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Epigenetic PTEN Silencing in Malignant Melanomas without *PTEN* Mutation

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A tumor suppressor gene at 10q 23.3, designated *PTEN*, encoding a dual specificity phosphatase with lipid and protein phosphatase activity, has been shown to play an important role in the pathogenesis of a variety of human cancers. Germline mutations in *PTEN* cause Cowden syndrome (CS), which is characterized by multiple hamartomas and a high risk of breast and thyroid cancers. Frequent loss of heterozygosity at 10q is found in both early and advanced-stage sporadic melanomas; however, mutations or deletions in *PTEN* are detected mainly in melanoma cell lines. In this study, we examined PTEN expression in 34 unselected sporadic melanomas (4 primary melanomas, 30 metastases) using immunohistochemistry and correlated this with the results of structural studies of this gene. Immunostaining of 34 melanoma samples revealed no PTEN expression in 5 (15%) and low PTEN expression in 17 (50%), whereas the rest of the tumors (35%) had high levels of expression. Hemizygous deletion was found in 32% of the tumors but neither intragenic *PTEN* mutation nor biallelic deletion was found in any of the samples. Of the 5 melanomas showing no PTEN expression, 4 had no mutation or deletion of *PTEN*. Of the 13 tumors having weak PTEN immunoreactivity and informative loss of heterozygosity results, 6 had evidence of hemizygous allelic loss of *PTEN* while the remaining 7 had intact *PTEN*. These results strongly support *PTEN* as a major tumor suppressor on 10q involved in melanoma tumorigenesis and suggest an epigenetic mechanism of biallelic functional inactivation not previously observed in other cancers where PTEN might be involved. ***(Am J Pathol 2000, 157:1123–1128)***

PTEN/MMAC1/TEP1 was recently identified as a tumor suppressor gene located at 10q23.3 and has been shown to be deleted or mutated in a variety of advanced tumors.[1–3] PTEN is a dual-specificity phosphatase with lipid and protein phosphatase activity. PTEN functions as a major lipid phosphatase, dephosphorylates PtdIns-3, 4, 5-triphosphate (PIP-3) and PtdIns-3, 4-diphosphate (PIP-2), which are required for the activation of PKB/Akt, an important mediator of cell survival protecting cells from apoptosis.[4–7] PTEN has been shown to be involved in cell migration, spreading, and focal adhesion formation through dephosphorylating focal adhesion kinase (FAK), presumably through its protein phosphatase activity.[8,9] Ectopic expression of PTEN results in cell cycle arrest at G1 and/or apoptosis in glioma and breast cancer cell line models.[5,10,11]

Germline mutations of *PTEN* have been found in the autosomal dominant hamartoma syndrome, Cowden Syndrome (CS), which is characterized by multiple hamartomas and an increased risk of malignant and benign breast and thyroid tumors, and Bannayan-Riley-Ruvalcaba (BRR) syndrome, a related disorder characterized by neonatal onset macrocephaly, lipomatosis, hemangiomas, and speckled penis, as well as some cancer risk.[12–15] Recently, a *Proteus*-like syndrome was found to result from germline and germline mosaic *PTEN* mutations.[16]

PTEN has been shown to be somatically deleted or mutated, to a greater or lesser extent, in a wide variety of sporadic advanced tumors, especially glioblastoma multiforme, endometrial carcinoma and advanced prostate cancers.[17–21] Most melanomas occur without any family history of melanocytic tumors and it is controversial whether melanomas are true component tumors of CS. Hereditary melanomas only account for approximately 10% of all clinical presentations; thus, the great majority are sporadic.[22] Involvement of genes that mediate growth arrest via cell cycle regulation, such as *p16*, has been found in a subset of hereditary and sporadic melanomas.[23] Loss of genetic material of the long arm of chromosome 10 has been detected in 30 to 50% of both early and advanced-stage sporadic melanomas, and has been associated with poor clinical outcome.[24,25] Given

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the frequent loss of heterozygosity (LOH) at 10q in melanomas, the 10q localization of the *PTEN* gene and the role of PTEN in cell cycle regulation and apoptosis, PTEN may be viewed as an excellent candidate melanoma growth suppressor to play a role in melanocytic oncogenesis. There are at least eight studies, comprising various numbers of tumors, examining the genomic status of *PTEN* in melanomas of various stages and principally, melanoma cell lines, with inconsistent results.[2,26–32] Despite the inconsistent results, it appeared that homozygous deletions and intragenic mutations of *PTEN* occurred, to an unknown extent, in the metastatic setting, especially in cell lines. Therefore, we sought to determine whether structural alterations in *PTEN* occurred with any frequency in noncultured cutaneous melanomas, especially in the metastatic setting, if loss of PTEN expression, detected by immunohistochemistry, is a mechanism of loss of function in melanomas, and if there is a correlation between PTEN protein expression levels and genomic structural alterations of this gene.

Materials and Methods

Melanoma Samples

Thirty-four unselected sporadic primary or metastatic melanoma samples were obtained from cases undergoing surgery in the Division of Surgical Oncology, James Cancer Hospital and Solove Research Institute, Ohio State University Comprehensive Cancer Center (Columbus, OH) during the period from 1997 to 1999, in an anonymized fashion in accordance with an IRB-approved protocol. Of these 34 samples, 4 were primary cutaneous melanomas and 30 were metastatic to various sites, including lymph nodes and other soft tissues. Tumor tissues were snap-frozen and kept at −80°C until nucleic acid extraction. Normal tissue from unaffected surrounding fat or muscle was obtained from 28 of these patients during the surgical procedure and served as a source of germline DNA. DNA was extracted from frozen normal and tumor tissue using Qiagen DNA-Mini kit according to the manufacturer's instructions (Qiagen, Valencia, CA).

Paraffin-embedded tissue blocks were available for all 34 melanomas. Four-micrometer sections were cut and mounted on Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA) for immunohistochemistry studies.

Immunohistochemistry

The specificity of PTEN monoclonal antibody 6H2.1 has been proven previously.[33,34] This antibody, raised against the last 100 C-terminal amino acids of human PTEN, was used essentially as previously described[33] with minor modifications. In brief, the sections were deparaffinized and hydrated by passing through xylene and a graded series of ethanol. Antigen retrieval was performed for 20 minutes at 98°C in 0.01 mol/L sodium citrate buffer, pH 6.4, in a microwave oven and incubating the sections in 0.3% hydrogen peroxide. After blocking for 30 minutes in 0.75% normal horse serum, the sections were incubated with 6H2.1 (dilution 1:100) overnight (or 16 hours) at 4°C. The sections were washed in PBS, pH 7.3, and then incubated with biotinylated horse anti-mouse IgG followed by avidin peroxidase using the Vectastain ABC elite kit (Vector Laboratories, Burlingame, CA). The chromogenic reaction was carried out with 3′−3′ diaminobenzidine using nickel cobalt amplification, which gives a black product. For sections with abundant melanin, 3′−3′−3′ triaminobenzidine was used, which produces a blue product. After counterstaining with nuclear Fast Red (Rowley Biochemical, Danvers, MA) and mounting, the slides were evaluated under a light microscope. The immunostaining patterns and intensities were determined by two independent observers (X.-P. Z. and C. E.) who each examined and independently scored the slides on two separate occasions. As previously described,[33,34] the vascular endothelium serves as an internal positive control, and the immunostaining of the endothelium is scored as ++. Levels of immunostaining in vascular endothelium are remarkably constant among various tissues, including breast,[33] thyroid,[34] pancreas (Perren A, Eng C, unpublished results) and colon (Zhou XP, Eng C, unpublished results). Immunostaining intensities equal to the vascular endothelium in a particular sample were scored as ++; weak or decreased staining intensity as +; and no immunostaining as −. Staining intensities greater than those of the internal control vascular endothelium were graded +++.

LOH Analysis

All 28 of the melanoma samples with matching germline DNA available were analyzed for LOH with markers D10S579, D10S2491, and D10S541, the former two of which are within 500 kb upstream of the transcriptional start site of *PTEN* and the latter within 300 kb of the translational stop site. In addition, LOH analysis was performed using intragenic intronic polymorphic markers IVS4 + 109 ins/del TCTTA and IVS8 + 32T/G within *PTEN*. Assessment of the status at IVS8 + 32T/G was performed by differential digestion with the restriction endonuclease *Hin*cII as described.[35] The status at IVS4 + 109 ins/delTCTTA was screened by PCR-based differential digestion with restriction endonuclease *Afl*II following the manufacturer's instructions (New England Biolabs, Beverly, MA). The primers used to amplify a 325-bp fragment containing exon 4 and this polymorphic site within intron 4 are E4-F (forward, 5′-CATTATAAAGATTCAGGCAAT-3′) and MMAC4-R (reverse, 5′-CTTTATGCAATACTTTTTCCTA-3′).

Mutation Analysis

PTEN mutation analysis of all 9 coding exons, exon-intron junctions, and flanking intronic sequences was performed using PCR-based denaturing gradient gel electrophoresis and semi-automated sequencing as previously described.[12,14,36]

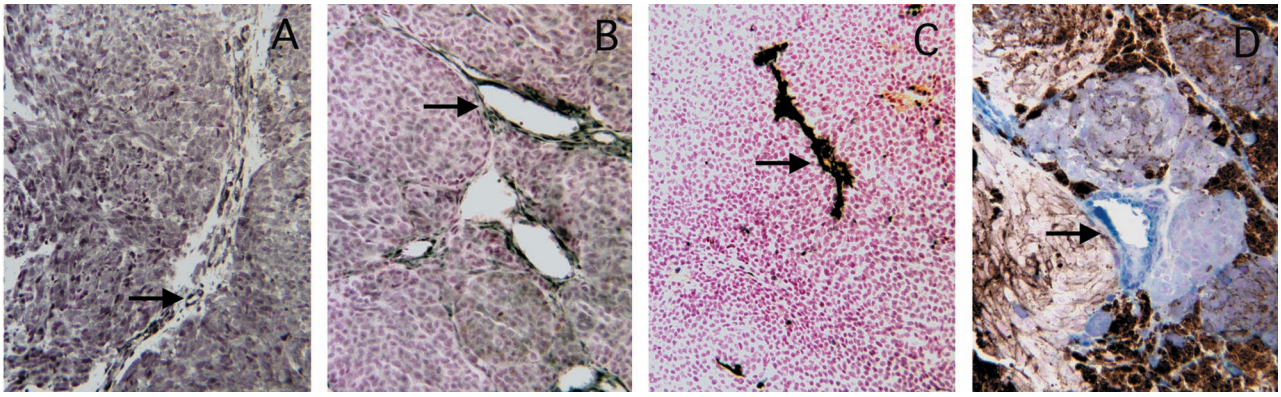

Figure 1. PTEN immunohistochemistry in melanomas. Positive staining (++) of vascular endothelial cells serves as an internal positive control (**arrows**). **A:** Melanoma exhibiting positive staining (++) for PTEN in all tumor cells. **B:** Melanoma exhibiting weak staining (+) in majority of tumor cells. **C:** Melanoma with negative staining (−) for PTEN in all tumor cells. **D:** Melanoma exhibiting absent staining for PTEN in tumor cells with abundant melanin. Original magnifications, ×20.

Results

PTEN Immunohistochemistry in Primary and Metastatic Melanomas

The expression of PTEN in 30 metastatic melanomas and 4 primary melanomas was evaluated by immunohistochemical analysis. All 34 melanoma sections had accompanying vascular endothelial cells present, which showed strong PTEN immunostaining in the cytoplasm and the nucleus, were graded ++, and served as internal positive controls as described previously.[33,34] Interestingly, the endothelial cells showed strong (++) PTEN immunostaining with a nuclear predominance (Figure 1). Nuclear and cytoplasmic staining intensity of fibrocytes varied from very strong (+++) to weak (+).

Overall, among all 34 melanomas, the neoplastic nuclei had weak (+) or no (−) PTEN immunostaining compared to the nuclear staining (++) of their respective vascular endothelial cells. Nearly two-thirds of the melanomas, 22 of 34 (65%), had weak (+) or absent (−) cytoplasmic PTEN staining (Figure 1, B and C; Table 1). Five (15%) melanomas lost all PTEN immunoreactivity (−) and 17 (50%) showed weak (+) cytoplasmic PTEN immunostaining. In general, the staining intensity or lack thereof within a sample was relatively uniform.

The remaining one-third of melanomas (12 of 34; 35%) showed moderate (++) cytoplasmic immunostaining and weak (+) nuclear staining compared to that of their respective vascular endothelial cells (Figure 1A; Table 1). However, two distinct staining patterns were noted among this group of 12 melanomas. Of these 12, 8 had uniform immunostaining of all tumor cells. The remaining 4 from this group had non-uniform staining of the tumor cells.

There were 8 tumors (7 metastatic; one primary) with abundant melanin in the cytoplasm of some or most tumor cells. Interestingly, most of the tumor cells with abundant melanin in their cytoplasm had weak (+) or no (−) immunohistochemical evidence of PTEN expression (Figure 1D). Of these 8 tumors with abundant melanin, 5 had weak immunostaining and 3 had no PTEN expression.

Table 1. Summary of PTEN Immunostaining and LOH Data

	PTEN staining		
	++	+	−
LOH	2	6	1
ROH	8	7	4
Total	10	13	5

LOH, loss of heterozygosity; ROH, retention of heterozygosity.

LOH and PTEN Mutation Analysis

PCR-based denaturing gradient gel electrophoresis revealed no intragenic *PTEN* mutations in any of the 34 melanomas (data not shown).

LOH analysis was performed on the 28 melanomas with corresponding germline DNA available using three microsatellite markers flanking *PTEN* (D10S579, D10S2491, and D10S541), and two polymorphic markers within *PTEN* (IVS4 + 109ins/delTCTTA and IVS8 + 32G/T). All 28 pairs were informative at a minimum of one of these markers and 26 of these pairs were informative at 2 or more loci (Figure 2). LOH at 10q23 was scored when one or more of the panel of 5 polymorphic loci showed LOH. Using these criteria, 10q23 LOH was seen in 9 tumors (32%; Figure 2). However, if the criteria were made more stringent, ie, LOH was scored only if 1 of the 2 intragenic *PTEN* polymorphic markers (IVS4 + 109ins/delTCTTA and/or IVS8 + 32G/T) showed LOH, then we found evidence for *PTEN* hemizygous deletion in only 5 tumors (18% of total, 31% of 16 cases informative for at least 1 of the 2 intragenic loci).

Comparison of Immunohistochemical and Structural Mutation Data

There were a total of 28 paired samples where both immunohistochemical data and LOH data are available. All but one were informative at 2 or more polymorphic loci within or flanking *PTEN* (Figure 2). Ten of the 28 samples were graded ++ for PTEN expressioin by immunohisto-

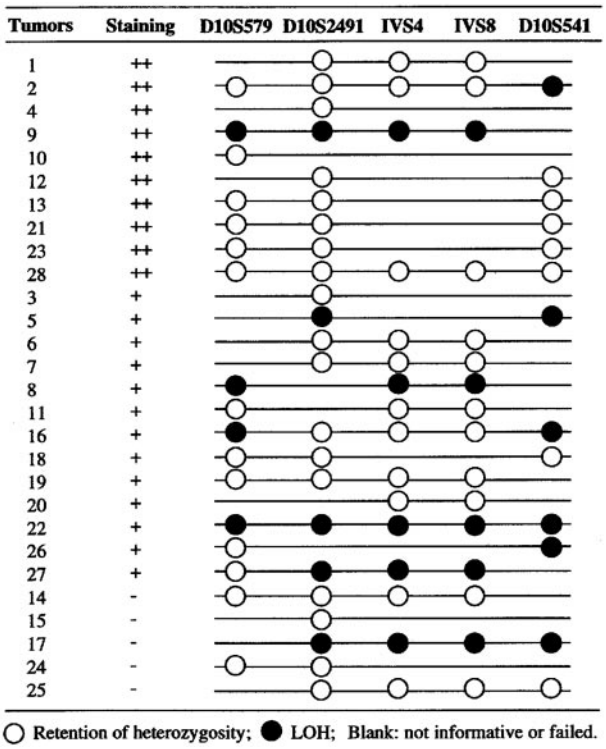

Figure 2. Correlation between PTEN immunostaining and LOH at 10q23 in melanomas.

chemistry, and 6 of these 10 did not show evidence of 10q23 or *PTEN* deletion. Two samples (Figure 2, samples 2 and 9, and Table 1) might be viewed as apparently discordant when LOH data are compared to those obtained from immunohistochemistry. Sample 2 showed LOH at D10S541 and retention of both alleles at the remaining 4 loci, notably those within *PTEN*. Thus, although this sample had LOH 3′ of *PTEN*, it is doubtful if it is deleted within *PTEN*. Hence, these data are concordant. Sample 9, on the other hand, showed LOH at all loci except for D10S541 where it is not informative (Figure 2). This melanoma showed a patchy, non-uniform staining pattern with weak (+) staining in some regions of the tumor section, although strong (++) staining was predominant.

Five melanomas were immunostain negative (−). Among these, 4 had no evidence of *PTEN* allele loss and 1 showed LOH involving 4 of the 5 markers (Figure 2).

Thirteen melanomas were immunostained weakly (+) and were informative at at least one polymorphic locus (Figure 2). Of these 13, 6 might be classified as having LOH representing hemizygous *PTEN* allelic loss that could correspond to the diminished immunostaining (Figure 2, samples 5, 8, 16, 22, 26, and 27). The remaining 7 with weak immunoreactivity retained heterozygosity at their respective informative loci.

In summary, the presence of LOH always correlated with decreased PTEN expression. In contrast, neither the stringent nor more relaxed criteria for scoring LOH in *PTEN* deletion revealed a correlation between the absence of allelic losses and PTEN expression.

Correlation between PTEN Immunohistochemistry and Clinicopathological Parameters

With a relatively small series comprising 30 metastatic tumors and 4 primary melanomas, we could not perform a rigorous statistical analysis of PTEN immunohistochemistry and clinicopathological features. However, it might be worth noting that all of the 5 melanomas with complete loss of PTEN expression (−) were metastatic melanomas. Among the only 4 primary tumors, 2 had hemizygous *PTEN* deletion and showed weak PTEN expression (+), whereas the remaining 2 had strong immunostaining (++).

Discussion

That loss of heterozygosity of markers along the long arm of chromosome 10 occurs in primary and metastatic melanomas is beyond doubt, however, to what extent *PTEN*, on 10q23.3, plays a role was questionable based on previous studies using mainly melanoma cell lines and some noncultured primary and metastatic melanomas.[2,26–32] In this study, we have found that somatic intragenic mutations of *PTEN* do not occur to a significant degree in non-cell line primary and metastatic melanomas. Power calculations suggest that if *PTEN* mutations occur at a frequency of 10% or more, the sample size of 34 used in this study should have a >95% likelihood of detecting at least one such mutation. Given that no mutations were noted, even in metastatic tumors, it might be reasonable to claim that intragenic *PTEN* mutation does not occur in more than 10% of melanomas and hence, is not considered a major mechanism of inactivation in this tumor type, at least in the midwestern United States population.

A relatively large study based on European melanoma samples[32] and a small United States study[28] have shown biallelic structural inactivation occurs by either homozygous deletion at 10q23 or somatic intragenic *PTEN* mutation plus loss of the remaining wild-type allele. In contrast, we have found that biallelic structural alteration does not occur in melanomas, even in the metastatic setting. Instead, even in the primary setting, hemizygous deletion at 10q23, encompassing *PTEN*, can occur with some frequency (50% of a small number) as an early event. In the metastatic setting, partial or complete expressional loss at the protein level was shown to occur in two-thirds of the melanomas, 75% of which are due to epigenetic silencing of PTEN (Figure 2). Indeed, of the 5 metastatic tumors with no PTEN expression, 4 had complete silencing of both *PTEN* alleles via mechanisms beyond structural alteration and one experienced loss of one allele and silencing of the remaining wild-type allele by epigenetic means (Table 1 and Figure 2). Similarly, among the 13 tumors (2 primary) with weak (+) PTEN immunostaining, 6 (2 primary) had hemizygous allelic loss that could account for the decreased protein expression. However, the remaining 7 had neither *PTEN* mutation nor deletion. Thus, these 7 might have monoallelic silencing via mechanisms other than structural alteration (mutation/deletion) of *PTEN*, which would also account for the decreased expression. All 10 melanomas with

strong immunostaining (++, Figure 2) have intact biallelic *PTEN* structure, although tumor 9 is worthy of comment. Tumor 9 has LOH involving all of *PTEN*. However, immunostaining is patchy: the majority of the tumor in the section had strong (++) immunostaining, but there were small areas of weak (+) staining. It is possible that the portion of this tumor used for LOH analysis is reflected by the areas of weak staining.

Epigenetic mechanisms of inactivation of *PTEN* were initially postulated for a subset of prostate cancer lines.[37] In this instance, hypermethylation of the promoter was shown to be the mechanism. Subsequently, epigenetic *PTEN* silencing was shown to be a major mechanism in hematological malignancy cell lines, where approximately 30 to 40% of these lines had a genomic alteration (deletion or mutation), 50% had no transcript, and up to 70% had no PTEN protein.[7] Thus, in these cell lines, transcriptional silencing, likely secondary to promoter methylation, as well as translational and post-translational mechanisms are pertinent.

High levels of PTEN expression, as demonstrated by immunohistochemistry, have been found in the developing human neural crest (graded +++), which is the precursor of melanocytes, and to a lesser extent (++) melanoblasts and normal adult melanocytes[34] (Zhou X-P, Eng C, unpublished findings). PTEN plays a role in G1 arrest and/or apoptosis (see Introduction). Conceivably, absent or decreased levels of such a molecule in melanocytes could lead to inability to cell cycle arrest and inability to undergo programmed cell death, thereby leading to melanoma formation. If PTEN's role in cell adhesion and migration can be confirmed, then absent or decreased PTEN could also lead to increased ability to metastasize.

With the accumulating knowledge of PTEN inactivation, it would appear that there are several mechanisms which lead to PTEN inactivation, and these might operate in a tissue-specific manner. Initial tumor cell line work almost uniformly suggested that *PTEN* intragenic mutations, homozygous deletions, and the two structural hits would be the rule across a large variety of tumors.[1,2,27] However, in retrospect, it would appear that these sorts of *PTEN* defects, which result in inability to arrest at G1 and/or inability to undergo apoptosis, are selected for in cell lines. Among noncultured neoplasias, in contrast, the high frequency of *PTEN* intragenic mutations and homozygous deletions have not been found in all tumor types, as suggested by early cell line work. Glioblastoma multiforme and endometrial adenocarcinomas seem to have a high frequency of intragenic *PTEN* mutations and deletions, such that in these two tumor types, the two structural hits can be observed.[17–20] Primary breast carcinomas have a relatively low ($\ll$5%) frequency of somatic intragenic *PTEN* mutation and a hemizygous deletion frequency of 30 to 40%.[38–40] However, we have shown that the second hit in primary breast carcinomas can be epigenetic as well.[33] Early work on sporadic noncultured nonmedullary thyroid tumors revealed a 10 to 25% LOH frequency, without any homozygous deletions, and a somatic frameshift mutation within *PTEN* in a single papillary thyroid carcinoma.[35,41] Recently, we have shown that although an epigenetic second hit can occur to functionally silence PTEN protein expression in thyroid tumors, differential subcellular compartmentalization between nucleus and cytoplasm might represent a novel mechanism of functional inactivation or modulation in nonmedullary thyroid cancers.[34] Noncultured malignant melanomas might be somewhat unique: though *PTEN* inactivation is seen to occur by one structural hit (LOH) followed by the second epigenetic hit, *PTEN* protein expression in melanomas can be biallelically silenced by epigenetic phenomena as well. Although numbers are relatively small, our study suggests that the latter might be a major mechanism of PTEN inactivation in the pathogenesis of melanomas.

In summary, we have found weak or absent PTEN protein expression with relatively high frequency in malignant melanomas. Our data not only strongly support PTEN as a major tumor suppressor on 10q involved in melanoma tumorgenesis, but also suggest a unique mechanism of biallelic functional inactivation not previously observed in other cancers where *PTEN* might be involved.

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References

1. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R: PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 1997, 275:1943–1947
2. Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T, Frye C, Hu R, Swedlund B, Teng DH, Tavtigian SV: Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 1997, 15:356–362
3. Li DM, Sun H: TEP1, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor beta. Cancer Res 1997, 57:2124–2129
4. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME: Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 1997, 91:231–241
5. Furnari FB, Huang HJ, Cavenee WK: The phosphoinositol phosphatase activity of PTEN mediates a serum-sensitive G1 growth arrest in glioma cells. Cancer Res 1998, 58:5002–5008
6. Myers MP, Pass I, Batty IH, Van der Kaay J, Stolarov JP, Hemmings BA, Wigler MH, Downes CP, Tonks NK: The lipid phosphatase activity of PTEN is critical for its tumor supressor function. Proc Natl Acad Sci USA 1998, 95:13513–13518
7. Dahia PL, Aguiar RC, Alberta J, Kum JB, Caron S, Sill H, Marsh DJ, Ritz J, Freedman A, Stiles C, Eng C: PTEN is inversely correlated with the cell survival factor Akt/PKB and is inactivated via multiple mechanisms in haematological malignancies. Hum Mol Genet 1999, 8:185–193
8. Tamura M, Gu J, Matsumoto K, Aota S, Parsons R, Yamada KM: Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. Science 1998, 280:1614–1617
9. Gu J, Tamura M, Pankov R, Danen EH, Takino T, Matsumoto K, Yamada KM: Shc and FAK differentially regulate cell motility and directionality modulated by PTEN. J Cell Biol 1999, 146:389–403
10. Li DM, Sun H: PTEN/MMAC1/TEP1 suppresses the tumorigenicity

and induces G1 cell cycle arrest in human glioblastoma cells. Proc Natl Acad Sci USA 1998, 95:15406–15411
11. Weng LP, Smith WM, Dahia PL, Ziebold U, Gil E, Lees JA, Eng C: PTEN suppresses breast cancer cell growth by phosphatase activity-dependent G1 arrest followed by cell death. Cancer Res 1999, 59: 5808–5814
12. Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z, Bose S, Call KM, Tsou HC, Peacocke M, Eng C, Parsons R: Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet 1997, 16:64–67
13. Marsh DJ, Dahia PL, Zheng Z, Liaw D, Parsons R, Gorlin RJ, Eng C: Germline mutations in PTEN are present in Bannayan-Zonana syndrome (letter). Nat Genet 1997, 16:333–334
14. Marsh DJ, Coulon V, Lunetta KL, Rocca-Serra P, Dahia PL, Zheng Z, Liaw D, Caron S, Duboue B, Lin AY, Richardson AL, Bonnetblanc JM, Bressieux JM, Cabarrot-Moreau A, Chompret A, Demange L, Eeles RA, Yahanda AM, Fearon ER, Fricker JP, Gorlin RJ, Hodgson SV, Huson S, Lacombe D, LePrat F, Odent S, Toulouse C, Olopade OI, Sobol H, Tishler S, Woods, CG, Robinson BG, Weber HC, Parsons R, Peacoche M, Long M, Eng C: Mutation spectrum and genotype-phenotype analyses in Cowden disease and Bannayan-Zonana syndrome, two hamartoma syndromes with germline PTEN mutation. Hum Mol Genet 1998, 7:507–515
15. Marsh DJ, Kum JB, Lunetta KL, Bennett MJ, Gorlin RJ, Ahmed SF, Bodurtha J, Crowe C, Curtis MA, Dasouki M, Dunn T, Feit H, Geraghty MT, Graham JM, Jr., Hodgson SV, Hunter A, Korf BR, Manchester D, Miesfeldt S, Murday VA, Nathanson KL, Parisi M, Pober B, Romano C, Tolmie JL, Trembath R, Winter RM, Zackai EH, Zori RT, Weng LP, Dahra PLM, Eng C: PTEN mutation spectrum and genotype-phenotype correlations in Bannayan-Riley-Ruvalcaba syndrome suggest a single entity with Cowden syndrome: Hum Mol Genet 1999, 8:1461–1472
16. Zhou XP, Marsh DJ, Hampel H, Mulliken JB, Gimm O, Eng C: Germline and germline mosaic PTEN mutations associated with a Proteus-like syndrome of hemihypertrophy, lower limb asymmetry, arteriovenous malformations and lipomatosis. Hum Mol Genet 2000, 9:765–768
17. Wang SI, Puc J, Li J, Bruce JN, Cairns P, Sidransky D, Parsons R: Somatic mutations of PTEN in glioblastoma multiforme. Cancer Res 1997, 57:4183–4186
18. Rasheed BK, Stenzel TT, McLendon RE, Parsons R, Friedman AH, Friedman HS, Bigner DD, Bigner SH: PTEN gene mutations are seen in high-grade but not in low-grade gliomas. Cancer Res 1997, 57: 4187–4190
19. Duerr EM, Rollbrocker B, Hayashi Y, Peters N, Meyer-Puttlitz B, Louis DN, Schramm J, Wiestler OD, Parsons R, Eng C, von Deimling A: PTEN mutations in gliomas and glioneuronal tumors. Oncogene 1998, 16:2259–2264
20. Tashiro H, Blazes MS, Wu R, Cho KR, Bose S, Wang SI, Li J, Parsons R, Ellenson LH: Mutations in PTEN are frequent in endometrial carcinoma but rare in other common gynecological malignancies. Cancer Res 1997, 57:3935–3940
21. Cairns P, Okami K, Halachmi S, Halachmi N, Esteller M, Herman JG, Jen J, Isaacs WB, Bova GS, Sidransky D: Frequent inactivation of PTEN/MMAC1 in primary prostate cancer. Cancer Res 1997, 57: 4997–5000
22. Welch DR, Goldberg SF: Molecular mechanisms controlling human melanoma progression and metastasis. Pathobiology 1997, 65:311–330
23. Kamb A, Herlin M: Malignant melanoma. The Genetic Basis of Human Cancer. Edited by Vogelstein B, Kinzler KW. New York, McGraw-Hill, 1998, pp 507–518
24. Herbst RA, Weiss J, Ehnis A, Cavenee WK, Arden KC: Loss of heterozygosity for 10q22–10qter in malignant melanoma progression. Cancer Res 1994, 54:3111–3114
25. Healy E, Belgaid C, Takata M, Harrison D, Zhu NW, Burd DA, Rigby HS, Matthews JN, Rees JL: Prognostic significance of allelic losses in primary melanoma. Oncogene 1998, 16:2213–2218
26. Guldberg P, thor Straten P, Birck A, Ahrenkiel V, Kirkin AF, Zeuthen J: Disruption of the MMAC1/PTEN gene by deletion or mutation is a frequent event in malignant melanoma. Cancer Res 1997, 57:3660–3663
27. Teng DH, Hu R, Lin H, Davis T, Iliev D, Frye C, Swedlund B, Hansen KL, Vinson VL, Gumpper KL, Ellis L, El-Naggar A, Frazier M, Jasser S, Langford LA, Lee J, Mills GB, Pershouse MA, Pollack RE, Tornos C, Troncoso P, Yung WK, Fujii G, Berson A, Steck PA, et al: MMAC1/PTEN mutations in primary tumor specimens and tumor cell lines: Cancer Res 1997, 57:5221–5225
28. Tsao H, Zhang X, Benoit E, Haluska FG: Identification of PTEN/MMAC1 alterations in uncultured melanomas and melanoma cell lines. Oncogene 1998, 16:3397–3402
29. Robertson GP, Furnari FB, Miele ME, Glendening MJ, Welch DR, Fountain JW, Lugo TG, Huang HJ, Cavenee WK: In vitro loss of heterozygosity targets the PTEN/MMAC1 gene in melanoma. Proc Natl Acad Sci USA 1998, 95:9418–9423
30. Boni R, Vortmeyer AO, Burg G, Hofbauer G, Zhuang Z: The PTEN tumour suppressor gene and malignant melanoma. Melanoma Res 1998, 8:300–302
31. Herbst RA, Podewski EK, Mommert S, Kapp A, Weiss J: PTEN and MXI1 allelic loss on chromosome 10q is rare in melanoma in vivo. Arch Dermatol Res 1999, 291:567–569
32. Birck A, Ahrenkiel V, Zeuthen J, Hou-Jensen K, Guldberg P: Mutation and Allelic Loss of the PTEN/MMAC1 gene in Primary and Metastatic Melanoma Biopsies. J Invest Dermatol 2000, 114:277–280
33. Perren A, Weng LP, Boag AH, Ziebold U, Thakore K, Dahia PL, Komminoth P, Lees JA, Mulligan LM, Mutter GL, Eng C: Immunohistochemical evidence of loss of PTEN expression in primary ductal adenocarcinomas of the breast. Am J Pathol 1999, 155:1253–1260
34. Gimm O, Perren A, Weng LP, Marsh DJ, Yeh JJ, Zeibold U, Gil E, Hinze R, Delbridge L, Lees JA, Mutter GL, Robinson BG, Komminoth P, Dralle H, Eng C: Differential nuclear and cytoplasmic expression of PTEN in normal thyroid tissue, and benign and malignant epithelial thyroid tumors. Am J Pathol 2000, 156:1693–1700
35. Dahia PL, Marsh DJ, Zheng Z, Zedenius J, Komminoth P, Frisk T, Wallin G, Parsons R, Longy M, Larsson C, Eng C: Somatic deletions and mutations in the Cowden disease gene, PTEN, in sporadic thyroid tumors. Cancer Res 1997, 57:4710–4713
36. Marsh DJ, Roth S, Lunetta KL, Hemminki A, Dahia PL, Sistonen P, Zheng Z, Caron S, van Orsouw NJ, Bodmer WF, Cottrell SE, Dunlop MG, Eccles D, Hodgson SV, Jarvinen H, Kellokumpu I, Markie D, Neale K, Phillips R, Rozen P, Syngal S, Vijg J, Tomlinson IP, Aaltonen LA, Eng C: Exclusion of PTEN and 10q22–24 as the susceptibility locus for juvenile polyposis syndrome. Cancer Res 1997, 57:5017–5021
37. Whang YE, Wu X, Suzuki H, Reiter RE, Tran C, Vessella RL, Said JW, Isaacs WB, Sawyers CL: Inactivation of the tumor suppressor PTEN/MMAC1 in advanced human prostate cancer through loss of expression. Proc Natl Acad Sci USA 1998, 95:5246–5250
38. Rhei E, Kang L, Bogomolniy F, Federici MG, Borgen PI, Boyd J: Mutation analysis of the putative tumor suppressor gene PTEN/MMAC1 in primary breast carcinomas. Cancer Res 1997, 57:3657–3659
39. Singh B, Ittmann MM, Krolewski JJ: Sporadic breast cancers exhibit loss of heterozygosity on chromosome segment 10q23 close to the Cowden disease locus. Genes Chromosomes Cancer 1998, 21:166–171
40. Feilotter HE, Coulon V, McVeigh JL, Boag AH, Dorion-Bonnet F, Duboue B, Latham WC, Eng C, Mulligan LM, Longy M: Analysis of the 10q23 chromosomal region and the PTEN gene in human sporadic breast carcinoma. Br J Cancer 1999, 79:718–723
41. Marsh DJ, Zheng Z, Zedenius J, Kremer H, Padberg GW, Larsson C, Longy M, Eng C: Differential loss of heterozygosity in the region of the Cowden locus within 10q22–23 in follicular thyroid adenomas and carcinomas. Cancer Res 1997, 57:500–503