Oncogene (2002) 21, 5975–5980

www.nature.com/onc

Two novel tumor suppressor gene loci on chromosome 6q and 15q in human osteosarcoma identified through comparative study of allelic imbalances in mouse and man

Michaela H Nathrath*[,1,2], Virginija Kuosaite[1], Michael Rosemann[1], Marcus Kremer[3], Christopher Poremba[4], Shigeharu Wakana[5], Masayuki Yanagi[1], Walter BJ Nathrath[6], Heinz Höfler[1,3], Kenji Imai[7] and Michael J Atkinson[1]

[1]Institute of Pathology, GSF-National Research Center for Environment and Health, 85764 Neuherberg, Germany; [2]Department of Pediatrics, Technical University of Munich, 80804 Munich, Germany; [3]Institute of Pathology, Technical University of Munich, 81675 Munich, Germany; [4]Institute of Pathology, Heinrich-Heine-University, 40225 Duesseldorf, Germany; [5]Mouse Functional Genomics Research Group, RIKEN Genomics Sciences Center, Yokohama 224-0804, Japan; [6]Institute of Pathology, Municipal Hospital München-Harlaching, 81545 Munich, Germany; [7]Institute of Mammalian Genetics, GSF-National Research Center for Environment and Health, 85764 Neuherberg, Germany

We have performed a comparative study of allelic imbalances in human and murine osteosarcomas to identify genetic changes critical for osteosarcomagenesis. Two adjacent but discrete loci on mouse chromosome 9 were found to show high levels of allelic imbalance in radiation-induced osteosarcomas arising in (BALB/c × CBA/CA) F1 hybrid mice. The syntenic human chromosomal regions were investigated in 42 sporadic human osteosarcomas. For the distal locus (OSS1) on mouse chromosome 9 the syntenic human locus was identified on chromosome 6q14 and showed allelic imbalance in 77% of the cases. Comparison between the human and mouse syntenic regions narrowed the locus down to a 4 Mbp fragment flanked by the marker genes ME1 and SCL35A1. For the proximal locus (OSS2) on mouse chromosome 9, a candidate human locus was mapped to chromosome 15q21 in a region showing allelic imbalance in 58% of human osteosarcomas. We have used a combination of synteny and microsatellite mapping to identify two potential osteosarcoma suppressor gene loci. This strategy represents a powerful tool for the identification of new genes important for the formation of human tumors.
Oncogene (2002) **21,** 5975–5980. doi:10.1038/sj.onc.1205764

Keywords: osteosarcoma; allelic imbalance; LOH; synteny relationship; inbred mouse strains

Introduction

Osteosarcoma is the most common bone tumor of childhood and adolescence, accounting for approximately 2–3% of all pediatric tumors. Despite advances in treatment, the outcome of the disease remains unpredictable (Fuchs *et al*., 1998; Provisor *et al*., 1997). Elucidation of the genetic changes critical to osteosarcoma formation may facilitate prognostic and therapeutic evaluation, but is hampered by the complex patterns of structural and numerical chromosomal abnormalities (Biegel *et al*., 1989; Bridge *et al*., 1997; Fletcher *et al*., 1994). As a result of this genetic instability alterations affecting multiple regions of the tumor cell genome are common in sporadic osteosarcoma. The most consistently observed changes are amplification of chromosome 12q13–15, affecting the MDM2, CDK4 and SAS genes (Brinkschmidt *et al*., 1998; Tarkkanen *et al*., 1995), and amplification of chromosome 8q23–25 containing the MYC gene (Brinkschmidt *et al*., 1998; Stock *et al*., 2000). Losses are most frequently seen at chromosomes 13q14 and 17p13, accompanied respectively by mutation of the Rb1 and p53 suppressor genes (Mulligan *et al*., 1990; Toguchida *et al*., 1988). Two additional loci, 3q26 and 18q21–22, show a high frequency of loss of heterozygosity (LOH) (Yamaguchi *et al*., 1992), but the identities of the relevant suppressor genes at these loci are now known (Kruzelock *et al*., 1997; Nellissery *et al*., 1998). Representational difference analysis identified small regions of DNA gain on chromosome 17 and chromosome 19 and DNA loss on chromosome 4, but the associated genes have not been identified either (Simons *et al*., 1997; 1999).

Osteosarcoma can be readily induced in mice by application of bone-seeking alpha-emitting radioisotopes, and are comparable to human osteosarcomas both histologically and pathobiologically (Gossner *et al*., 1976; Luz *et al*., 1991). Murine osteosarcomas are detectable at an early stage, limiting the opportunity for the development of complex genomic alterations, and increasing the likelihood that detected genetic alterations are biologically relevant. We have therefore carried out a comparative microsatellite-based screen

*Correspondence: M Nathrath, Institute of Pathology, GSF-National Research Center for Environmental and Health, Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany; E-mail: nathrath@gsf.de
Received 15 February 2002; revised 12 June 2002; accepted 14 June 2002

for allelic imbalance (AI) in mouse and human osteosarcomas. Through the combination of these data with genome mapping data from both species we have been able to identify two new candidate suppressor gene loci.

Results

Allelic imbalance affects two loci, OSS1 and OSS2, on chromosome 9 in mouse osteosarcoma

The genome-wide screen of AI in mouse osteosarcoma was performed with a set of 18 osteosarcomas using a panel of 177 microsatellite markers. Allelic imbalance was found affecting multiple loci. Those represented in more than 50% of the tumors were deemed to be major sites of AI, whilst those affecting less than 25% of the tumors were considered to represent the background genomic instability of the tumors. Two of the nine major sites identified in this screen co-localized with the loci of genes (p53 and RB1) already known to be involved in the formation of mouse and human osteosarcomas. These results will be described elsewhere in detail (manuscript in preparation). Here we present the pattern of allelic loss on mouse chromosome 9 using 16 microsatellite markers. We identified two adjacent but discrete loci on mouse chromosome 9 that show high levels of AI (78%), and which have not been described to date. These loci are both located within an interval defined by the markers D9Mit144 and D9Mit182 (Figure 1). The order of the microsatellite markers shown in Figure 1 is determined from T31 radiation hybrid mapping data from our own typing study (D9Mit307, D9mit113, D9Mit134, D9Mit291, D9Mit10, D9Mit110, D9Mit272) and from published data (D9Mit144, D9Mit133, D9Mit271 (Van Etten *et al*., 1999) and D9Mit196, D9Mit182 (McCarthy *et al*., 1997)). Because D9Mit271 was not polymorphic between the strains of interest, it was substituted in our study by D9Mit9, which is physically linked to D9Mit271 at less than 1 kb distance (unpublished observation).

The more distal locus on chromosome 9, provisionally named OSS1, was flanked by the markers D9Mit9 and D9Mit291. Each of these two markers shows AI in 12/18 cases, with potential overlapping deletions present in 78% (14/18) of osteosarcomas. The distal border is defined by tumors 2 and 12, which show retention of heterozygosity of markers up to and including D9Mit291. The proximal boundary is defined by tumors 4 and 8, which show retention of markers up to and including D9Mit9 (Figure 1).

For the more proximal locus on chromosome 9 (OSS2) marker D9Mit307 shows AI in 78% (14/18) of tumors. The distal boundary of this locus is defined by tumors 8, 12 and 15, which all show AI affecting D9Mit307, but not the flanking distal marker D9Mit133. The proximal boundary of this locus could not be precisely defined due to the presence of a large chromosomal segment lacking polymorphic markers beyond D9Mit307. D9Mit144, the first informative marker proximal to this non-polymorphic section, shows retention of heterozygosity in four additional cases, thus representing a conservative limit for the proximal end of OSS2 (Figure 1).

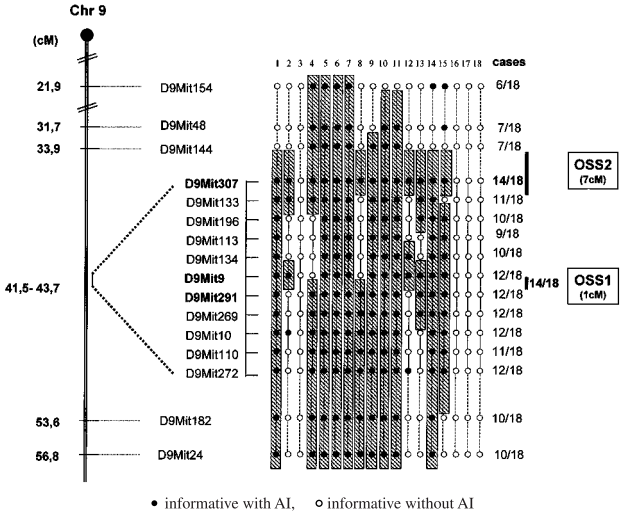

Figure 1 Allelic imbalance (AI) mapping using 16 polymorphic markers on mouse chromosome 9 in 18 cases of murine osteosarcoma. Marker position is depicted to the left of the ideogram and is listed according to the MIT/Whitehead Institute genetic map. The order of the markers on RH map was defined using radiation hybrid panel. The case numbers are depicted in the top row. The frequencies of AI are listed in the right column. Hatched boxes indicate the affected regions defining the interval of interest. The regions commonly affected (OSS1 and OSS2) are indicated by vertical bars with genetic distances marked

Allelic imbalance in human osteosarcoma loci syntenic to OSS1 and OSS2 affects human chromosomes 6 and 15

Using the mouse–human synteny map provided by the Mouse Genome Database (http://www.informatics.jax.org/menus/homolog_menu.shtml) we were able to place OSS1 within a region of synteny on human chromosome 6q including TBX18 (Figure 2). AI was found in 69% (29/42) of the human osteosarcomas to affect this region of chromosome 6q. The highest frequency of AI occurred at marker D6S1627, which shows AI in 77% of informative cases (23/30). The proximal boundary of the interval affected by AI is defined by tumor 33, showing retention of heterozygosity at marker D6S1609. The distal boundary is defined by tumor 34, with retention of heterozygosity at marker D6S434. These two tumors define an interval of approximately 18 Mbp that encompases the marker D6S1627, which shows the highest level of AI (Figure 3).

Due to the complex synteny pattern of the region containing mouse OSS2 we were unable to unequivocally identify the corresponding human region. The loci on human chromosome 6q12–11 (COL12A1), 6p (BMP5) or 15q21 (SCG3) are all potential syntenic sites of OSS2 (Figure 2). In the human osteosarcoma, a high level of AI was found on chromosome 15q21, making this region the most likely candidate region for

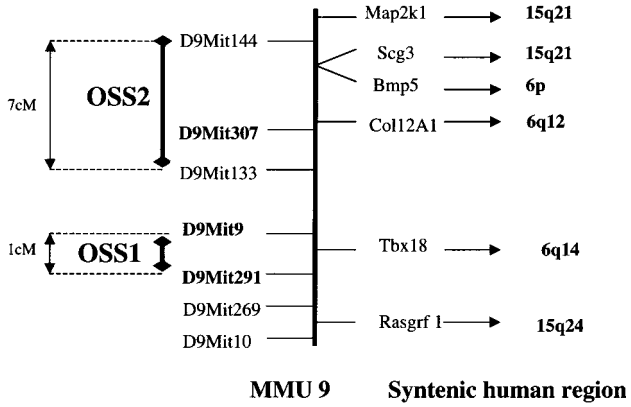

Figure 2 Mouse–human synteny interval spanning OSS1 and OSS2. Mouse genes and STS markers on chromosome 9 were mapped using the T31 RH panel. Locus order was determined using the RHMAP program. Raw data vectors were submitted to NCBI GeneMap99 (http://www.ncbi.nlm.nih.gov/genemap99/) or the Jackson laboratory RH database (http://www.jax.org/resources/documents/cmdata/rhmap/). The genes depicted are those included in the synteny intervals between MMU 9 and HSA15, HSA6p and HSA6q

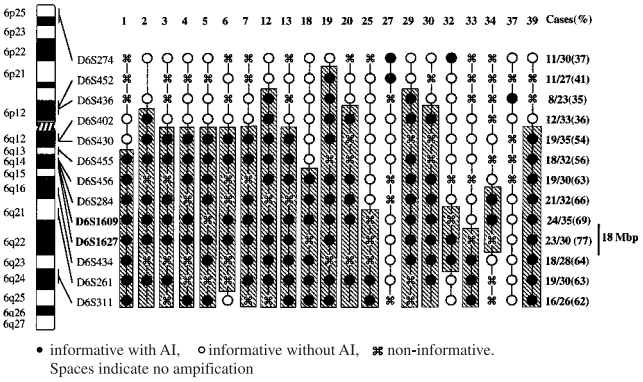

Figure 3 Allelic imbalance (AI) mapping using 13 polymorphic markers on human chromosome 6 in 21 of 42 cases of high-grade human osteosarcoma. The case numbers are shown in the top row. Ten cases with AI at every informative locus and 11 cases without AI at any informative locus are not shown. Hatched boxes indicate the regions with allelic imbalance spanning chromosome 6q14–16. A vertical bar indicates the region commonly affected with physical distance marked. The frequencies of AI in informative cases are listed in the right column. Locations of the markers used are depicted to the right of the ideogram. Cytogenetic bands at the 400-band level are present on the left

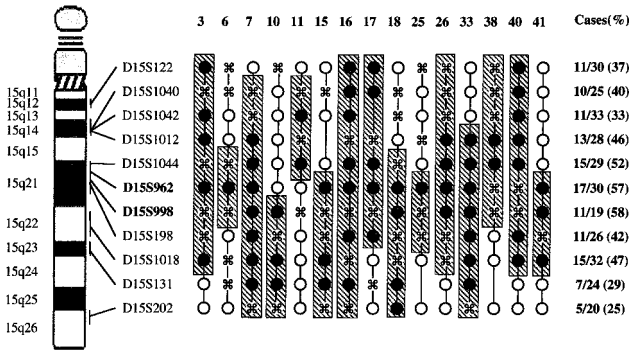

Figure 4 Allelic imbalance (AI) mapping using 11 polymorphic markers on chromosome 15 in 15 of 42 cases of high-grade human osteosarcoma. The case numbers are shown in the top row. Nine cases with AI at every informative locus and 18 cases without AI at any informative locus are not shown. Hatched boxes indicate the regions with allelic imbalance spanning chromosome 15q21. The frequencies of AI in informative cases are listed in the right column. Locations of the markers used are depicted on the right of the ideogram. Cytogenetic bands at the 400-band level are present on the left

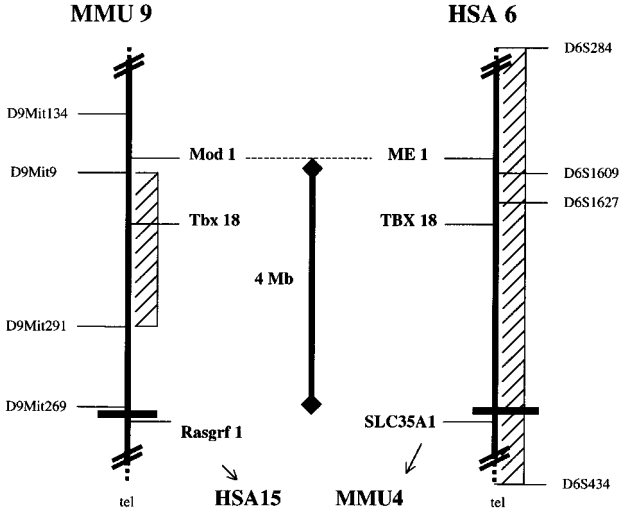

Figure 5 Synteny mapping identifies a 4 Mbp region containing the OSS1 tumor suppressor gene in human osteosarcoma. Shown is the region of allelic imbalance (AI) common to both mouse chromosome 9 and human 6q14. Markers D9Mit9 and D9Mit291 define the minimal region affected by AI in mouse. Mod1 defines the proximal border in mouse; analogously orthologous ME1 defines the proximal border of the region affected by AI in human osteosarcomas. Distal border is defined by SCL35A1, which in the mouse is located on chromosome 4 and therefore excluded from the OSS1 locus in mouse. OSS1 is therefore located between ME1 and SCL35A1, a region of approximately 4 Mbp

OSS2. Markers in this region show AI in 58% (11 of 19 informative cases with marker D15S998) and 57% (17 of 30 informative cases with marker D15S962) (Figure 4).

Mouse/human synteny mapping places OSS1 between ME1 and SCL35A1

ME1, the human ortholog of mouse *Mod1*, can be used to define the proximal border of the minimal region affected by AI on chromosome 6q14. Because *Mod1*, which is located proximal to D9Mit9, lies outside the proximal border defined for mouse OSS1 we deduce that the proximal border of the human locus can be refined to exclude ME1 on chromosome 6q (Figure 5).

SLC35A1 defines the distal boundary of the human OSS1 locus. This gene maps to the distal portion of the region affected by AI in human osteosarcomas on

chromosome 6q. Since it is placed on MMU4 by radiation hybrid mapping it is clearly not part of the murine OSS1 locus and can thus be used as the distal boundary marker of human OSS1 (Figure 5).

These comparisons between mouse and human sequences permit us to refine the human OSS1 locus to that portion of chromosome 6q between ME1 and SCL35A1, a region of approximately 4 Mbp.

Mouse/human synteny relationships place the OSS2 region between HDC and D15S198

Employing an analogue procedure to that for the mapping of OSS1 we were able to define the proximal border of OSS2 using the HDC locus. This gene, located within the region of chromosome 15 affected by AI in the human osteosarcomas, is located on MMU2. Since this region does not exhibit significant AI in mouse osteosarcoma (data not shown), HDC can be used to define the proximal border of the human OSS2 region. This distal boundary of OSS2 is defined by marker D15S198, which lies outside the region affected by AI in the human osteosarcoma. In this way, we can define a region of chromosome 15q21 corresponding to mouse OSS2, located between HDC and the microsatellite marker D15S198, a region spanning approximately 8 Mbp.

Discussion

Genetic and biological similarities in the neoplastic process between mouse and man have been exploited to identify the general molecular mechanism of tumorigenesis. Indeed, studies of mouse tumorigenesis models demonstrate that proto-oncogene activation and loss of function of tumor suppressor genes are molecular mechanisms resembling those in human tumors (Balmain and Harris, 2000). Here we present an example where data obtained in a mouse tumor model can be meaningfully extrapolated to man. By combining AI data from the mouse and human osteosarcoma with established data of the mouse/human synteny relationship we have been able to define two novel tumor suppressor gene loci in osteosarcoma. Invesigation of the allelic status of mouse osteosarcomas revealed frequent AI affecting two loci on chromosome 9. Synteny mapping placed one of these loci, OSS1, onto HSA6q14. In this region a high frequency of AI (77%) was revealed in human osteosarcomas. Synteny mapping of the regions of interest in murine and human tumors placed OSS1 in an approximately 4 Mbp interval between ME1 and SCG35A1, and, using an analogous procedure, OSS2 in the region between HDC and the microsatellite marker D15S198, an interval of 8 Mbp.

No genes previously shown to be involved in the formation of malignant tumors have been mapped to the OSS1 and OSS2 regions on mouse chromosome 9. In a study of spontaneous and chemically induced liver tumors in mice frequent loss of heterozygosity was found in a region indistinguishable from the region of OSS2 in our study, but the associated gene is not yet identified (Davis *et al*., 1994).

In man, allelotype analysis in osteosarcoma has revealed allelic loss in up to 50% of the tumors at chromosome 15q (Yamaguchi *et al*., 1992), and comparative genomic hybridization studies have revealed that both the chromosome 6q and chromosome 15q loci identified in our study exhibit losses of genomic material in up to 40% of osteosarcoma (Brinkschmidt *et al*., 1998; Tarkkanen *et al*., 1995). This suggests that the AI we observed is the result of LOH and therefore indicative of the presence of tumor suppressor genes at these loci. Chromosomal losses affecting these regions on chromosome 6q and 15q have also been described in a number of other malignant tumors, but when they were mapped in detail they did not correspond to either OSS1 or OSS2 (Balsara *et al*., 1999; Bernues *et al*., 1999; Park *et al*., 2000; Reardon *et al*., 1999).

Multiple molecular events are likely to underlie the development of osteosarcoma. Thus, involvement of a number of oncogenes in osteosarcomas has been reported including MDM2, CDK4 and SAS on chromosome 12q13–15 (Kanoe *et al*., 1998; Ladanyi *et al*., 1993; Lonardo *et al*., 1997; Wei *et al*., 1999) and MYC on chromosome 8q24 (Gamberi *et al*., 1998; Pompetti *et al*., 1996). On the other hand, losses of 13q (Belchis *et al*., 1996; Hansen *et al*., 1985; Toguchida *et al*., 1988; Wadayama *et al*., 1994; Yamaguchi *et al*., 1992) and 17p (Goto *et al*., 1998; Mulligan *et al*., 1990; Toguchida *et al*., 1992; Yamaguchi *et al*., 1992) in up to 60–77% of the tumors suggest that disturbances of the Rb- and p53-dependent growth-regulatory pathways are critically involved in the pathogenesis of sporadic osteosarcomas. The importance of these tumor suppressor genes is emphasized by the fact that patients with germline mutations in both p53 and RB are predisposed to osteosarcoma development. As only a minority of these patients develop osteosarcoma (Draper *et al*., 1986; Malkin *et al*., 1990), additional genetic factors are obviously required for the formation of osteosarcoma. Of the other known genetic changes the high frequency of allelic loss at 3q and 18q (Kruzelock *et al*., 1997; Nellissery *et al*., 1998; Yamaguchi *et al*., 1992) suggests the involvement of at least two additional tumor suppressor genes in the development of osteosarcoma. The AI frequency affecting OSS1 on chromosome 6q14 approaches that described for p53 and RB1. This suggests that the tumor suppressor gene we assume to be in the OSS1 region may play a similarly important role in osteosarcoma tumorigenesis.

It has already been shown that disturbances of developmental control genes are associated with tumorigenesis. Interestingly, TBX18, a member of the T-Box gene family, maps to this region of interest. T-Box genes are transcription factors involved in the regulation of developmental processes, e.g. TBX5 has been observed to have a profound inhibitory effect on cell proliferation during cardiogenesis (Hatcher *et al*., 2001). TBX genes

have also been shown to regulate bone and extremity development. Indeed, TBX2 seems to play a role in osteogenesis in the human (Chen *et al*., 2001), and *tbx18* expression was demonstrated in developing limb buds in mice (Kraus *et al*., 2001). From all this is may be speculated that TBX18 is a good candidate for the 6q OSS1 tumor suppressor gene.

To our knowledge, this is the first study demonstrating the power of comparing murine and human tumors, which takes advantage of the synteny relationship for fine mapping of tumor suppressor gene loci. Considering the similarities of tumorigenic mechanisms in both species and the current progress of genomic sequencing in mouse and man, exploitation of the synteny relationship could improve the identification of proto-oncogenes and tumor-suppressor genes.

Materials and methods

Mouse osteosarcoma

A genomic-wide screen for AI was performed on 18 osteosarcomas induced in female (BALB/c × CBA/CA) F1 hybrid mice through a single i.p. injection of 185 Bq/g body weight ^{227}Th-citrate. Genomic DNA was isolated directly from 12 fresh-frozen tumors (>5 mm diameter) using a genomic DNA extraction kit (Qiagen, 40724 Hilden, Germany) and from cells microdissected from six smaller tumors (<5 mm in diameter) using the QIAamp tissue extraction kit (Qiagen). DNA from non-tumorous tissue was obtained by the same method and provided reference DNA for each tumor.

Human osteosarcoma

Forty-two high-grade intramedullary osteosarcomas were analysed. Genomic DNA was obtained from tissue microdissected from 29 formalin-fixed paraffin-embedded tumors using the QIAamp tissue kit, and from 13 fresh-frozen biopsy samples using the Puregene DNA extraction protocol (Biozyme, 31833 Hess. Oldendorf, Germany), followed by treatment with RNase A. Non-tumorous formalin-fixed tissues or peripheral blood lymphocytes were extracted to provide reference DNA for each patient.

Analysis of allelic imbalance in mouse tissues

A panel of 177 informative microsatellite markers was used. Microsatellite markers showing at least a 2 bp difference between BALB/c and CBA/CA alleles were amplified using published PCR primer sequences (Dietrich *et al*., 1994). PCR reactions contained 20 ng template DNA, 5 pmol of primer oligonucleotide, 1.25 mM dNTPs, 1 U Taq polymerase (Amersham Pharmacia) and PCR reaction buffer containing 1.5 mM $MgCl_2$ in a final volume of 20 μl. Thirty PCR cycles were performed (1 min 94°C, 1 min 55°C, 1 min 72°C). After gel electrophoresis the ratio of maternal and paternal alleles was determined by digital analysis of Ethidium Bromide stained 8% polyacrylamide gels (IQ software, BioImage Systems Corp, Ann Arbour, MI, USA). AI was defined as a reduction of one allele in tumor tissue by at least 50% compared to the corresponding allele in non-tumor tissue. Reductions of between 40 and 50% were also considered as AI if both flanking markers showed an AI of more than 50%.

Analysis of allelic imbalance in human tissues

High polymorphic microsatellite markers mapping to chromosome 6p22.3–6q23.3 and 15q11.1–15q26.1 were selected from the Human Genome Database (http://www.genome.wi.mit.edu). PCR amplification was performed under standard conditions in a 15 μl reaction mixture containing 2 μl of template DNA, 15 mM $MgCl_2$ PCR buffer, 1.25 mM dNTPs, 20 pmol of each primer oligonucleotide (forward primers were fluorescently labeled) and 1.5 U Taq polymerase (Amersham Pharmacia). Thirty amplification cycles were performed (0.5 min 94°C, 0.5 min 55–60°C and 1.5 min 72°C). Reaction products were resolved and quantified using an automated ABI 377 sequencer and ABI Prism Gene Scan software. AI was defined as above.

Mapping of mouse–human syntenic regions

The synteny relationship between mouse chromosome 9 (MMU9) and human chromosome 6 (HSA6) was refined by radiation hybrid mapping of anchor genes (*Scg3*, *Bmp5*, *Col12a1*, *Mod1*, *Tbx18* and *Rasgrf1*) relative to a number of microsatellite markers using the mouse T31 radiation hybrid panels (Research Genetics). Our T31 mapping data of markers, including ones shown in Figure 1 as well as others which will be described elsewhere, have been deposited at The Jackson Laboratory T31 Mouse Radiation Hybrid Database (http://www.jax.org/resources/document/cmdata/rhmap/rh.html). The best locus order for the microsatellite markers and anchor genes we mapped was determined at LOD 2.5 using the RHMAP program (Boehnke *et al*., 1991). The order for the markers on human chromosomes and the physical distances were taken from the Human Genome working draft (http://genome.ucsc.edu).

Acknowledgements
We thank Utz Linzner for oligonucleotide preparation and Wibke Wistrich for excellent technical assistance. Parts of this project were funded by grant F14P-CT95-0008 from the Radiation protection program of the European Union (M Rosemann) and from the Land Bayern (M Nathrath: HSP III) and the clinical research fund 'KKF' of the Technical University Munich (M Nathrath).

References

Balmain A and Harris CC. (2000). *Carcinogenesis,* **21,** 371–377.

Balsara BR, Bell DW, Sonoda G, De Rienzo A, du Manoir S, Jhanwar SC and Testa JR. (1999). *Cancer Res.,* **59,** 450–454.

Belchis DA, Meece CA, Benko FA, Rogan PK, Williams RA and Gocke CD. (1996). *Diagn. Mol. Pathol.,* **5,** 214–219.

Bernues M, Casadevall C, Caballin MR, Miro R, Ejarque MJ, Chechile G, Gelabert A and Egozcue J. (1999). *Cancer Genet. Cytogenet.,* **112,** 42–45.

Biegel JA, Womer RB and Emanuel BS. (1989). *Cancer Genet. Cytogenet.,* **38,** 89–100.

Boehnke M, Lange K and Cox DR. (1991). *Am. J. Hum. Genet.,* **49,** 1174–1188.

Bridge JA, Nelson M, McComb E, McGuire MH, Rosenthal H, Vergara G, Maale GE, Spanier S and Neff JR. (1997). *Cancer Genet. Cytogenet.,* **95,** 74–87.

Brinkschmidt C, Blasius S, Burger H, Simon R, Diallo R, Battmann A, Winkelmann W, Bocker W and Dockhorn-Dworniczak B. (1998). *Verh. Dtsch. Ges. Pathol.,* **82,** 184–188.

Chen J, Zhong Q, Wang J, Cameron RS, Borke JL, Isales CM and Bollag RJ. (2001). *Mol. Cell Endocrinol.,* **177,** 43–54.

Davis LM, Caspary WJ, Sakallah SA, Maronpot R, Wiseman R, Barrett JC, Elliott R and Hozier JC. (1994). *Carcinogenesis,* **15,** 1637–1645.

Dietrich WF, Miller JC, Steen RG, Merchant M, Damron D, Nahf R, Gross A, Joyce DC, Wessel M, Dredge RD, Marquis A, Stein LD, Goodman N, Page DC and Lander ES. (1994). *Nat. Genet.,* **7,** 220–245.

Draper GJ, Sanders BM and Kingston JE. (1986). *Br. J. Cancer,* **53,** 661–671.

Fletcher JA, Gebhardt MC and Kozakewich HP. (1994). *Cancer Genet. Cytogenet.,* **77,** 81–88.

Fuchs N, Bielack SS, Epler D, Bieling P, Delling G, Korholz D, Graf N, Heise U, Jurgens H, Kotz R, Salzer-Kuntschik M, Weinel P, Werner M and Winkler K. (1998). *Ann. Oncol.,* **9,** 893–899.

Gamberi G, Benassi MS, Bohling T, Ragazzini P, Molendini L, Sollazzo MR, Pompetti F, Merli M, Magagnoli G, Balladelli A and Picci P. (1998). *Oncology,* **55,** 556–563.

Gossner W, Hug O, Luz A and Muller WA. (1976). *Rec. Res. Cancer Res.,* **54,** 36–49.

Goto A, Kanda H, Ishikawa Y, Matsumoto S, Kawaguchi N, Machinami R, Kato Y and Kitagawa T. (1998). *Jpn. J. Cancer Res.,* **89,** 539–547.

Hansen MF, Koufos A, Gallie BL, Phillips RA, Fodstad O, Brogger A, Gedde-Dahl T and Cavenee WK. (1985). *Proc. Natl. Acad. Sci. USA,* **82,** 6216–6220.

Hatcher CJ, Kim MS, Mah CS, Goldstein MM, Wong B, Mikawa T and Basson CT. (2001). *Dev. Biol.,* **230,** 177–188.

Kanoe H, Nakayama T, Murakami H, Hosaka T, Yamamoto H, Nakashima Y, Tsuboyama T, Nakamura T, Sasaki MS and Toguchida J. (1998). *Anticancer Res.,* **18,** 2317–2321.

Kraus F, Haenig B and Kispert A. (2001). *Mech. Dev.,* **100,** 83–86.

Kruzelock RP, Murphy EC, Strong LC, Naylor SL and Hansen MF. (1997). *Cancer Res.,* **57,** 106–109.

Ladanyi M, Cha C, Lewis R, Jhanwar SC, Huvos AG and Healey JH. (1993). *Cancer Res.,* **53,** 16–18.

Lonardo F, Ueda T, Huvos AG, Healey J and Ladanyi M. (1997). *Cancer,* **79,** 1541–1547.

Luz A, Muller WA, Linzner U, Strauss PG, Schmidt J, Muller K, Atkinson MJ, Murray AB, Gossner W, Erfle V and Höfler H. (1991). *Radiat. Environ. Biophys.,* **30,** 225–227.

Malkin D, Li FP, Strong LC, Fraumeni Jr JF, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA and Friend SH. (1990). *Science,* **250,** 1233–1238.

McCarthy LC, Terrett J, Davis ME, Knights CJ, Smith AL, Critcher R, Schmitt K, Hudson J, Spurr NK and Goodfellow PN. (1997). *Genome Res.,* **7,** 1153–1161.

Mulligan LM, Matlashewski GJ, Scrable HJ and Cavenee WK. (1990). *Proc. Natl. Acad. Sci. USA,* **87,** 5863–5867.

Nellissery MJ, Padalecki SS, Brkanac Z, Singer FR, Roodman GD, Unni KK, Leach RJ and Hansen MF. (1998). *Am. J. Hum. Genet.,* **63,** 817–824.

Park WS, Park JY, Oh RR, Yoo NJ, Lee SH, Shin MS, Lee HK, Han S, Yoon SK, Kim SY, Choi C, Kim PJ, Oh ST and Lee JY. (2000). *Cancer Res.,* **60,** 70–73.

Pompetti F, Rizzo P, Simon RM, Freidlin B, Mew DJ, Pass HI, Picci P, Levine AS and Carbone M. (1996). *J. Cell. Biochem.,* **63,** 37–50.

Provisor AJ, Ettinger LJ, Nachman JB, Krailo MD, Makley JT, Yunis EJ, Huvos AG, Betcher DL, Baum ES, Kisker CT and Miser JS. (1997). *J. Clin. Oncol.,* **15,** 76–84.

Reardon DA, Entrekin RE, Sublett J, Ragsdale S, Li H, Boyett J, Kepner JL and Look AT. (1999). *Genes Chromosomes Cancer,* **24,** 230–237.

Simons A, Janssen IM, Suijkerbuijk RF, Veth RP, Pruszczynski M, Hulsbergen-van de Kaa CA, du Manoir S and Geurts van Kessel A. (1997). *Genes Chromosomes Cancer,* **20,** 196–200.

Simons A, Schepens M, Forus A, Godager L, van Asseldonk M, Myklebost O and van Kessel AG. (1999). *Genes Chromosomes Cancer,* **26,** 115–124.

Stock C, Kager L, Fink FM, Gadner H and Ambros PF. (2000). *Genes Chromosomes Cancer,* **28,** 329–336.

Tarkkanen M, Karhu R, Kallioniemi A, Elomaa I, Kivioja AH, Nevalainen J, Bohling T, Karaharju E, Hyytinen E, Knuutila S and Kallioniemi OP. (1995). *Cancer Res.,* **55,** 1334–1338.

Toguchida J, Ishizaki K, Sasaki MS, Ikenaga M, Sugimoto M, Kotoura Y and Yamamuro T. (1988). *Cancer Res.,* **48,** 3939–3943.

Toguchida J, Yamaguchi T, Ritchie B, Beauchamp RL, Dayton SH, Herrera GE, Yamamuro T, Kotoura Y, Sasaki MS, Little JB, Weichselbaum RR, Ishizaki K and Yandell DW. (1992). *Cancer Res.,* **52,** 6194–6199.

Van Etten WJ, Steen RG, Nguyen H, Castle AB, Slonim DK, Ge B, Nusbaum C, Schuler GD, Lander ES and Hudson TJ. (1999). *Nat. Genet.,* **22,** 384–387.

Wadayama B, Toguchida J, Shimizu T, Ishizaki K, Sasaki MS, Kotoura Y and Yamamuro T. (1994). *Cancer Res.,* **54,** 3042–3048.

Wei G, Lonardo F, Ueda T, Kim T, Huvos AG, Healey JH and Ladanyi M. (1999). *Int. J. Cancer,* **80,** 199–204.

Yamaguchi T, Toguchida J, Yamamuro T, Kotoura Y, Takada N, Kawaguchi N, Kaneko Y, Nakamura Y, Sasaki MS and Ishizaki K. (1992). *Cancer Res.,* **52,** 2419–2423.