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BRIEF COMMUNICATIONS

# Characterization of Chromosome 1 Abnormalities in Malignant Melanomas

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Chromosome 1 abnormalities are the most commonly detected aberrations in many cancers including malignant melanomas. Specific breakpoints are reported for malignant melanomas throughout the chromosome but especially at 1p36 and at several sites throughout 1p22-q21. In addition, partial deletions and loss of heterozygosity have been found on 1p indicating the possible location of tumor suppressor genes. Here we have characterized the involvement of chromosome 1 in a series of seven malignant melanoma cell lines. Initial chromosome painting studies revealed that six of the cell lines had chromosome 1 rearrangements. Deletions involving 1p10-32, 1q11-44, and 1q25-44 were observed. The other rearrangement breakpoints included three in the 1q10-p11 region with the rest at 1p36, 1p34, 1p32, 1p31, 1p12-13, 1q21, and 1q23. The breaks at 1q10-p11 were investigated further using an alpha-satellite 1 centromere probe and yeast artificial chromosomes (YACs) from the region. Two of the 1q10-p11 breaks mapped in the centromeric region, while the others mapped to variable sites. This suggests that the role of these rearrangements in the pathogenesis of melanomas does not involve the alteration of specific oncogenes in the breakpoint region. During the YAC mapping a previously undetected, small (<1 Mbp) del(1)(p10p11) was identified. This deletion lies within minimal overlapping deleted regions reported in head and neck as well as breast carcinomas and it could therefore facilitate the isolation of a carcinoma-associated tumor suppressor gene. *Genes Chromosomes Cancer 28: 121–125, 2000.* © 2000 Wiley-Liss, Inc.

Malignant melanoma is an aggressive cancer of increasing incidence, with UV-radiation playing an important role in the genetic alterations that lead to tumor development (Maestro and Boiocchi, 1995). However, very little is known about the molecular mechanisms underlying the development of this disease, and the identification of associated genetic changes would help to advance our understanding of this process. Cytogenetic analysis of 158 patients has shown that clonal structural abnormalities involving chromosomes 1, 3, 6, 7, 9, and 11 are seen in 59, 25, 46, 34, 26, and 27% of cases, respectively, with breakpoints clustering at 1p36, throughout 1p22-q21, 6p11-q21, 9p, 11q23-ter, 13p, and 19q13 (Thompson et al., 1995). Consistent deletions and loss of heterozygosity (LOH) in 1p (especially distally), 6q, and 9p suggest the involvement of tumor suppressor genes in these regions. The LOH at distal 1p sites is associated with a later, metastatic stage (Walker et al., 1995). Abnormalities in the p34cdc2-related PITSLRE protein kinase gene complex (CDC2L) at 1p36 have recently been detected in a series of melanoma cell lines, suggesting that one or more of these genes may be the putative tumor suppressor gene/cancer susceptibility gene mapping to this region (Nelson et al., 1999). Inactivation of a tumor suppressor gene in 6q12-21, in some cases associated with t(1;6)(q21;q14), has also been suggested as a common mechanism in melanomas (Trent et al., 1990; Welch et al., 1994; Zhang et al., 1995) and candidate genes have been isolated (Ray et al., 1996). *CDKN2A* at 9p21, encoding an inhibitor (p16) of cyclin dependent kinase 4 (CDK4), has been identified as a tumor suppressor gene involved in melanomas as well as other tumor types (Kamb et al., 1994; Weaver-Feldhaus et al., 1994).

Cytogenetic studies have revealed that chromosome 1 is also nonrandomly involved in a number of other malignancies (Schwab et al., 1996; Mitelman, 1998). Although this has generally been interpreted as indicating the location of oncogenes or tumor suppressor genes, the evidence for this is scarce with only limited, detailed associated molecular investigations and few genes from the region(s) have been implicated. Specific chromosome translocations and associated gene fusions are fre-

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quently found in hematological malignancies and sarcomas but have not been identified in carcinomas with the exception of rare examples such as the t(X;1)(p11.1;q21) and variant rearrangements associated with papillary renal cell carcinoma (Sidhar et al., 1996; Clark et al., 1997). In this study, we investigated the potential role of chromosome 1 alterations in the tumorigenesis of malignant melanoma that may have implications for the study of other tumor types. This has been achieved by characterizing the chromosome 1 abnormalities in a series of seven malignant melanoma cell lines by fluorescence in situ hybridization (FISH) in order to determine whether consistent breaks or deletions involving specific oncogenes/tumor suppressor genes were present in 1p36 and other regions of chromosome 1, such as the previously reported clusters throughout 1p22-q21.

The human malignant melanoma cell lines were obtained from various sources and some have been described previously: MM253 and MM96 (Pope et al., 1979) were supplied by Dr. P Parsons (Queensland Institute of Medical Research, Australia). RPMI 7932 (Quinn et al., 1977) was obtained from Professor G.E. Moore (Dept. of Health and Hospitals, Denver, CO, USA). WM1604, WM1480, WM1687, and WM1433 were all derived at the Wistar Institute. Cells were cultured in RPMI 1640 medium containing fetal bovine serum and antibiotics. Metaphase slides were prepared as previously described (Birdsall et al., 1992). Fluorescein isothiocyanate (FITC)-labeled chromosome 1 paint (Cambio, Cambridge, UK) and FITC-labeled alpha-satellite centromere 1 probe (Oncor, Gaithersburg, MD) were used according to the manufacturer's instructions. YAC clones derived from 1p11-q21 and originating from the CEPH Megayac library at the Genethon Corporation were also used. DNA from whole yeast cells containing the individual YACs was isolated as previously reported (Knight et al., 1992). One microgram of YAC DNA was directly labeled by nick translation with fluorescein-11-dUTP (Amersham, Arlington Heights, IL) and the DNA purified by passing through a Sephadex G50 column. Two hundred nanograms of YAC DNA was mixed with 5 μg of human CotI DNA (Gibco BRL, Gaithersburg, MD) and hybridized, in a volume of 11 μl, to metaphases cells from a normal control and the cell lines as described previously (Weber-Hall et al., 1996). Metaphase images were captured using a digital imaging system with a cooled CCD camera (Photometrics, Tucson, AZ) and specialist software (Vysis, Richmond, UK).

TABLE 1. Abnormal Chromosomes Detected by Chromosome 1 Painting in a Series of Seven Malignant Melanoma Cell Lines

| Cell line | Aberrations involving chromosome 1 |
|---|---|
| RPMI 7932 | none |
| MM253 | del(1)(p10-11p32) |
| WM1604 | der(1)t(1;2)(p10-11;q10-11) |
| | der(2)t(1;2)(p10-11;q10-11) |
| WM1480 | add(1)(p36) |
| | der(?)t(?;1)(?;p36) |
| | der(?)t(?;1)(?;q21) |
| WM1687 | add(1)(p10-11) |
| | ins(1;?)(p31;?) |
| | der(?)t(?;1)(?;p34) |
| MM96 | i(1)(q10) or idic(1)(p11) |
| | del(1)(q11) |
| WM1433 | der(1)inv(1)(p12-13q23)del(1)(q25) |
| | der(1)inv(1)(p12-13q23)del(1)(q25)add(1)(p32) |
| | der(?)t(?;1)(?;p32) |

The results of the chromosome painting experiments are summarized in Table 1 and examples shown in Figure 1. All the cell lines except RPMI 7932 showed chromosome 1 abnormalities. Multiple chromosome 1 rearrangements were seen for WM1480, WM1687, MM96, and WM1433 and a large variety of aberrations were observed including deletions, translocations, insertions, isochromosomes, and inversions. The deleted segments were p10/11-p32, q11-44, and q25-44. For the other rearrangements, three of the breaks were within q10-p11 with the rest at 1p36, 1p34, 1p32, 1p31, 1p12-13, 1q21, and 1q23.

The q10-p11 segment was the most commonly involved and these breaks were investigated further using CEPH YACs mapping at this locus and an alpha-satellite 1 centromere probe (Figs. 1 and 2). YACs 799e1 and 758a5 are the most proximal YACs on 1p from available contig data (http://www-genome.wi.mit.edu) and our own mapping studies, and were found to consistently hybridize to 1p11 and the distal end of the heterochromatin (1q12-21) on normal metaphase material. This pattern of hybridization has been reported previously for another YAC that overlaps these two (Jin et al., 1998) and probably indicates the presence of homologous regions on proximal 1p and 1q, evidence for which was recently reported in a mapping study of the Fcγ receptor family (Maresco et al., 1998). The add(1)(p11) breakpoint in WM1687 mapped between YACs 721e10 and 754d5. The del(1)(p11p32) in MM253 was observed to break in 1p11 between

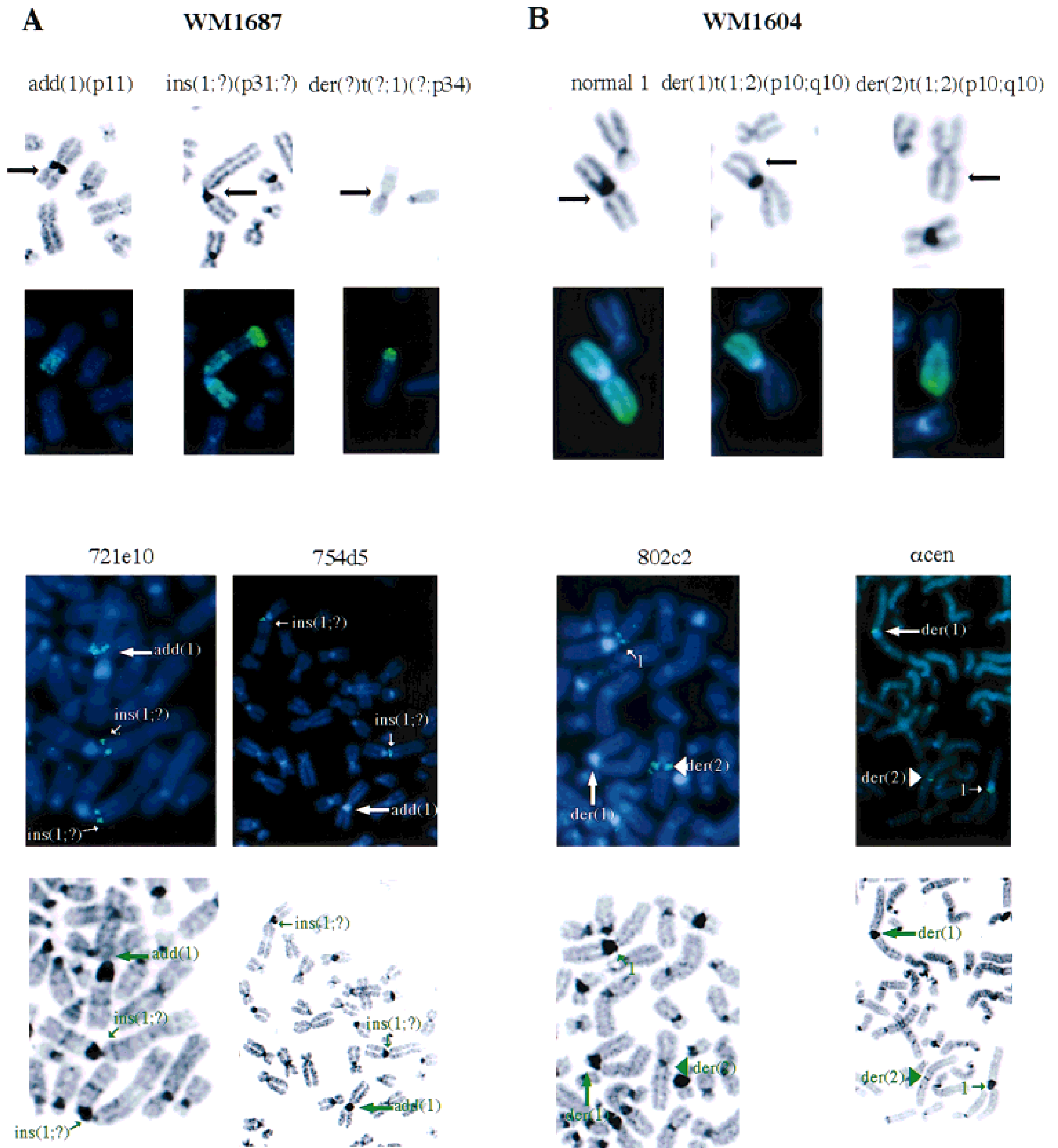


Figure 1. Representative examples of chromosome 1 painting and YAC FISH analysis of the 1q10-p11 region in malignant melanoma cell lines **(A)** WM1687 and **(B)** WM1604. The upper panels show inverted DAPI-banded images and painting of chromosome 1 material. The lower panels illustrate the results of FISH analysis using YACs 721e10, 754d5, 802c2, and an α-satellite chromosome 1 centromere probe. For WM1687 the add(1)(p11) breakpoint was mapped between YACs 721e10 and 754d5. In cell line WM1604, the t(1;2)(p10;q10) breakpoint mapped proximal to YAC 802c2, within the centromeric region.

YACs 820d6 and 799e1. During the YAC mapping of MM253, an apparently normal chromosome 1 was detected that hybridized the centromere probe and all YACs as expected, except that none of the 1p11 signal from YACs 799e1 and 758a5 was present. This indicates the presence of a very small deletion, which we estimate to be <1 Mbp from the available YAC contig data (http://www-genome.wi.mit.edu). As the centromere probe signal is small, it is hard to establish whether one

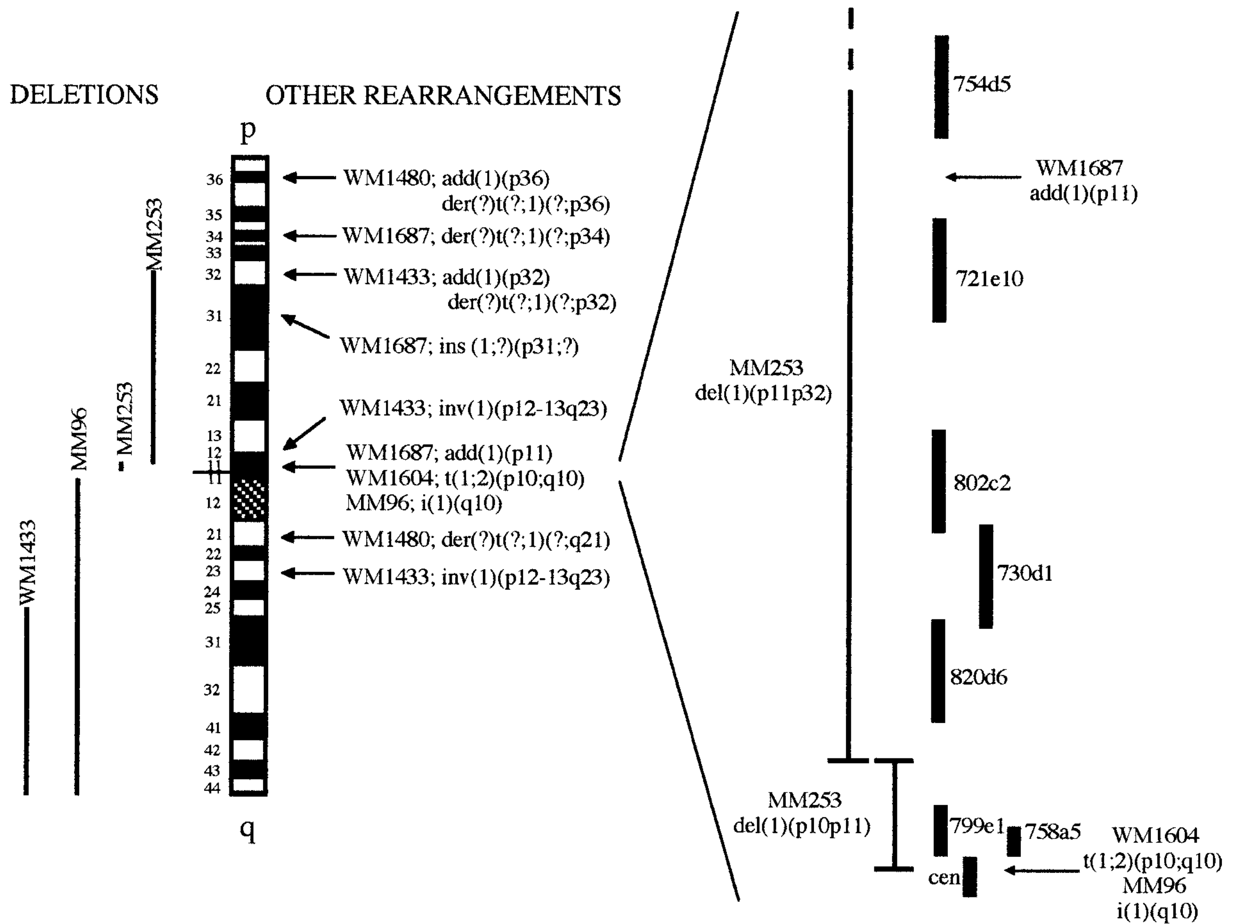


Figure 2. Schematic representation of the chromosome 1 aberrations detected in a series of seven malignant melanoma cell lines. Deletions are represented on the left of the chromosome and breakpoints for the other rearrangements are indicated to the right. On the far right side a magnified view of the 1p10-11 region is shown representing the mapping of breakpoints in cell lines WM1687, WM1604, MM96, and MM253 using YACs 754d5, 721e10, 802c2, 730d1, 820d6, 799e1, 758a5, and an α-satellite 1 centromere probe.

break is actually in the centromere, but taken together with the YAC mapping this appears likely. The other two breakpoints (t(1;2)(p10;q10) in WM1604 and i(1)(q10) in MM96) mapped centromeric to the most proximal 1p YACs (799e1 and 758a5), but centromere probe signal was observed on the der(1) for WM1604 and i(1q) for MM96. Again it is difficult to assess whether breaks were within the centromere or to one side using the small centromere probe, but it is most likely from the YAC results that the breaks are truly centromeric.

This study confirms the previous, cytogenetically reported clusters of breakpoints in melanomas at 1p36 and throughout 1p22-q21 (Thompson et al., 1995) by fluorescence in situ hybridization (FISH) on a series of seven malignant melanoma cell lines. In our study, a break at 1p36 was observed in only one cell line (WM1480). Four of the seven lines showed breaks in the 1p11/centromeric region. However, further investigation revealed no consistent break outside the centromeric region. Two breaks were observed at 1p32, but as one of the rearrangements was a deletion and the other a translocation it is again unlikely that a consistent gene is involved. The results suggest that the role of the 1p breaks in the pathogenesis of melanomas do not involve the alteration of oncogenes at particular breakpoints as seen for many hematological malignancies and some solid tumors. It is possible that the importance of the rearrangements in the development of melanomas is the gain or loss of genes outside the breakpoint regions arising from the resulting genomic imbalances. A similar conclusion was reached following a cytogenetic and FISH study of head and neck carcinomas that also

frequently display chromosome 1 rearrangements affecting the centromeric and proximal 1p regions (Jin et al., 1998). Alternatively, the clustering of breaks may be a symptom of the genomic instability around the centromere.

The small (<1 Mbp), interstitial del(1)(p10p11) in MM253 was of interest as such deletions can be useful in narrowing down the location of tumor suppressor genes. Although previous melanoma studies have detected LOH throughout 1p, the distal region of 1p is the most commonly involved (Dracopoli et al., 1989). However, small minimal overlapping regions of deletion in head and neck as well as breast carcinomas map to this same site in 1p11 (Biéche et al., 1993; Hoggard et al., 1995; Loupart et al., 1995; Jin et al., 1998), suggesting a carcinoma-associated tumor suppressor gene may lie within this region. The fact that the del(1)(p10p11) in MM253 is smaller and overlapping with those reported in these other carcinomas may facilitate the search for this gene.

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## REFERENCES

Biéche I, Champème M-H, Matifas F, Cropp CS, Callahan R, Lidereau R. 1993. Two distinct regions involved in 1p deletion in human primary breast cancer. Cancer Res 53:1990–1994.

Birdsall SH, McLennan KA, Gusterson BA. 1992. t(6;12)(q23;q13) and t(10;16)(q22;p11) in a phyllodes tumor of breast. Cancer Genet Cytogenet 60:74–77.

Clark J, Lu YJ, Sidhar SK, Parker C, Gill S, Smedley D, Hamoudi R, Linehan WM, Shipley J, Cooper CS. 1997. Fusion of splicing factor genes PSF and NonO (p54nrb) to the TFE3 gene in papillary renal cell carcinoma. Oncogene 15:2233–2239.

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.

Hoggard N, Brintnell B, Howell A, Weissenbach J, Varley J. 1995. Allelic imbalance on chromosome 1 in human breast cancer. II. Microsatellite repeat analysis. Genes Chromosomes Cancer 12: 24–31.

Jin Y, Jin C, Wennerberg J, Mertens F, Höglund M. 1998. Cytogenetic and fluorescence *in situ* hybridization characterization of chromosome 1 rearrangements in head and neck carcinomas delineate a target region for deletions within 1p11-1p13. Cancer Res 58:5859–5865.

Kamb A, Gruis NA, Weaver-Feldhaus J, Liu Q, Harshman K, Tavtigian SV, Stockert E, Day RS, Johnson BE, Skolnick MG. 1994. A cell cycle regulator potentially involved in genesis of many tumor types. Science 264:436–440.

Knight JC, Reeves BR, Kearney L, Monaco AP, Lehrach H, Cooper CS. 1992. Localization of the synovial sarcoma t(X;18)(p11.2; q11.2) breakpoint by fluorescence *in situ* hybridization. Hum Mol Genet 1:633–637.

Loupart M-L, Armour J, Walker R, Adams S, Brammar W, Varley J. 1995. Allelic imbalance on chromosome 1 in human breast cancer. I. Microsatellite and RFLP analysis. Genes Chromosomes Cancer 12:16–23.

Maestro R, Boiocchi M. 1995. Sunlight and melanoma: An answer from *MTS1* (p16) (Letter with comment). Science 267:15–16.

Maresco DL, Blue LE, Culley LL, Kimberly RP, Anderson CL, Theil KS. 1998. Localization of FCGR1 encoding Fcγ receptor class I in primates: Molecular evidence for two pericentric inversions during the evolution of human chromosome 1. Cytogenet Cell Genet 82:71–74.

Mitelman F. 1998. Catalog of chromosome aberrations in Cancer '98. CD-ROM. Version 1. New York: Wiley-Liss.

Nelson MA, Ariza ME, Yang JM, Thompson FH, Taetle R, Trent JM, Wymer J, Massey-Brown H, 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 15:91–99.

Ray ME, Su YA, Meltzer PS, Trent JM. 1996. Isolation and characterization of genes associated with chromosome-6 mediated tumor suppression in human malignant melanoma. Oncogene 12:2527–2533.

Schwab M, Praml C, Amler LC. 1996. Genomic instability in 1p and human malignancies. Genes Chromosomes Cancer 16:211–229.

Sidhar SK, Clark J, Gill S, Hamoudi R, Crew AJ, Gwilliam R, Ross M, Linehan WM, Birdsall S, Shipley J, Cooper CS. 1996. The t(X;1)(p11.2;q21.2) translocation in papillary renal cell carcinoma fuses a novel gene PRCC to the TFE3 transcription factor gene. Hum Mol Genet 5:1333–1338.

Thompson FH, Emerson J, Olson S, Weinstein R, Leavitt SA, Leong SPL, Emerson S, Trent JM, Stanbridge EJ, McBride HL, Meese EU, Casey G, Araujo De, Witkowski CM, Nagle RB. 1990. Tumorigenicity in human melanoma cell lines controlled by introduction of human chromosome 6. Science 246:258–271.

Trent JM, Nelson MA, Salmon SE, Taetle R. 1995. Cytogenetics of 158 patients with regional or disseminated melanoma. Cancer Genet Cytogenet 83:93–104.

Walker GJ, Palmer JM, Walter MK, Hayward NK. 1995. A genetic model of melanoma tumorigenesis based on allelic losses. Genes Chromosomes Cancer 12:134–141.

Weaver-Feldhaus J, Gruis NA, Neuhausen S, Le Paslier D, Stockert E, Skolnick M, Kamb A. 1994. Localization of a putative tumor suppressor gene by using homozygous deletions in melanoma. Proc Natl Acad Sci USA 91:7563–7567.

Weber-Hall S, McManus A, Anderson J, Nojima T, Abe S, Pritchard-Jones K, Shipley J. 1996. Novel formation and amplification of the *PAX7-FKHR* fusion gene in a case of alveolar rhabdomyosarcoma. Genes Chromosomes Cancer 17:7–13.

Welch DR, Chen P, Miele ME, McGary CT, Bower JM, Stanbridge EJ, Weismann BE. 1994. Microcell-mediated transfer of chromosome 6 into metastatic human C8161 melanoma cells suppresses metastasis but does not inhibit tumorigenicity. Oncogene 9: 255–262.

Zhang J, Cui P, Glatfelter AA, Cummings LM, Meltzer PS, Trent JM. 1995. Microdissection based cloning of a translocation breakpoint in a human malignant melanoma. Cancer Res 55:4640–4645.