)t(13;20)(q14;q11), der(20)t(12;20)(q15;q11). −22 (modal number = 70)
9. UACC 929
50–75, XY, der(1)t(1;11)(p11;q11), der(1)t(1;16)(p11;q21), del(1)(p36), −1, der(3)t(3;9)(p11;p21), −4, −6, der(7)t(7;17)(p15; q22), +7, −8, der(9)t(3;9)(p12–21;p11), −10, del(10)(q23), der(11)t(1;11)(q12;p11), −11, i(12p), del(12)(q15), −13, del(16)(q12), −17, t(7;17)(q12;p21), −19, +20, del(20)(q11), −X, −Y (modal number = 71)
10. UACC 1237
63–68, X, −X, der(1)t(1;6)(p11;p21), del(1)(q32), −1, del(2)(p21), −2, der(3)t(1;3)(q21;q27–29), −4, −5, −6, −9, der(10)t(9; 10)(q13;q11), −12, −13, der(13)t(6;13)(p11;p11), −14, del(14)(q23), −15, −16, −17, −X (modal number = 67)
11. UACC 903
41–50, X, −X, del(1)(p34), der(1)t(1;1)(q31;p36), der(1)t(1;11)(p34;q23), del(2)(p16), del(3)(p21), der(4)t(4;15)(q22;q15), +7, del(7)(q31), der(8)t(8;15)(q21;q15), del(10)(q23), der(11)t(1;11)(q32–42;q22), der(12)t(12;20)(q22;q11), del(13)(q21), del(14)(q21), −15, add(17)(q21–23), der(19)t(14;19)(q22;q13), der(20)t(2;20)(q31;q11), +21 (modal number = 44)
12. UACC 1065
43–75, X, −X, del(1)(p21), del(1)(p36), −2, del(2)(p14), del(3)(p14), del(3)(p21), der(4)t(4;8)(q22;q21), −4, i(6p), del(6)(q22), i(8q), der(8)t(8;4)(q13;q24), del(7)(q31), +8, −9, del(9)(p21), −10, del(10)(q24), −12, del(12)(q21), −13, del(14)(q21), −16, der(17)t(16;17)(p11;p11), −19, del(19)(q13), +20, del(X)(q22) (bimodal cell number, modal number = 43, and modal number = 66)
13. UACC 612
65–123 XXX, del(1)(p35), −1, −2, del(2)(p11), del(3)(p21), −3, der(3)t(3;5)(p21;p14), del(4)(p15), −4, i(6p), −6, del(7)(q11), −9, −10, −11, −12, −13, −14, der(16)t(16;17)(q11;q21) two copies, −17, −18, +20, add(20)(q11) (modal number = 68)
14. UACC 457
55–69 XX, i(1q), del(1)(p36), −2, del(3)(p21), der(3)t(3;5)(p21;p14), −4, −5, del(6)(q22), der(7)t(7;15)(p11;q11), der(9)t(9; 20)(p24;q11),del(10)(q23), −11, −12, der(12)t(12;X)(q22;q24), del(12)(q15), −13, −16, −18, −19, −19, i(20q) (modal number = 68)
15. UACC 2565
42–50 XY, der(1)t(1;6)(p11;p11), der(1)t(1;15)(p36;q22), +1, der(2)t(2;6)(p13;q22), del(2)(p16), del(3)(p21), der(3)t(3;15)(p21; q22), i(3p), del(4)(p15), dup(4)(p15), der(5)t(5;17)(p15;q23), der(6)t(6;2)(q22;p12), del(6)(q22), +7, del(7)(q31), der(7)t(7;15)(q31;q22), der(7)t(3;7)(p11;p14), der(9)t(9;13)(p21;q22), del(9)(p21), der(9)t(1;9)(p36;p22), der(10)t(1;4;10)(q24; p14;p11), der(10)t(6;10)(p22;q21), del(10)(q22), del(11)(q22), del(12)(p12), del(12)(q21), t(12;20)(q15;20q11)(50.0% of the cells), −13, der(14)t(14;19)(p11;q13), der(15)t(15;3)(q21;q14), del(16)(q22), del(17)(p11), del(19)(q13) (modal number = 49)

Modal karyotype and chromosome number present in the melanoma cell lines. At least 20 metaphase cells of good banding and spectral hybridization quality were analyzed for each tumor. Only aberrations that were observed in at least 40.0% of the cells were included in the karyotype.

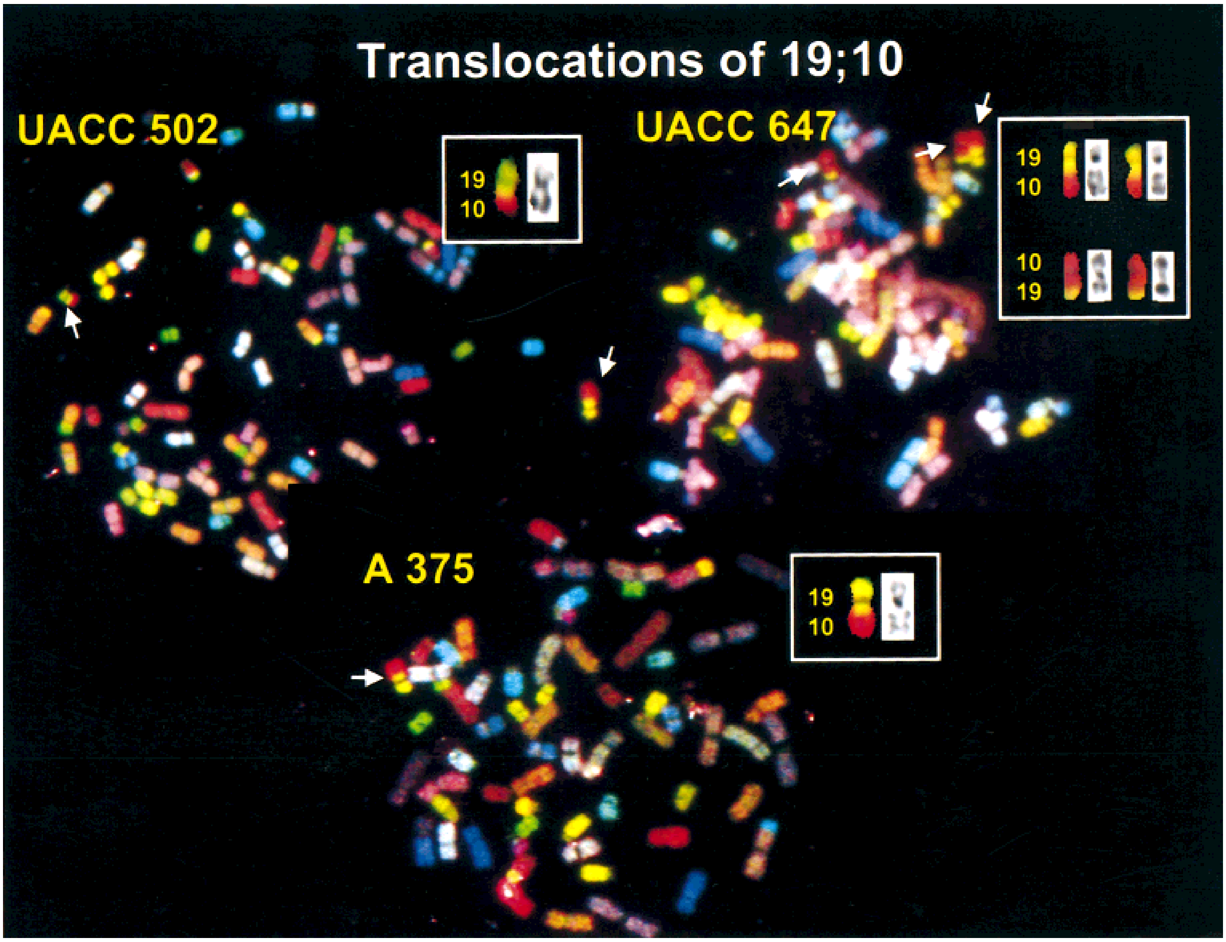

Figure 1. A composite of three spectral images of tumor cell lines UACC 502, UACC 647, and A 375. The translocation of 19 and 10 is separated in a white box to the right of the spectral image. The location of the translocation of 19 and 10 in the spectral image is indicated by a white arrow.

the 9p21 region in both sporadic and familial cases (Hussussian et al., 1994; Kumar et al., 1998).

A high incidence of chromosome 10 loss in malignant melanoma has been reported previously (Thompson et al., 1995; Bastian et al., 1998; Nelson et al., 1999). The loss of 10q in dysplastic nevus indicates that this occurs early in the progression of the disease (Parmiter et al., 1988). The *MXI1* tumor suppressor gene has been mapped to chromosome band 10q25, and this gene has been demonstrated to be lost in desmoplastic melanoma. The loss of *MXI1* in malignant melanoma is independent of tumor stage, indicating that this is an early event (Rao et al., 1999). Significant loss of chromosome 10 has also been reported in gliomas and prostate carcinomas (Gray et al., 1995; Steck et al., 1999). Other genes within this region of chromosome 10 have been implicated as tumor suppressor genes, including *MMAC/PTEN* and *DMBT1* (Steck et al., 1999). The loss of 10q in all of the melanoma cell lines further emphasizes the importance of the deletion of this region. Translocations involving chromosome 10 with other chromosomes have also been reported in dysplastic nevi (Parmiter et al., 1988; Nourse et al., 1990). The observation of the translocation der(10)t(10;19)(q23;q13) indicates not only that the loss of genetic material may be important, but also that a fusion gene may be involved in the progression of the disease. Further investigations will determine whether the previously unidentified translocation of 10 and 19 is an early or a late event in the disease.

The deletion of 12p13 and 12q13–24 has been reported in colon, head and neck, and lung cancer as well as testicular germ cell tumors and malignant gliomas (Becher et al., 1997; Bigner and Schrock, 1997) and malignant melanoma (Mertens et al., 1997). Germline mutations in the *CDK4* region of chromosome arm 12q have been reported in familial melanoma (Zuo et al., 1996). The identification

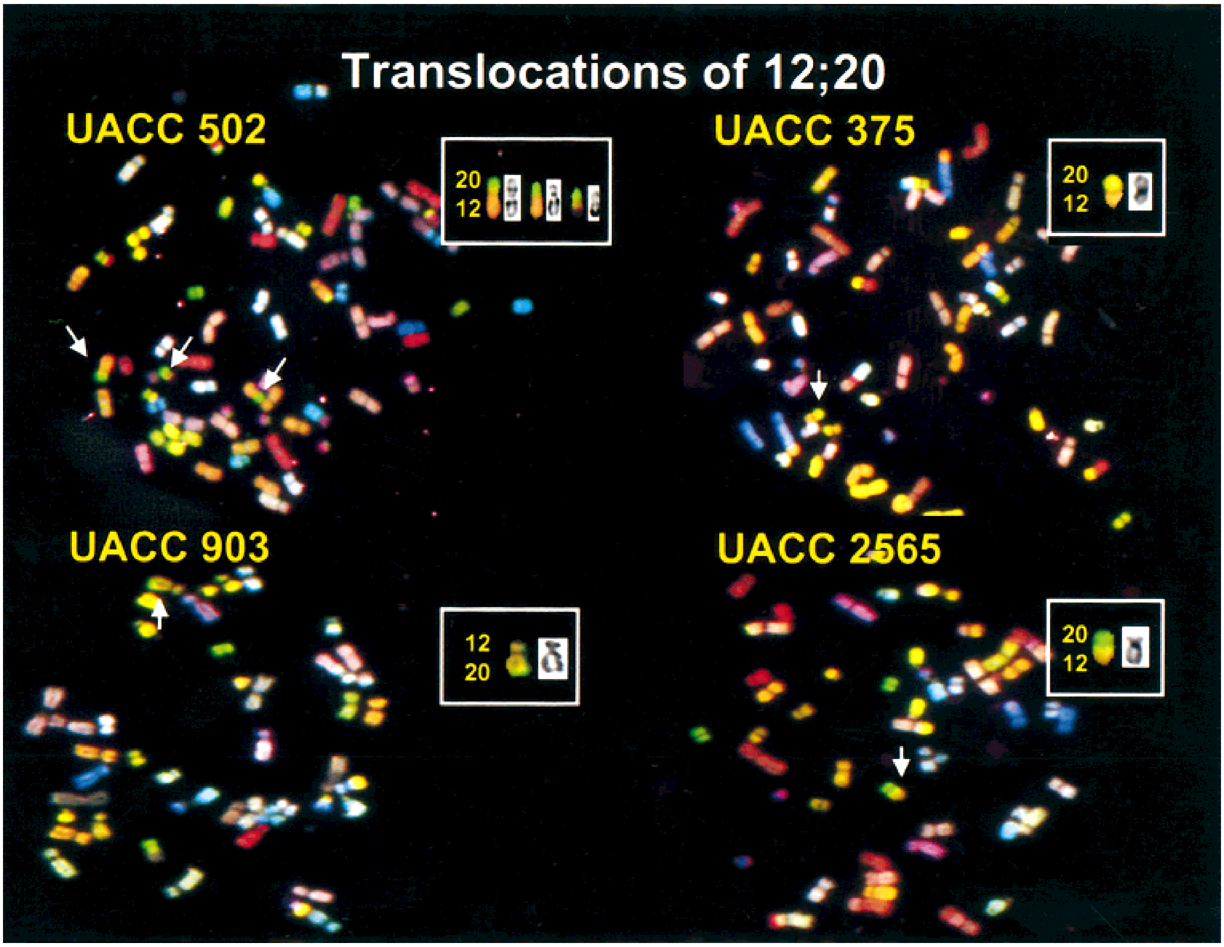

Figure 2. The figure is a composite of the spectral images of tumor cell lines UACC 502, UACC 375, UACC 903, and UACC 2565 demonstrating the translocation of 12 and 20. The location of the translocation in the spectral image is indicated by a white arrow. The translocation is separated by a white box to the right of the spectral image.

of more than one translocation of chromosome 12 indicates that genes in this region may be involved in sporadic melanoma.

Although a clustering of breaks was reported in metaphase chromosomes prepared from metastatic melanoma samples from 158 patients (Thompson et al., 1995), the rearrangement of chromosome 19 was missed in previous studies because of the difficulty of identifying the two translocations der(12)t(12;19)(q13;q13) and der(10)t(10;19)(q23;q13) by traditional Giemsa banding analysis. Interestingly, a similar translocation, t(10;19)(q24;q13), was reported in malignant glioma cells (Chernova and Cowell, 1998).

An increased copy number of the long arm of chromosome 20, q11–13, has been reported in malignant melanoma (Barks et al., 1997). The consistent rearrangement der(12)t(12;20)(q15;q11) had not been characterized previously. Amplification of chromosome 20 has been reported in breast cancer, ovarian cancers, and erythroleukemia (Tanner et al., 1994; Saito et al., 1995). The amplification of chromosome arm 20q in a number of tumors indicates the presence of a gene or genes that contribute to tumor progression.

Conventional banding analysis has previously demonstrated alterations of chromosomes 1, 6, 10, 12, and 20 in malignant melanoma. Previous identification of small rearrangements of chromosomes 10, 12, 19, and 20 were not possible with standard banding analysis. In this study, we have been able to identify a high frequency of alteration of chromosome 19 in malignant melanoma that was not detected before. In addition, we have been able to demonstrate three unique translocations that had not been identified by other cytogenetic methods. The mapping of the critical genes in melanoma has been hindered by the large regions of deletion.

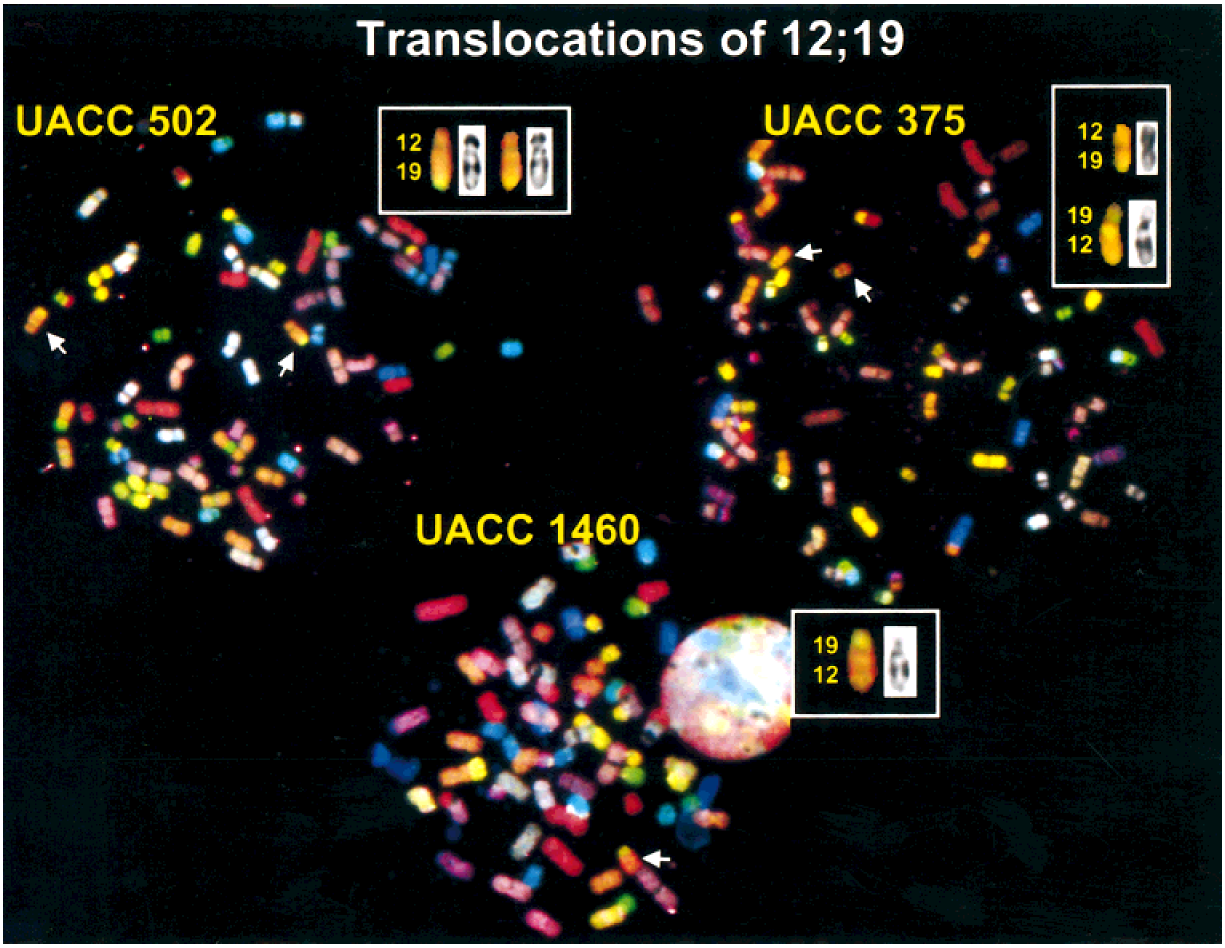

Figure 3. The figure is a composite of the spectral images from tumor cell lines UACC 502, UACC 375, and UACC 1460. The location of the translocations of chromosomes 12 and 19 in the spectral image is indicated by a white arrow. The banded and spectral image of the translocation is separated by a white box to the right of the spectral image.

The mapping of specific translocations will facilitate the identification of these critical genes. Further studies will examine the minimal break points as well as the possibility of fusion genes that are unique to melanoma.

REFERENCES

Ariza ME, Broome-Powell M, Lahti JM, Kidd VJ, Nelson MA. 1991. Fas-induced apoptosis in human malignant melanoma cell lines is associated with the activation of the p34(cdc2)-related PITSLRE protein kinases. J Biol Chem 274:28505–28513.

Balazs M, Adam Z, Begany A, Tukruri AT, Adany R. 1999. Involvement of chromosome losses in the progression and metastasis formation of a human malignant melanoma. Cancer Genet Cytogenet 109:114–118.

Barks JH, Thompson FH, Taetle R, Yang JM, Stone JF, Wymer JA, Khavari R, Guan XY, Trent JM, Pinkel D, Nelson MA. 1997. Increased chromosome 20 copy number detected by fluorescence in situ hybridization (FISH) in malignant melanoma. Genes Chromosomes Cancer 19:278–285.

Bastian BC, LeBoit PE, Hamm H, Brocker EB, Pinkel D. 1998. Chromosomal gains and losses in primary cutaneous melanomas detected by comparative genomic hybridization. Cancer Res 58: 2170–2175.

Becher R, Korn WM, Prescher G. 1997. Use of fluorescence in situ hybridization and comparative genomic hybridization in the cytogenetic analysis of testicular germ cell tumors and uveal melanomas. Cancer Genet Cytogenet 93:22–28.

Bigner SH, Schrock E. 1997. Molecular cytogenetics of brain tumors. J Neuropathol Exp Neurol 56:1173–1181.

Brozena SF, Fenske NA, Perez IR. 1993. Epidemiology of malignant melanoma, worldwide incidence, and etiologic factors. Semin Surg Oncol 9:165–167.

Chernova O, Cowell JK. 1998. Molecular definition of chromosome translocations involving 10q24 and 19q13 in human malignant glioma cells. Cancer Genet Cytogenet 105:60–68.

Cowan JM, Halaban R, Francke U. 1988. Cytogenetic analysis of melanocytes from premalignant nevi and melanomas. J Natl Cancer Inst 80:1159–1164.

Dracopoli NC, Harnett P, Bale SJ, Stanger BZ, Tucker MA, Housman DE, Kefford RF. 1989. Loss of alleles from the distal short arm of chromosome 1 occurs late in melanoma tumor progression. Proc Natl Acad Sci USA 86:4614–4618.

Everall JD, Dowd PM. 1978. Influence of environmental factors excluding ultra violet radiation on the incidence of skin cancer. Bull Cancer 65:241–247.

Goldstein AM, Goldin LR, Dracopoli NC, Clark WH Jr, Tucker MA. 1996. Two-locus linkage analysis of cutaneous malignant melanoma/dysplastic nevi. Am J Hum Genet 58:1050–1056.

Gray IC, Phillips SM, Lee SJ, Neoptolemos JP, Weissenback J, Spurr NK. 1995. Loss of the chromosomal region 10q23–25 in prostate cancer. Cancer Res 55:4800–4803.

Guan XY, Zhang H, Yang JM, Wang J, Taetle R, Meltzer PS, Trent JM. 1998. Detection of chromosome 6 abnormalities in melanoma cell lines by chromosome arm painting probes. Cancer Genet Cytogenet 107:89–92.

Herlyn M. 1993. Molecular and cellular biology for melanoma. Austin, TX: RG Landes Co. p 1–102.

Hussussian CJ, Struewing JP, Goldstein AM, Higgens PA, Ally DS, Sheahan MD, Clark WH Jr, Tucker MA, Dracopoli NC. 1994. Germline p16 mutations in familial melanoma. Nat Genet 8:15–21.

Kakazu N, Taniwaki M, Horlike S, Nishida K, Tatekawa T, Nagai M, Takahashi T, Akaogi T, Inazawa J, Ohki M, Abe T. 1999. Combined spectral karyotyping and DAPI banding analysis of chromosome abnormalities in myelodysplastic syndrome. Genes Chromosomes Cancer 26:336–345.

Kumar R, Lundh Rozell B, Louhelainen J, Hemminki K. 1998. Mutations in the CDKN2A (p16INK4a) gene in microdissected sporadic primary melanomas. Int J Cancer 75:193–198.

Loomis D, Browning SR, Schenck AP, Gregory E, Savitz DA. 1997. Cancer mortality among electric utility workers exposed to polychlorinated biphenyls. Occup Environ Med 54:720–728.

Mertens F, Johansson B, Hoglund M, Mitelman F. 1997. Chromosomal imbalance maps of malignant solid tumors: a cytogenetic survey of 3185 neoplasms. Cancer Res 57:2765–2780.

Nelson MA, Ariza ME, Yang JM, Thompson FH, Taetle R, Trent JM, Wymer J, Massey-Brown K, Broome-Powell M, Easton J, Lahti JM, Kidd VJ. 1999. Abnormalities in the p34cdc2-related PITSLRE protein kinase gene complex (CDC2L) on chromosome band 1p36 in melanoma. Cancer Genet Cytogenet 108:91–99.

Nourse J, Mellentin JD, Galili N, Wilkinson J, Stanbridge E, Smith SD, Cleary ML. 1990. Chromosomal translocation t(1;19) results in synthesis of a homeobox fusion mRNA that codes for a potential chimeric transcription factor. Cell 60:535–545.

Parmiter AH, Balaban G, Clark WH Jr, Nowell PC. 1988. Possible involvement of the chromosome region 10q24–q26 in early stages of melanocytic neoplasia. Cancer Genet Cytogenet 30:313–317.

Philipp R, Hughes AO, Robertson MC, Mitchell TF. 1983. Malignant melanoma incidence and association with arsenic. Bristol Med Chir J 98:165–169.

Rao UN, Bakker A, Swalsky PA, Finkelstein SD. 1999. Max interacting protein 1: loss of heterozygosity is frequent in desmoplastic melanoma. Mod Pathol 12:344–350.

Rushton L, Alderson MR. 1981. An epidemiological survey of eight oil refineries in Britain. Br J Ind Med 38:225–234.

Saito M, Helin K, Valentine MB, Griffith BB, Willman CL, Harlow E, Look AT. 1995. Amplification of the E2F1 transcription factor gene in the HEL erythroleukemia cell line. Genomics 25:130–138.

Schrock E, du Manior S, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA, Ning Y, Ledbetter DH, Bar-Am I, Soenksen D, Garini Y, Ried T. 1996. Multicolor spectral karyotyping of human chromosomes. Science 273:494–497.

Steck PA, Lin H, Langford LA, Jasser SA, Koul D, Yung WK, Pershouse MA. 1999. Functional and molecular analyses of 10q deletions in human gliomas. Genes Chromosomes Cancer 24:135–143.

Su YA, Bittner ML, Chen Y, Tao L, Jiang Y, Zhang Y, Stephan DA, Trent JM. 2000. Identification of tumor-suppressor genes using human melanoma cell lines UACC903, UACC903(+6), and SRS3 by comparison of expression profiles. Mol Carcinog 28:119–127.

Tanner MM, Tirkkonen M, Kallioniemi A, Collins C, Stokke T, Karhu R, Kowbel D, Shadravan F, Hintz M, Kuo WL, Waldman FM, Iola JJ, Gray JW, Kallioniemi O-P. 1994. Increased copy number at 20q13 in breast cancer: defining the critical region and exclusion of candidate genes. Cancer Res 54:4257–4260.

Thompson FH, Emerson J, Olson S, Weinstein R, Leavitt SA, Leong SPL, Emerson S, Trent JM, Nelson MA, Salmon SE, Teatle R. 1995. Cytogentics of 158 patients with regional or disseminated melanoma. Cancer Genet Cytogenet 83:93–104.

Zuo L, Weger J, Yang Q, Goldstein AM, Tucker MA, Walker GJ, Hayward N, Dracopoli NC. 1996. Germline mutations in the p16INK4a binding domain of CDK4 in familial melanoma. Nat Genet 12:97–99.