Virchows Arch (2000) 436:487–493

ORIGINAL ARTICLE

**Julia Reifenberger · Marietta Wolter · Jan Boström
Rainer Büschges · Klaus W. Schulte · Mosaad Megahed
Thomas Ruzicka · Guido Reifenberger**

Allelic losses on chromosome arm 10q and mutation of the *PTEN* (*MMAC1*) tumour suppressor gene in primary and metastatic malignant melanomas

Received: 1 September 1999 / Accepted: 22 December 1999

Abstract Malignant melanomas frequently show loss of alleles on the long arm of chromosome 10. The *PTEN* (*MMAC1*) gene has been identified as a tumour suppressor gene at 10q23.3 that is mutated in various types of advanced human cancers. We have investigated a series of 40 sporadic melanomas from 37 patients (15 primary cutaneous melanomas and 25 melanoma metastases) for allelic losses on chromosome 10, as well as for deletion and mutation of the *PTEN* gene. Microsatellite analysis revealed loss of heterozygosity at loci located on 10q in tumours from 15 of 34 patients investigated (44%). Somatic *PTEN* mutations were identified in melanomas from 4 of 37 patients (11%), all of whom had metastatic disease. In two of these patients, the tumours had additionally lost one *PTEN* allele, indicating complete loss of wild-type *PTEN* in the tumour cells. Our findings corroborate that loss of heterozygosity on chromosome 10 is a frequent aberration in malignant melanomas and implicate *PTEN* as a tumour suppressor gene inactivated by somatic mutation in a fraction of these tumours.

Key words Chromosome 10 · Loss of heterozygosity · Molecular genetics · *PTEN* · Skin tumours

Introduction

Cutaneous malignant melanomas have shown a dramatic increase in both incidence and mortality over the past decades [35]. The genetic principles that underlie the development and progression of these tumours are only beginning to emerge. Cytogenetic and molecular genetic studies have revealed that a number of chromosomal and genetic alterations are involved in melanoma pathogenesis (for review see [19]). A common alteration in sporadic melanomas is the loss of genetic information on the long arm of chromosome 10, which has been found in between 30% and more than 60% of the cases [2, 14, 15, 18, 47]. The *PTEN* gene ("phosphatase and tensin homologue deleted on chromosome ten") [24], also known as *MMAC1* ("mutated in multiple advanced cancers") [42] or *TEP1* ["transforming growth factor (TGF)β-regulated and epithelial cell-enriched phosphatase"] [22], has been identified as a tumour suppressor gene at 10q23.3. Somatic mutations of *PTEN* have been detected in different types of malignant tumours, including carcinomas of the breast and prostate, and glioblastomas of the brain [24, 42]. In addition, germline mutations in the *PTEN* gene have been shown to be responsible for Cowden's disease, a hereditary syndrome predisposing to multiple hamartomas and various tumours [26]. The most common malignant tumours associated with Cowden's disease are carcinomas of the breast and thyroid gland [26]. However, individual patients with both Cowden's disease and cutaneous melanoma have also been reported [11, 40].

Recent studies have shown that the Pten protein is important in the regulation of cell cycle progression [10, 23], cell migration and spreading [44], and apoptosis [7, 25, 41]. The cell cycle regulatory and anti-apoptotic functions appear to be closely linked to the function of Pten as a negative regulator of the protein kinase B (PKB/Akt) pathway [7, 25, 32, 41, 50]. The tumour suppressor properties of Pten have been substantiated by the finding that *PTEN* heterozygous ($PTEN^{+/-}$) mice are highly susceptible for the development of various types of tumours [6, 31, 43]. In addition, $PTEN^{+/-}$ mice show an impaired Fas-mediated apoptosis and may develop a lethal autoimmune disorder [7].

With respect to the role of *PTEN* in sporadic malignant melanomas, mutations or homozygous gene dele-

J. Reifenberger · K.W. Schulte · M. Megahed · T. Ruzicka
Department of Dermatology, Heinrich-Heine University, Moorenstraße 5, D-40225 Düsseldorf, Germany

M. Wolter · R. Büschges · G. Reifenberger (✉)
Department of Neuropathology, University of Bonn Medical Center, Sigmund-Freud Straße 25, D-53105 Bonn, Germany
e-mail: g.reifenberger@uni-bonn.de
Tel.: +49-228-2875775, Fax: +49-228-2874331

J. Boström
Department of Neurosurgery, University of Bonn Medical Center, Sigmund-Freud Straße 25, D-53105 Bonn, Germany

tions have been detected in 15 of 35 melanoma cell lines [12]. The current data on melanomas in vivo are controversial: one study reported on *PTEN* alterations in 2 of 17 melanoma metastases [46], while other investigators detected no mutations in a series of 23 primary melanomas and 17 melanoma metastases [3]. In order to further evaluate the significance of chromosome-10 losses and *PTEN* alterations in sporadic melanomas in vivo, we have analysed 15 primary cutaneous melanomas and 25 melanoma metastases from 37 patients for allelic losses on chromosome 10, as well as for deletion and mutation of the *PTEN* gene. Our data confirm that loss of heterozygosity on chromosome 10 is a common abnormality in melanomas, which was detected in 44% of our cases. In addition, we found somatic *PTEN* mutations in melanomas from four patients. Thus, our data indicate that mutation of *PTEN* is an important molecular mechanism contributing to the pathogenesis of a fraction of sporadic melanomas.

Materials and methods

The 40 melanomas investigated were from 12 male and 25 female patients (mean age at operation 67 years) operated on either at the Department of Dermatology or the Department of Neurosurgery, Heinrich-Heine University, Düsseldorf, Germany. From three patients, primary and metastatic tumours could be studied (M15 and M16, M24 and M25, M29 and M63; Table 1). One patient had a giant polypoid melanoma (M55) that originated from a congenital nevus [9]. None of the patients had a family history of Cowden's disease. The tumours included 15 primary cutaneous melanomas [8 nodular melanomas (NM), 4 acral lentiginous melanomas (ALM), 2 superficial spreading melanomas (SSM), and 1 polypoid melanoma (PM)] and 25 melanoma metastases [18 cutaneous melanoma metastases (CMM), 1 regional lymph-node melanoma metastasis, 1 spinal and 5 intracerebral melanoma metastases]. The Clark level and tumour thickness of the primary melanomas are listed in Table 1. Parts of each tumour were frozen immediately after operation and stored at –80°C. Histological evaluation of these tumour pieces showed an estimated tumour cell content of at least 60% in two specimens and 70% or more in the remaining specimens (Table 1). Peripheral blood samples for the extraction of constitutive (leukocyte) DNA were available from 34 patients.

Extraction of high-molecular-weight DNA and RNA from frozen tumour tissue was carried out by means of ultracentrifugation, as described previously [16]. Extraction of high-molecular-weight DNA from peripheral blood leukocytes was performed according to a standard protocol [38]. From three tumours (M15, M16, M37), DNA was additionally isolated from formalin-fixed paraffin-embedded specimens after microdissection of areas with a high tumour cell content (>90%). DNA from the microdissected specimens was purified as described previously [34].

The following six microsatellite loci were selected from the Généthon microsatellite map [13]: *D10S249* (10p15-pter), *D10S215* (10q23, proximal to *PTEN*), *D10S541* (10q23, distal to *PTEN*), *D10S209* (10q25), *D10S587* (10q25-q26) and *D10S212* (10q26). Polymerase chain reaction (PCR) was performed as described previously [4, 5]. PCR products were separated on 10% denaturing polyacrylamide gels containing 8 M urea and were visualised by means of silver staining. Assessment of allelic loss was done as described previously [4]. An interstitial retention of heterozygosity at *D10S209* was found in tumour M37, and this was confirmed by duplex PCR using primers for *D10S209* together with primers for either *D4S413* or *D4S1607* in the same reaction tube (Fig. 1).

All tumours were screened for homozygous deletion of *PTEN* using duplex PCR with genomic primer pairs for either exon 1 or exon 9 [42], together with primers for the glyceraldehyde phosphate dehydrogenase (*GAPDH*) gene on 12p [5]. Duplex PCR was performed with 100 ng genomic DNA as a template in 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.5 µM each *GAPDH* primer, 0.5 µM each *PTEN* primer, and 1 U *Taq* DNA polymerase (Eurogentec, Seraing, Belgium). Three minutes of initial denaturation at 94°C and 27 cycles of 1 min at 94°C, 1 min at 56°C and 1 min at 72°C were followed by a final extension at 72°C for 5 min. To assure that the PCR products were obtained in the exponential phase, PCR reactions with different cycle numbers (from 25 to 30 cycles) were performed. Twenty-seven cycles turned out to be optimal. The PCR products were separated on 2% agarose gels and ethidium bromide-stained bands were recorded by the Gel-Doc 1000 system (Bio-Rad Lab., Hercules, Calif.). Quantitative densitometric analysis of the signal intensities obtained for the target gene (*PTEN*) and the reference locus (*GAPDH*) was performed using the Molecular Analyst version 2.1 software (Bio-Rad). Densitometrically determined ratios of 1.6 or more were regarded as retention of two gene copies, ratios between 0.7 and 1.5 as loss of one gene copy and ratios of less than 0.6 as loss of both gene copies.

For single-strand conformation polymorphism (SSCP) analysis, 3 µg total RNA from each tumour was reverse transcribed into cDNA in a total volume of 50 µl using random hexanucleotide primers and Superscript reverse transcriptase (Gibco BRL, Eggenstein, Germany). The entire coding region of *PTEN* was then amplified using reverse-transcription PCR with a set of seven overlapping primer pairs, as described previously [5]. The PCR products were screened for mutations after electrophoresis on 8% non-denaturing polyacrylamide gels [5]. Each PCR product was analysed at room temperature and at 4°C. The SSCP band patterns were visualised by silver staining of the gels. PCR products with aberrant SSCP patterns were sequenced in both directions by manual sequencing using the USB Sequenase PCR product sequencing kit (Amersham Life Sciences, Cleveland, Ohio.). All tumours showing *PTEN* mutations at the transcript level, as well as one melanoma from which no RNA was available (M48), were analysed for mutations at the genomic level using primers described by Steck et al. [42]. The somatic origin of the *PTEN* mutations was confirmed by sequencing of leukocyte DNA from the respective patients.

Results

Analysis of six highly polymorphic microsatellite loci from chromosome 10 revealed loss of heterozygosity (LOH) at one or more loci in 16 tumours from 15 patients (Table 1, Fig. 1). No instance of microsatellite instability was seen. Allelic loss was found in six primary melanomas (2 of 8 NM, 2 of 4 ALM, 1 of 2 SSM and 1 of 1 PM) and ten melanoma metastases. In three instances, primary and metastatic tumours from the same patient could be investigated for allelic loss. In each case, the metastases showed identical allelic losses or retentions as the respective primary melanomas. One tumour (M47) demonstrated retention of both alleles at *D10S249* (10p15-pter) but allelic loss at all informative markers from 10q. Another tumour (M37) showed LOH at all informative loci except for an interstitial retention of heterozygosity at *D10S209* (10q25). In order to exclude the possibility of homozygous deletion at *D10S209*, we performed two independent duplex-PCR reactions using primers for *D10S209* together with primers for either *D4S413* or *D4S1607*. These experiments confirmed retention of heterozygosity at *D10S209* in M37 (Fig. 1).

Table 1 Summary of patient data and molecular genetic results. *ALM* acral lentiginous melanoma; *SSM* superficial spreading melanoma; *NM* nodular melanoma; *PM* polypoid melanoma; *CMM* cutaneous melanoma metastasis; *l.n. MM* lymph-node melanoma metastasis; *spinal MM* spinal melanoma metastasis; *i.c. MM* intracerebral melanoma metastasis; *n.a.* not analysed; *ins* insertion; *del* deletion; *dupl* duplication

Tumour-No.[a]	Tumour type	Clark level/ thickness (mm)	Age	Gender	Localization	Tumour cell content (%)	LOH on chromosome 10[b]	*PTEN* copy number[c]	*PTEN* mutation
M5	ALM	IV/4.0	68	Female	Plantar	70	–	2	–
M13	ALM	III/1.4	67	Female	Plantar	60	+	1	–
M15	ALM	IV/5.5	80	Female	Plantar	>90[d]	+	1	c.164ins45 (dupl. exon 3)
M48	ALM	IV/1.7	75	Female	Plantar	70	–	2	–
M21	SSM	IV/1.7	55	Female	Leg	80	–	2	–
M37	SSM	IV/1.1	87	Female	Neck	>90[d]	+	1	–
M9	NM	IV/4.5	70	Male	Leg	70	–	2	–
M12	NM	IV/3.9	74	Female	Shoulder	80	–	2	–
M19	NM	V/4.6	75	Female	Leg	90	–	2	–
M24	NM	IV/4.9	57	Female	Foot	80	–	2	c.1002–1003CC>TT (R335X)
M29	NM	IV/2.9	29	Female	Abdomen	80	–	2	–
M39	NM	IV/6.0	68	Male	Abdomen	80	+	1	–
M47	NM	IV/2.2	72	Female	Neck	80	+	1	–
M54	NM	IV/1.5	77	Male	Shoulder	70	–	2	c.730C>T (P244S)
M55	PM	V/35	64	Female	Abdomen	90	+	1	–
M1	CMM	–	65	Male	Chest	90	–	2	–
M2	CMM	–	63	Female	Leg	80	–	2	–
M3	CMM	–	85	Female	Scalp	80	–	2	–
M4	CMM	–	72	Female	Leg	80	–	2	–
M10	CMM	–	78	Female	Inguinal	80	+	1	–
M14	CMM	–	66	Male	Arm	80	–	2	–
M16	CMM	–	80	Female	Plantar	>90[d]	+	1	c.164ins45 (dupl. exon 3)
M17	CMM	–	71	Male	Supraclavicular	70	–	2	–
M18	CMM	–	59	Female	Abdomen	90	–	2	–
M23	CMM	–	86	Female	Labia majora	80	–	2	–
M25	CMM	–	57	Female	Leg	60	–	2	c.1002–1003CC>TT (R335X)
M26	CMM	–	69	Female	Chest	80	n.a.	1	–
M38	CMM	–	83	Female	Leg	80	+	1	–
M45	CMM	–	40	Male	Leg	80	+	1	–
M46	CMM	–	63	Female	Abdomen	80	n.a.	2	–
M50	CMM	–	88	Female	Leg	70	+	1	–
M63	CMM	–	31	Female	Back	90	–	2	–
M64	CMM	–	72	Male	Axilla	90	–	2	–
M11	l.n. MM	–	43	Male	Axilla	80	–	2	–
M32	spinal MM	–	76	Female	Spinal	80	+	1	–
M33	i.c. MM	–	69	Male	Cerebral	80	n.a.	1	–
M34	i.c. MM	–	53	Female	Cerebral	90	+	1	–
M35	i.c. MM	–	76	Female	Cerebral	70	+	1	–
M36	i.c. MM	–	60	Male	Cerebral	80	+	1	c.843–850delAGGACCAG (frame shift)
M44	i.c. MM	–	72	Male	Cerebral	90	+	1	–

[a] From three patients, the primary melanoma and a cutaneous melanoma metastasis were investigated (M15 and M16, M24 and M25, M29 and M63)

[b] + loss of heterozygosity (LOH) at one or more loci from chromosome 10; – retention of heterozygosity at all informative loci from 10q

[c] *PTEN* gene copy number as calculated by duplex PCR for exon 1 and exon 9: *2* two gene copies; *1* one gene copy (hemizygous deletion)

[d] Estimated tumour cell content of the microdissected tumour areas used for LOH analysis

Fourteen tumours showed LOH at all informative loci, suggesting loss of an entire copy of chromosome 10. The polypoid melanoma M55 showed LOH at all informative loci from 10q, while the congenital nevus from which M55 originated had generally retained heterozygosity on 10q (data not shown).

Duplex-PCR analysis of *PTEN* copy number revealed reduced signal intensities for exons 1 and 9 corresponding to the loss of one gene copy in all 16 tumours with LOH on 10q (Table 1). In addition, two further tumours, M26 and M33, which were not investigated using microsatellite analysis (because no corresponding constitutional DNA was available) showed loss of one *PTEN* copy using duplex-PCR analysis (Table 1). None of the 40 melanomas investigated showed evidence of homozygous *PTEN* deletion by duplex-PCR analysis (Table 1).

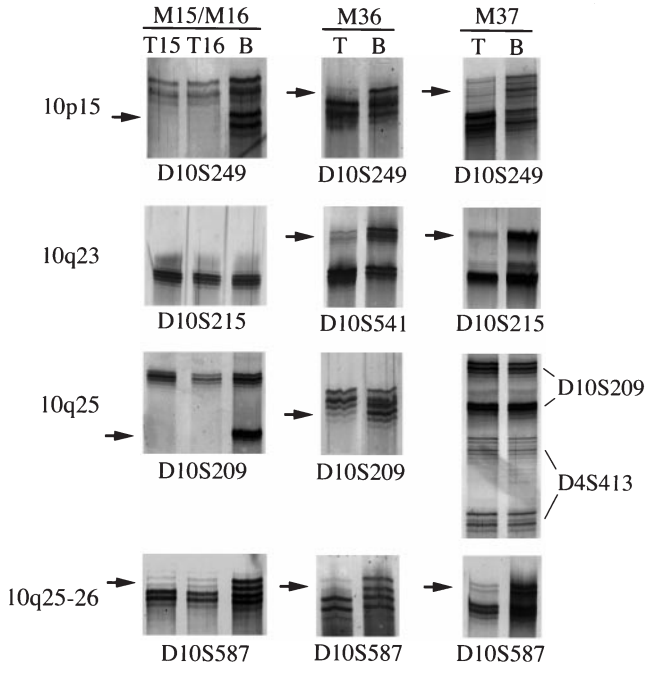

Fig. 1 Microsatellite analysis for loss of heterozygosity (LOH) on chromosome 10 in melanomas. Shown are results obtained for M15 and M16 (primary and metastatic melanoma from one patient), M36 and M37. *T* tumour DNA; *B* corresponding blood (leukocyte) DNA. Microsatellite analysis of M15, M16 and M37 was performed with DNA isolated from microdissected tumour areas. Note that M15, M16 and M36 demonstrated LOH at all informative markers. These tumours also carried *PTEN* mutations in the remaining allele. Tumour M37 had retained heterozygosity at *D10S209*, while all informative loci located proximally and distally showed LOH. To confirm an interstitial retention of heterozygosity at *D10S209*, this marker was amplified in a duplex-polymerase chain reaction together with a reference locus (*D4S413*). *Arrows* indicate lost alleles in the tumour DNA. M15 and M16 are not informative at *D10S215*

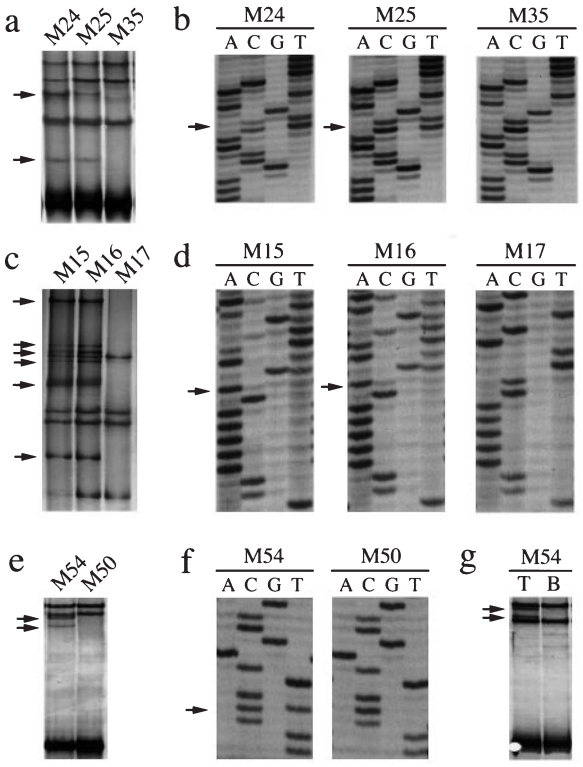

Fig. 2 Mutational analysis of *PTEN* in malignant melanomas from three patients. **a** Single-strand conformation polymorphism (SSCP) analysis of *PTEN* transcripts from M24 and M25 demonstrated an aberrant band pattern (*arrows*) relative to M35. **b** Sequencing revealed a CC-to-TT transition at nucleotides 1002–1003 (*arrow*), resulting in the introduction of a premature stop codon at codon 335 (c.1002–1003CC>TT: R335X). **c** SSCP analysis of M15 and M16 showed an aberrant band pattern relative to M17 (*arrows*). **d** These tumours carried an insertion of 45 bp, corresponding to exon 3 at nucleotide 164, i.e. an in-frame duplication of exon 3. The *arrows* indicate the start point of the insertion. The sequence of the non-coding strand is shown. **e** Aberrant SSCP bands detected for *PTEN* transcripts in tumour M54 (*arrows*). **f** M54 showed a missense mutation (c.730C>T, *arrow*) that resulted in the substitution of the conserved amino acid proline by serine at position 244. **g** At the genomic level, SSCP for exon 7 detected aberrant bands only in the tumour DNA of M54 (*arrows*) but not in the corresponding leukocyte DNA

Mutational analysis of the entire coding region of *PTEN* at the transcript level revealed mutations in tumours from four patients, all of whom had metastatic disease (Table 1). In two of these patients, identical mutations were found in the primary tumours and the respective metastases. The *PTEN* alteration detected in melanomas M24 and M25 was a CC-to-TT transition at nucleotides 1002–1003 (nucleotide numbering from the transcriptional start site, GenBank accession number U93051), resulting in the introduction of a premature stop codon at codon 335 (Fig. 2a, b). The same mutation (c.1002–1003CC>TT: R335X) was found after sequencing genomic DNA from tumours M24 and M25, while the patient's constitutional DNA showed the wild-type sequence. Tumours M15 and M16 expressed aberrant transcripts carrying an in-frame duplication of exon 3 (c.164ins45, Fig. 2c, d). Sequence analysis of these tumours at the DNA level revealed no mutations in the limited intronic regions flanking exon 3 that could be analysed using the available primers. In tumour M54, one *PTEN* allele carried a somatic missense mutation that resulted in the substitution of proline by serine at codon 244 (c.730C>T: P244S; Fig. 2e–g). An 8-bp deletion in exon 8 (c.843–850delAGGACCAG) was found in tumour M36. This mutation resulted in a frame shift and the introduction of a premature stop codon 13 codons downstream of the mutation site. Sequencing of this patient's constitutional DNA showed the wild-type sequence.

Discussion

The frequency of LOH on chromosome 10 determined for our series of melanomas (44%) corresponds well with several previous investigations showing allelic loss on this chromosome in 30–50% of the cases [14, 15, 17, 18, 47]. A study employing comparative genomic hy-

bridisation (CGH) for the analysis of cutaneous melanomas reported a somewhat higher incidence of 63% for deletions involving chromosome 10 [2]. We found allelic losses on chromosome 10 in both primary melanomas and melanoma metastases. Among the primary tumours, LOH was detected in all subtypes, i.e. NMs, ALMs, SSMs and PMs. There was no obvious correlation to tumour thickness or Clark level. However, our tumour series did not include melanomas of Clark levels I or II, since these lesions are usually required in toto for establishment of the histological diagnosis.

Mutational analysis of the *PTEN* tumour suppressor gene revealed mutations in melanomas from 4 of the 37 patients investigated (11%). This result is in line with data reported by Tsao et al. [46], who identified *PTEN* alterations in 2 of 17 metastatic melanomas (12%). The actual percentage of melanomas with *PTEN* aberrations is probably somewhat higher, because SSCP analysis may have missed some mutations. The sensitivity of SSCP analysis in detecting single base substitutions has been shown to range from 97% for fragments of 150 bp to about 70% for fragments of 250 bp [39]. We estimate that our SSCP analysis with PCR products ranging from 185 bp to 266 bp in size [5] detects about 75% of the *PTEN* mutations present. Nevertheless, it appears that *PTEN* alterations are less frequent in primary and metastatic melanomas in vivo than in melanoma cell lines. Two previous studies have investigated melanoma cell lines for *PTEN* aberrations and identified mutations or homozygous deletions in 43% and 29% of the cell lines investigated [12, 46]. The most likely explanation for this difference is that in vitro cultivation of melanomas selects for tumour cells having inactivated *PTEN* by homozygous deletion or mutations. In line with this assumption, Robertson et al. [36] reported that introduction of a normal chromosome 10 into *PTEN* mutant melanoma cells results in in vitro LOH at 10q23 and concomitant loss of the introduced wild-type *PTEN* gene.

Two of the *PTEN* mutations detected in our tumour series predict the expression of truncated proteins. One intracerebral melanoma metastasis showed an 8-bp intragenic deletion causing a frame shift and the introduction of a premature stop codon within exon 8. A CC-to-TT transition leading to the replacement of arginine at codon 335 by a stop codon (resulting in a protein lacking 68 amino acids from the C terminus) was found in the primary and metastatic tumour of another patient. This type of mutation has been reported as a typical alteration following ultraviolet irradiation of DNA [45]. Both truncating mutations translate into proteins lacking parts of the C-terminal C2 domain [21]. This domain is important for the association of Pten with the plasma membrane, and mutations within this domain have been shown to reduce Pten's ability to suppress tumour cell growth [21]. A duplication of exon 3, which codes for a highly conserved part of the Pten protein with extensive homology to auxilin and tensin [22, 24, 42], was found in another patient's primary (M15) and metastatic melanoma (M16). We have previously reported on duplications of entire coding exons in two glioblastomas [5]. In one of these tumours, a mutation in the intronic branch site consensus sequence was found, while no intronic mutations were identified in the second case. Analysis of the intronic splice sites and intronic regions flanking exon 3 showed no sequence alterations in tumours M15 and M16. The missense mutation identified in tumour M54 results in a substitution of proline at position 244 by serine. This mutation maps within the C2 domain and, thus, likely causes conformational changes that compromise the proper function of Pten.

The fact that *PTEN* mutations were detected in malignant melanomas from only 2 of 15 patients carrying tumours with losses on 10q is striking and deserves further investigation. One possibility is that the loss of one *PTEN* allele (without alteration of the second allele) is sufficient to provide a selective growth advantage to melanoma cells. In line with this hypothesis, a recent study on *PTEN* heterozygous mice revealed that inactivation of one *PTEN* allele increases survival and proliferation of certain cell types [7]. In addition, it remains to be investigated whether *PTEN* is inactivated in melanomas by mechanisms other than coding region mutations or homozygous deletion, e.g. transcriptional silencing by mutation or hypermethylation of promotor sequences, mutation of non-coding sequences that reduce mRNA stability, or increased protein degradation. Studies on other human tumour types have suggested that loss of Pten expression is an alternative mechanism to *PTEN* mutation or homozygous deletion in subsets of breast [30] and prostate [49] carcinomas.

Finally, one or more additional, yet unknown, melanoma-associated tumour suppressor gene(s) may be located on chromosome 10. Unfortunately, neither our microsatellite data nor previous LOH analyses of melanomas [14, 15, 17, 18, 47] have revealed a consistent region of common deletion that does not involve the *PTEN* locus. A cytogenetic study reported translocations with breakpoints at 10q24 and 10q25 in two malignant melanomas, suggesting alteration of one or more genes distal to *PTEN* [29]. Supporting this hypothesis, studies on other human tumour types, including glioblastomas, prostate carcinomas and endometrial carcinomas, have revealed common regions of deletion mapping to 10q24-q26, i.e. distal to the *PTEN* locus [1, 20, 28, 33]. One candidate gene located at 10q24-q25 is *MXI1*, which encodes a basic helix-loop-helix protein of the Mad protein family that can counteract the function of Myc oncoproteins as transcriptional activators [48, 51]. Mutations of *MXI1* have been reported in prostate carcinomas [8]. Another putative tumour suppressor gene, designated *DMBT1*, has been cloned from 10q25.3-q26.1 and reported to carry intragenic homozygous deletions in glioblastomas and medulloblastomas [27]. Since neither *MXI1* nor *DMBT1* have been thoroughly investigated in melanomas, it is currently unknown whether one of these genes is altered in these tumours. A recent study suggested that a further melanoma-associated tumour suppressor gene is located on 10p15.3 [37]. Thus, the com-

bined loss of 10q23 (*PTEN*) and 10p15.3 may provide a significant growth advantage over an individual loss of either region, thereby explaining the frequent finding of monosomy 10 in melanomas.

In conclusion, our findings corroborate that mutation of *PTEN* represents an important genetic alteration in a subset of malignant melanomas. However, the frequency of *PTEN* alterations in melanomas in vivo appears to be lower than in melanoma cell lines. The absence of detectable *PTEN* mutations and homozygous deletions in the majority of tumours with chromosome 10 losses raises interesting hypotheses that need to be addressed in further studies: (i) loss of a single *PTEN* allele may already provide a selective growth advantage to melanoma cells due to *PTEN* haploinsufficiency, (ii) complete inactivation of *PTEN* in melanomas may be achieved by mechanisms other than coding region mutations or homozygous gene deletions and (iii) one or more additional, not yet identified, melanoma-associated tumour suppressor gene(s) may be located on chromosome 10.

Acknowledgements This study was supported by grants from the Deutsche Forschungsgemeinschaft (SFB400 C5), the Deutsche Krebshilfe/Dr. Mildred Scheel Foundation (10-1361-Re2) and the Schäfersnolte Foundation.

References

1. Albarosa R, Colombo BM, Roz L, Magnani I, Pollo B, Cirenei N, Giani C, Conti AM, DiDonato S, Finocchiaro G (1996) Deletion mapping of gliomas suggest the presence of two small regions for candidate tumour-suppressor genes in a 17-cM interval on chromosome 10q. Am J Hum Genet 58:1260–1267
2. 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
3. Böni R, Vortmeyer AO, Burg G, Hofbauer G, Zhuang Z (1998) The *PTEN* tumour suppressor gene and malignant melanoma. Melanoma Res 8:300–302
4. Boström J, Mühlbauer A, Reifenberger G (1997) Deletion mapping of the short arm of chromosome 1 identifies a common region of deletion distal to *D1S496* in human meningiomas. Acta Neuropathol 94:479–485
5. Boström J, Cobbers JMJL, Wolter M, Tabatabei G, Weber RG, Lichter P, Collins VP, Reifenberger G (1998) Mutation of the *PTEN* (*MMAC1*) tumour suppressor gene in a subset of glioblastomas but not in meningiomas with loss of chromosome arm 10q. Cancer Res 58:29–33
6. Di Cristofano A, Pesce B, Cordon-Cardo C, Pandolfi PP (1998) Pten is essential for embryonic development and tumour suppression. Nat Genet 19:348–355
7. Di Cristofano A, Kotsi P, Peng YF, Cordon-Cardo C, Elkon KB, Pandolfi PP (1999) Impaired Fas response and autoimmunity in *PTEN*$^{+/-}$ mice. Science 285:2122–2125
8. Eagle LR, Yin X, Brothman AR, Williams BJ, Atkin NB, Prochownik EV (1995) Mutation of the *MXI1* gene in prostate cancer. Nat Genet 9:249–255
9. Franke W, Neumann NJ, Reifenberger J, Megahed M, Ruzicka T, Schulte KW (1998) Melanoma giganteum vom polipoiden Typ. Zeitschrift für Hautkrankheiten H+G 73:705–707
10. Furnari FB, Huang HJS, Cavenee WK (1998) The phosphoinositol phosphatase activity of PTEN mediates a serum-sensitive G_1 growth arrest in glioma cells. Cancer Res 58:5002–5008
11. Greene SL, Thomas JR, Doyle JA (1984) Cowden's disease with associated malignant melanoma. Int J Dermatol 23: 466–467
12. Guldberg P, thor Straten P, Birck A, Ahrenkiel V, Kirkin AF, Zeuthen J (1997) Disruption of the *MMAC1/PTEN* gene by deletion or mutation is a frequent event in malignant melanoma. Cancer Res 57:3660–3663
13. Gyapay G, Morisette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, Bernardi G, Lathrop M, Weissenbach J (1994) The 1993–94 Généthon human genetic linkage map. Nat Genet 7:250–255
14. Healy E, Belgaid CE, Takata M, Vahlquist A, Rehman I, Rigby H, Rees JL (1996) Allelotypes of primary cutaneous melanoma and benign melanocytic nevi. Cancer Res 56:589–593
15. Herbst RA, Weiss J, Ehnis A, Cavenee WK, Arden KC (1994) Loss of heterozygosity for 10q22–10qter in malignant melanoma progression. Cancer Res 54:3111–3114
16. Ichimura K, Schmidt EE, Goike HM, Collins VP (1996) Human glioblastomas with no alterations of the *CDKN2A* (*p16^{INK4a}*, *MTS1*) and *CDK4* gene have frequent mutations of the retinoblastoma gene. Oncogene 13:1065–1072
17. Indsto JO, Holland EA, Kefford RF, Mann GJ (1998) 10q deletions in metastatic cutaneous melanoma. Cancer Genet Cytogenet 100:68–71
18. Isshiki K, Elder DE, Guerry D, Linnenbach AJ (1993) Chromosome 10 allelic loss in malignant melanoma. Genes Chromosomes Cancer 8:178–184
19. Kamb A, Herlyn M (1998) Malignant melanoma. In: Vogelstein B, Kinzler KW (eds) The genetic basis of human cancer. McGraw-Hill, New York, pp 507–518
20. Komiya A, Suzuki H, Ueda T, Yatani R, Emi M, Ito H, Shimazaki J (1996) Allelic losses at loci on chromosome 10 are associated with metastasis and progression of human prostate cancer. Genes Chromosomes Cancer 17:245–253
21. Lee J-O, Yang H, Georgescu M-M, Di Christofano A, Maehama T, Shi Y, Dixon JE, Pandolfi P, Pavletich NP (1999) Crystal structure of the PTEN tumour suppressor: implications for its phosphoinositide phosphatase activity and membrane association. Cell 99:323–334
22. Li DM, Sun H (1997) TEP1, encoded by a candidate tumour suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor beta. Cancer Res 57:2124–2129
23. Li DM, Sun H (1998) PTEN/MMAC1/TEP1 suppresses the tumourigenicity and induces G1 cell cycle arrest in human glioblastoma cells. Proc Natl Acad Sci U S A 95:15406–15411
24. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, Mc Combie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R (1997) *PTEN*, a putative tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275:1943–1947
25. Li J, Simpson L, Takahashi M, Miliaresis C, Myers MP, Tonks N, Parsons R (1998) The PTEN/MMAC1 tumour suppressor induces cell death that is rescued by the AKT/protein kinase B oncogene. Cancer Res 58:5667–5672
26. Liaw D, Marsh DJ, Li J, Dahia PLM, Wang SI, Zheng Z, Bose S, Call KM, Tsou HC, Peacocke M, Eng C, Parsons R (1997) Germline mutations of the *PTEN* gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet 16: 64–67
27. Mollenhauer J, Wiemann S, Scheuerlen W, Korn B, Hayashi Y, Wilgenbus KK, von Deimling A, Poustka A (1997) *DMBT1*, a new member of the SRCR superfamily, on chromosome 10q25.3–26.1 is deleted in malignant brain tumours. Nat Genet 17:32–39
28. Nagase S, Yamakawa H, Sato S, Yajima A, Horii A (1997) Identification of a 790-kilobase region of common allelic loss in chromosome 10q25-q26 in human endometrial cancer. Cancer Res 57:1630–1633
29. 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
30. Perren A, Weng LP, Boag AH, Ziebold U, Thakore K, Dahia PLM, Komminoth P, Lees JA, Mulligan LM, Mutter GL, Eng C (1999) Immunohistochemical evidence of loss of PTEN expression in primary ductal adenocarcinomas of the breast. Am J Pathol 155:1253–1260
31. Podsypanina K, Ellenson LH, Nemes A, Gu J, Tamura M, Yamada KM, Cordon-Cardo C, Catoretti G, Fisher PE, Parsons R (1999) Mutation of *PTEN/MMAC1* in mice causes neoplasia in multiple organ systems. Proc Natl Acad Sci U S A 96:1563–1568
32. Ramaswamy S, Nakamura N, Vazquez F, Batt DB, Perera S, Roberts TM, Sellers WR (1999) Regulation of G1 progression by the PTEN tumour suppressor protein is linked to inhibition of the phosphatidylinositol 3-kinase/Akt pathway. Proc Natl Acad Sci U S A 96:2110–2115
33. Rasheed BKA, McLendon RE, Friedman HS, Friedman AH, Fuchs HE, Bigner DD, Bigner SH (1995) Chromosome 10 deletion mapping in human gliomas: a common deletion region in 10q25. Oncogene 10:2243–2246
34. Reifenberger J, Ring GU, Gies U, Cobbers JMJL, Oberstraß J, An H-X, Niederacher D, Wechsler W, Reifenberger G (1996) Analysis of *p53* mutation and epidermal growth factor receptor amplification in recurrent gliomas with malignant progression. J Neuropathol Exp Neurol 55:824–833
35. Rigel DS, Frieman RJ, Kopf AW (1996) The incidence of malignant melanoma in the United States: issues as we approach the 21st century. J Am Acad Dermatol 34:839–847
36. Robertson GP, Furnari FB, Miele ME, Glendening MJ, Welch DR, Fountain JW, Lugo TG, Huang H-JS, Cavenee WK (1998) In vitro loss of heterozygosity targets the *PTEN/MMAC1* gene in melanoma. Proc Natl Acad Sci U S A 95:9418–9423
37. Robertson GP, Herbst RA, Nagane M, Huang HJ, Cavenee WK (1999) The chromosome 10 monosomy common in human melanomas results from loss of two separate tumour suppressor loci. Cancer Res 59:3596–3601
38. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, USA, pp 9.17–9.19
39. Sheffield VC, Beck JS, Kwitek AE, Sandstrom DW, Stone EM (1993) The sensitivity of single-strand conformation polymorphism analysis for the detection of single base substitutions. Genomics 16:325–332
40. Siegel JM, Reed WB (1976) Cowden's syndrome: report of a case with malignant melanoma. Pahlavi Med J 7:262–269
41. Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, Sasaki T, Rulnd J, Penninger JM, Siderovski DP, Mak TW (1998) Negative regulation of PKB/Akt-dependent cell survival by the tumour suppressor PTEN. Cell 95:29–39
42. Steck PA, Pershouse MA, Jasser SA, Yung WKA, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T, Frye C, Hu R, Swedlund B, Teng DHF, Tavtigian SV (1997) Identification of a candidate tumour suppressor gene, *MMAC1*, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 15:356–362
43. Suzuki A, de la Pompa JL, Stambolic V, Elia AJ, Sasaki T, Barrantes IB, Ho A, Wakeham A, Itie A, Khoo W, Fukumoto M, Mak TW (1998) High cancer susceptibility and embryonic lethality associated with mutation of the *PTEN* tumour suppressor gene in mice. Curr Biol 8:1169–1178
44. Tamura M, Gu J, Matsumoto K, Aota S, Parsons R, Yamada KM (1998) Inhibition of cell migration, spreading, and focal adhesions by tumour suppressor PTEN. Science 280: 1614–1617
45. Thomas DC, Kunkel TA (1993) Replication of UV-irradiated DNA in human cell extracts: evidence for mutagenic bypass of pyrimidine dimers. Proc Natl Acad Sci U S A 90:7744–7748
46. Tsao H, Zhang X, Benoit E, Haluska FG (1998) Identification of *PTEN/MMAC1* alterations in uncultured melanomas and melanoma cell lines. Oncogene 16:3397–3402
47. Walker GJ, Palmer JM, Walters MK, Hayward NK (1995) A genetic model of melanoma tumourigenesis based on allelic losses. Genes Chromosomes Cancer 12:134–141
48. Wechsler DS, Shelly CA, Dang CV (1996) Genomic organization of human *MXI1*, a putative tumour suppressor gene. Genomics 32:466–470
49. Whang YE, Wu X, Suzuki H, Reiter RE, Tran C, Vessella RL, Said JW, Isaacs WB, Sawyers CL (1998) Inactivation of the tumour suppressor *PTEN/MMAC1* in advanced human prostate cancer through loss of expression. Proc Natl Acad Sci U S A 95:5246–5250
50. Wu X, Senechal K, Neshat MS, Whang YE, Sawyers CL (1998) The PTEN/MMAC1 tumour suppressor phosphatase functions as a negative regulator of the phosphoinositide 3-kinase/Akt pathway. Proc Natl Acad Sci U S A 95:15587–15591
51. Zervos AS, Gyuris J, Brent R (1993) Mxi1, a protein that specifically interacts with Max to bind Myc-Max recognition sites. Cell 72:223–232