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REVIEW

Genetic alterations of *PTEN* in human melanoma

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Abstract The *PTEN* gene is one of the most frequently inactivated tumor suppressor genes in sporadic cancers. Inactivating mutations and deletions of the *PTEN* gene are found in many types of cancers, including melanoma. However, the exact frequency of *PTEN* alteration in melanoma is unknown. In this study, we comprehensively reviewed 16 studies on *PTEN* genetic changes in melanoma cell lines and tumor biopsies. To date, 76 *PTEN* alterations have been reported in melanoma cell lines and 38 *PTEN* alterations in melanoma biopsies. The rate of *PTEN* alterations in melanoma cell lines, primary melanoma, and metastatic melanoma is 27.6, 7.3, and 15.2%, respectively. Three mutations were found in both melanoma cell lines and biopsies. These mutations are scattered throughout the gene, with the exception of exon 9. A mutational hot spot is found in exon 5, which encodes the phosphatase activity domain. Evidence is also presented to suggest that numerous homozygous deletions and missense variants exist in the *PTEN* transcript. Studying *PTEN* functions and implications of its mutations and other genes could provide insights into the precise nature of *PTEN* function in melanoma and additional targets for new therapeutic approaches.

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Introduction

*P*hosphatase and *ten*sin homolog (*PTEN*) is 47-kDa protein and was first identified as a candidate tumor suppressor gene in 1997 after its positional cloning from a region of chromosome 10q23 known to exhibit loss in a wide spectrum of tumor types [1–3]. Since then, mutations of *PTEN* have been detected in a variety of human cancers including breast, thyroid, glioblastomas, endometrial, prostate, and melanoma [4–14]. Inherited mutations in this gene also predispose carriers to develop Cowden's disease, a heritable cancer risk syndrome, and several related conditions [15–17]. PTEN is classified as a tumor suppressor because its activity is lost by deletion, mutation, or through epigenetic changes [18–21]. Molecular mechanistic studies of PTEN have provided a great deal of insight into the basis for its involvement in tumor suppression. The PTEN protein has both protein phosphatase and lipid phosphatase activity [22, 23]. Although the tumor suppressive function of PTEN has mainly been attributed to its lipid phosphatase activity, a role for PTEN protein phosphatase activity in cell-cycle regulation and inhibition of cell invasion in vitro has been suggested as well [24–28]. Loss of PTEN function seems to be responsible for many of the phenotypic features of melanoma, thus PTEN may serve as a potential target for drug development. However, most types of tumors with *PTEN* alteration also carry other genetic changes, making the role of PTEN more ambiguous. As discussed below, *PTEN* homozygous deletions and missense mutations alone is sufficient to cause tumorigenesis in certain tissues but not in others. However, even when

mutation of *PTEN* alone has minimal effects, it frequently contributes to tumorigenesis in the context of other genetic alterations. In this review, signaling pathways mediated by the lipid and protein phosphatase activities of PTEN, and the implication of PTEN loss in melanoma tumorigenesis, will be discussed.

PTEN, a tumor suppressor gene

The most convincing initial insight into the potential involvement of chromosome 10 in melanoma was reported by Fountain et al. [29]. Many Studies on the relative frequency of chromosomal aberrations revealed that several chromosomes were more commonly altered than chromosome 10; however, chromosomes 9 and 10 were unique in their early alteration and dysplastic lesions. The presence of a tumor suppressor gene(s) on chromosome 10q had long been suspected, since loss of heterozygosity (LOH) on regions of chromosome 10q was observed frequently in a number of cancer types [30–34]. In melanoma, loss of chromosome 10 was first reported by Parmiter et al. [35]. Since then, LOH of chromosome 10q has been studied extensively and a frequency of 30–50% has been found in melanoma, suggesting that the presence of tumor suppressor gene(s) on chromosome 10q is critical for inhibiting melanoma tumorigenesis [32, 33]. However, LOH studies in melanoma did not eventually yield the identification of a tumor suppressor gene on chromosome 10q. In 1997, by homozygous deletion mapping in gliomas and breast tumors, *PTEN* was finally identified as a candidate tumor suppressor gene on chromosome 10q. That year, three research groups independently reported the cloning of *PTEN*, *MMAC1* and *TEP1*, which turned out to be the same tumor suppressor gene. In 1997, Li et al. [1] first isolated *PTEN* by mapping of homozygous deletions on chromosome 10q23 in breast tumors. The predicted *PTEN* protein contained the phosphatase consensus motif and had ~40% homology with the focal adhesion protein tensin. It was named *PTEN* (*p*hosphatase and *ten*sin homolog deleted in from chromosome *ten*). Similarly, *MMAC1* was cloned based on homozygous deletion studies in glioma tumor cells by Steck and colleagues [2]. Coding region mutations of this gene were observed in numerous cancer types including glioblastomas, prostate, kidney and breast cancers, thus it was named *MMAC1* (*m*utated in *m*ultiple *a*dvanced *c*ancers). *TEP1*, on the other hand, was identified as a protein tyrosine phosphatase by searching Genebank sequences containing phosphatase consensus motifs. The expression level of this gene was found to be altered in a number of tumor cells and it was rapidly downregulated by transforming growth factor-β (TGF-β). Therefore, it was called *TEP1* (*T*GF-β-regulated and *e*pithelial cell-enriched *p*hosphatase) [3]. Sequence identity between *PTEN*, *MMAC1*, and *TEP1* confirmed that they were of the same gene. Subsequently, a high frequency of *PTEN* mutations have been reported in malignant melanoma, squamous cell carcinoma, endometrial, and thyroid tumors in addition to glioma, prostate, and breast tumors [4, 5, 7, 36]. These findings placed *PTEN* among the most mutated tumor suppressor genes in human cancers.

PTEN signaling

The *PTEN* gene spans 105 kb and includes nine exons (Fig. 1a). PTEN was first predicted to be a protein phosphatase since it contained (I/V)-H-C-X-A-G-X-X-R-(S/T)-G, the critical motif found in protein tyrosine phosphatases (PTPs) and dual-specificity phosphatases (DSPs) [1, 37, 38]. However, the recombinant PTEN protein exhibited higher catalytic activity towards negatively charged phosphorylated polypeptides than phosphoproteins [39]. Phosphatidylinositol (3,4,5)-tris-phosphate [PtdIns(3,4,5)P_3] was then identified as a substrate of PTEN [40]. In 1999, the crystal structure of PTEN was unraveled, showing an overall phosphatase domain structure similar to that of the DSP Vaccinia Hi-related phosphatase (VHR). However, the active site pocket of PTEN appeared deeper and wider, and two basic residues (Lys125 and Lys128) were localized within the active site loop, which were absent in PTP and VHR [41]. It was suggested that PTEN, as a phosphatase, might have preference towards PtdIns(3,4,5)P_3 and highly acidic residues present in polypeptides, although it could use both protein and lipid as substrates.

As a phosphatase, PTEN acts to remove phosphates from lipids. The best described substrate of PTEN is PtdIns(3,4,5)P3. PTEN removes the phosphate in PtdIns(3,4,5)P_3 to generate PtdIns(4,5)P_2. PTEN serves to counter-balance the effects of phosphoinositide 3′ kinase (PI3K), which normally adds a phosphate to PtdIns(4,5)P_2 to generate PtdIns(3,4,5)P_3. PtdIns(3,4,5)P_3 recruits kinases such as phosphoinositide-dependent kinase 1 (PDK1), which in turn phosphorylates Akt that phosphorylates other downstream proteins involved in regulation of apoptosis and cell-cycle progression. PTEN removal of the phosphate from PtdIns(3,4,5)P_3 inhibits this pathway by preventing localization of proteins with pleckstrin homology domains to the cell membrane. In addition to this activity, other functions could be affected following alterations (deletion/mutation) of the *PTEN* gene.

The PTEN tumor suppressor function requires both the phosphatase and the lipid membrane-binding domains (Fig. 1b), and the lipid phosphatase activity of PTEN dephosphorylates the 3-phosphoinositide products of PI3K.

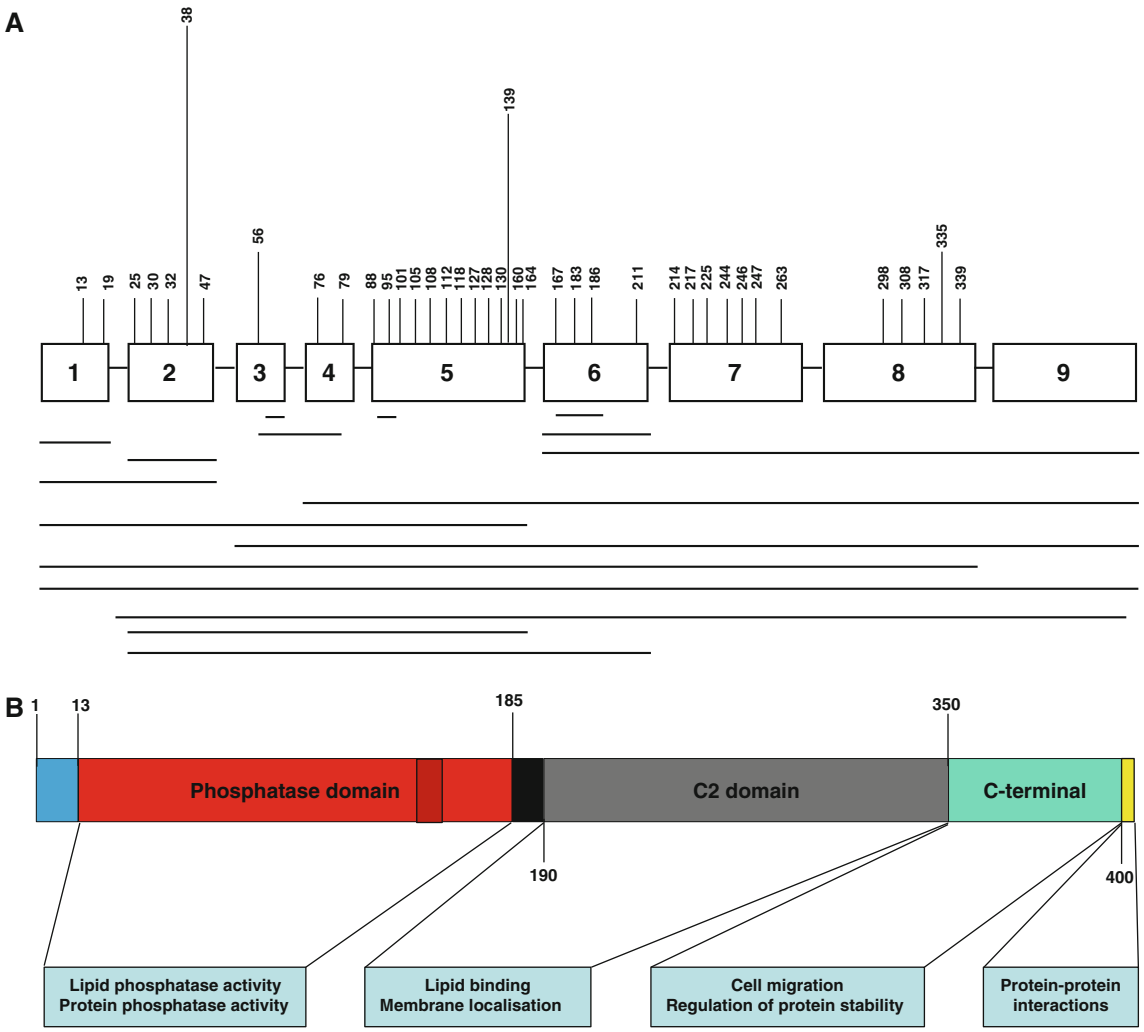

Fig. 1 Schematic representation of *PTEN* gene and genetic alterations identified in melanoma. **a** Structural representation of *PTEN* with 9 exons represented by *boxes*. The exon 5 represents the hot spot of mutations and the different numbers show the mutated codons. The *vertical lines* correspond to the localization of *PTEN* mutations in the exons and the *height of the lines* indicates the mutation frequencies. The *horizontal lines* refer to different extent of deletions. **b** Schematic representation of PTEN protein and its biological functions. PTEN contains two key domains: the phosphatase domain (in *red*; amino acids 14–185), which possesses lipid and protein phosphatase activities; and the C2 domain (in *grey*; amino acids 190–350), which is responsible for lipid binding and membrane localization. There are two other important domains; the carboxy-terminal region (amino acids 351–400), which is involved in cell migration and regulation of protein stability; and the PDZ-binding domain (in *yellow*; amino acids 401–403), which is important for protein–protein interactions

3-phosphoinositides can activate important survival kinases, such as PDK1 and Akt, as well as other proteins that are not kinases. PTEN therefore negatively regulates the Akt pathway, leading to decreased phosphorylation of Akt substrates such as tuberous sclerosis 2 (TSC2) and PRAS40 (encoded by *AKT1S1*) that control mTOR activity, p27 (encoded by *CDKN1B*), p21 (encoded by *CDKN1A*), glycogen synthase kinase 3 (GSK3α and GSK3β), BCL-2-associated agonist of cell death (BAD), apoptosis signal regulating kinase 1 (ASK1, also known as MAP3K5), WT1 regulator PAWR (also known as PAR4) and CHK1, as well as members of the forkhead transcription factor family (for example, FOXO1, FOXO3, and FOXO4) [42]. Changes in phosphorylation alter the activity and/or localization of these proteins, which in turn affects processes such as cell cycle progression, metabolism, migration, apoptosis, transcription, and translation.

Mechanism of PTEN regulation

There are multiple mechanisms for the regulation of PTEN, including transcription, mRNA stability, microRNA (miRNA) targeting, translation, and protein stability. *PTEN* is transcriptionally silenced by promoter methylation in endometrial, gastric, lung, thyroid, breast and ovarian tumors, as well as glioblastoma [43–49]. In glioma, lung, and prostate cancer, PTEN expression is decreased by overexpression of miRNA-26a (miR-26a), miR-106b-25

cluster, or the miR-21 [50–52]. PTEN can also be post-translationally regulated by phosphorylation, ubiquitylation, oxidation, acetylation, proteosomal degradation, and subcellular localization [53, 54]. Although many of these post-translational changes in PTEN have been shown to alter various cellular phenotypes in vitro, most have not been validated as key regulators of PTEN in human cancers or mouse models. PTEN amino acids Lys13 and Lys289 are monoubiquitinated, which leads to nuclear import in vitro, and mutations at Lys289 have been observed in Cowden syndrome and associated with nuclear exclusion [55]. Recently, a regulatory role has been reported by Poliseno et al., [56] showing that mRNA molecules from PTENP1 (PTEN pseudogene) can act as competitive endogenous RNA sequestering miRNA molecules. In a given tissue, there would be a balance in the level of expression from both PTEN and PTENP1; if PTENP1 transcription decreases, more miRNAs are able to target PTEN. On the other hand, an increase in PTENP1 transcription implies that less miRNA will target PTEN. Therefore, PTENP1 indirectly regulates PTEN by competing for binding to the miRNA. In conclusion, PTENP1 transcripts can act as indirect post-transcriptional regulators decoding miRNAs that target the *PTEN* gene. However, the role of PTENP1 in disease (tumorigenesis, in particular) has not been proven.

Other criteria for PTEN regulation is the importance of its gene dosage in tumorigenesis events. Indeed, a generated complete or partial loss of *PTEN* knockout mice allowed to understand its tumor suppressive activity in specific cells and/or tissues in vivo, [57]. Using mouse genetic engineering, several studies have contributed to show the impact of partial *PTEN* level reduction in cancer. A number of cancers, including mammary, prostate, and uterine were found in *PTEN* heterozygous mice [58–60]. Also, studies on knockout mice showed that complete *PTEN* loss results in exhaustion of the hematopoietic stem cell compartment prior to leukemia development [61, 62] or a novel cellular senescence program [63]. As *BRAF* mutation induces senescence in melanocytes, a process that is important for melanomagenesis, the cooperation between PTEN and BRAF on cellular senescence warrants further investigation.

PTEN, a mutated gene in human cancers

Numerous mutations have been reported throughout this gene (Fig. 1a). Mutations resulting in the loss of function or reduced levels of PTEN, as well as *PTEN* deletions or alteration are found in many sporadic tumors [64]. *PTEN* mutations are found throughout most of the *PTEN* coding region, with the exception of exon 9, which encodes the carboxy-terminal 63 amino acids [65]; more than 40% occur within exon 5, which encodes the phosphatase domain [65]. Allelic or total deletion of *PTEN* is a frequent occurrence in cancers such as breast, prostate cancer, and melanoma. A subset of patients with melanoma carries mutations in the *PTEN* promoter or in potential splice donor and acceptor sites [5, 66–69]. Splicing alterations can lead to exon skipping that alters PTEN functions. In mice, decreasing PTEN level correlates with increasing tumor susceptibility [70, 71]. This suggests that reduced levels of normal PTEN are insufficient for its tumor suppressor function and raises the possibility that reduction of *PTEN* activity could be an important driving mechanism for cancer.

PTEN mutations have been extensively characterized and found in three linked autosomal dominant cancer predisposition syndromes: Cowden's disease (CD), Lhermitte–Duclos disease, and Bannayan-Zonana syndrome. These cancer syndromes share similar phenotypic characteristics including mental retardation, gastrointestinal hamartomas, thyroid adenomas, breast fibroadenomas, macrocephaly, and mucocutaneous lesions [72, 73]. Over 80% of patients with CD harbored germline *PTEN* mutations. LOH studies in 20 hamartomas using markers flanking and within *PTEN* showed that wild-type *PTEN* locus was indeed lost in two breast fibroadenomas, one thyroid adenoma, and one pulmonary hamartoma, confirming that *PTEN* functions as a tumor suppressor gene in CD [74]. Somatic *PTEN* alteration is common in many sporadic tumor types [75]. Various tissue-specific and/or inducible homozygous deletions of *PTEN* have been generated in mice to model sporadic PTEN loss in tumorigenesis. In the endometrium [76], mammary gland [77], prostate [78], and in T cells [79], homozygous deletion of *PTEN* led to rapid tumor formation in the targeted tissue. Similarly, *PTEN*-deficient mice developed tumors in the liver [80], bladder [81], and lung [82]. By contrast, when *PTEN* was deleted the intestine [83], no malignant tumors developed, although intestinal polyps were common, as observed in Cowden syndrome. Loss of other tumor suppressors or the activation of oncogenes can nonetheless combine with *PTEN* loss to cause cancer in these organs.

PTEN mutations in melanoma

PTEN mutations in melanoma were reported shortly after its cloning. Initial studies demonstrated a mutation rate of ~30–40% in melanoma cell lines and ~10% in primary melanomas [5, 66]. Functional studies supported the hypothesis that PTEN played an important role in melanoma. Indeed, in *PTEN*-deficient melanoma cells, ectopic expression of *PTEN* was able to reduce melanoma tumorigenicity and metastasis [3, 84], implicating PTEN as a critical tumor suppressor in melanoma tumorigenesis.

To elucidate the role of PTEN loss in melanoma tumorigenesis, a thorough understanding of the functions of PTEN on the structural/molecular level and PTEN-mediated signaling events is necessary.

The *PTEN* mutations are scattered along the length of the gene, with the exception of exon 9 (no mutation reported). A mutational hot spot is found in exon 5 (33%), which encodes the phosphatase catalytic core motif (Fig. 1), and recurrent mutations are also found at CpG dinucleotides suggesting deamination-induced mutations. The genetic alterations included point missense mutations, insertions, splice site mutations, small and gross deletions of the gene (Tables 1, 2). The majority of these alterations lead to premature termination with small transcripts or functional inactivation of the protein in some cases.

A similar mutational profile has been found for *PTEN* mutations in human cancers. The highest frequency of *PTEN* mutations is found in endometrial carcinomas and glioblastomas. *PTEN* mutations are also found in lymphoma, thyroid, breast, prostate carcinomas, and melanomas [85]. In melanoma, *PTEN* mutation rates of 27.6% in melanoma cell lines (Table 3) and 12.0% in melanoma biopsies have been reported (Table 4). In 1997, Guldberg et al. [5] first reported that 42.9% (15/35) of examined melanoma cell lines harbored *PTEN* mutations. Nine of these cell lines showed homozygous deletion of *PTEN* gene, and six lines had mutations in one allele in combination with the loss of the second. Tsao et al. [66] examined 45 melanoma cell lines and found *PTEN* mutations in 28.9% of the melanoma cell lines, including 20.0% (9/45) homozygous deletions and 8.9% (4/45) frameshift, nonsense, and intronic splice mutations. Teng et al. [86] examined seven melanoma cell lines and found that four cell lines contained homozygous deletions in the *PTEN* gene. Pollock et al. [87] reported that 22.8% (13/57) of melanoma cell lines carried mutations in *PTEN*. Eight of these cell lines showed mutations in one allele and five had homozygous deletion of *PTEN*. In total, 76 different alterations were found in all the cell lines studied (Tables 1, 3). Among these alterations, there are 20 deletions for which the exact regions were not determined. All information on *PTEN* mutational status in melanoma biopsies was obtained from a single tumor of the patients. To expand our knowledge on PTEN functions, it would be important to study its status in multiple tumors from the same patient.

PTEN mutations are uncommon in uncultured melanoma biopsies. Tsao et al. [66] examined 17 uncultured metastatic melanoma samples; only one case of homozygous deletion and another case of premature stop mutation were identified (Tables 2, 4). When Boni et al. [88] tried to identify mutations within the exons 5, 6, 7, and 8 of the *PTEN* gene, no mutations were found. Teng et al. [86] found 10% (1/10) missense mutation of *PTEN* in primary melanoma tumors. Birck et al. screened a panel of 77 melanoma biopsies including 16 primary and 61 metastatic tumors. *PTEN* mutations were identified in 6.6% (4/61) of the metastatic tumors, while no mutation was found in primary melanoma [13]. In this study, they have detected a nonsense mutation (L139X) that had already been reported by Guldberg et al. [5]. By examining two intragenic biallelic polymorphisms, 53.8% (21 out of 39) informative specimens showed loss of one *PTEN* allele [13]. Reifenberger et al. [89] examined 40 melanomas and found 20% (3/15) of primary melanomas and 12% (3/25) of metastatic melanomas contained *PTEN* mutations. Two different mutations were found in both primary and metastatic melanomas. Celebi et al. [14] also detected *PTEN* sequence alterations in four of 21 (19%) metastatic melanoma samples. Two other mutations were found in the putative splice site of *PTEN*, making the total alterations at 28.6%. Similar results were reported by Poetsch et al. [90] and Abdel-Rahman et al. [91] for metastatic melanomas (Tables 2, 4). Taken together, these data supported the notion that *PTEN* alterations occur in melanomas and loss of *PTEN* may contribute to melanoma development.

Functional studies support a role for *PTEN* in melanoma tumor suppression. An in vitro LOH study by Robertson et al. [92] showed that *PTEN* was indeed targeted for LOH in melanoma. A melanoma cell line UACC903 with duplicated mutant chromosome 10 was used to build the in vitro LOH model. A wild-type chromosome 10 was transferred into the cells and underwent spontaneous breakage and deletions over time in culture. During this process, the introduced wild-type copy of *PTEN* was lost. In parallel, another melanoma cell line with wild-type *PTEN* gene maintained a transferred 10q23–24 region that contained the exogenous *PTEN* gene. Ectopic expression of *PTEN* into UACC903 cells was also demonstrated to suppress tumor cell growth [92]. Similar findings have been reported by Tsao et al. [67] as well. In a number of melanoma cell lines, overexpression of *PTEN* uniformly inhibited colony formation, implicating a tumor-suppressive function of *PTEN* in melanoma [67].

In contrast, three groups have failed to detect significant *PTEN* mutation rates in melanoma. Boni et al. [88] used two microsatellite markers flanking *PTEN* gene to search for LOH surrounding the *PTEN* locus, and found no LOH for either of the markers in 40 (23 primary and 17 metastatic) melanoma tissue. Further SSCP analysis for exons of *PTEN* gene did not yield any abnormal bands [88]. Herbst et al. [93] analyzed LOH at loci closely linked or intragenic to *PTEN* in 65 melanomas. A rate of LOH of lower than 16% with eight different polymorphism markers led to the conclusion that it rather represented random genetic events than indicating that *PTEN* was the target in melanoma [93]. Poetsch et al. [90] screened 25 primary and 25 metastatic melanomas for *PTEN* mutation, and found two missense and eight silent mutations (Table 2).

Table 1 *PTEN* alterations in melanoma cell lines

Cell line	Exon	Codon	Mutations	Effect on PTEN expression	References
Ma-Mel-45a	1	13	K13X	Premature stop	[147]
TCL11D11	1	–	Del E1	ND	[86]
G-mel	1	–	Del E1	No initiation	[67]
MM472	2	30	P30L	ND	[87]
Ma-Mel-85	2	32	Del I32	ND	[147]
FM76	2	38	P38S	ND	[5]
FM81	2	38	P38S	ND	[5]
4686M	2	38	P38S	PP	[148]
Ma-Mel-150a	2	38	P38S	ND	[147]
Ma-Mel-32	2	38	P38L	ND	[147]
NK14	2	38	P38L	ND	[87]
D35	2	47	R47R	PP	[149]
TCL11E3	2	–	Del E2	ND	[86]
Sk-Mel-24	2	–	Del E2	NP	[5]
HS944	2	–	Del E2	Premature stop	[67]
MGH-BO-1	2	–	Del E2	Premature stop	[66, 67]
Sk-Mel-37	2	–	Del E2	Premature stop	[66, 67]
HS944	2	–	Del E2	Premature stop	[68]
MGH-BO-1	2	–	Del E2	Premature stop	[68]
Sk-Mel-37	2	–	Del E2	Premature stop	[68]
MM200	3	56	F56I	NP	[87, 149]
Sk-Mel-28	3	56	A56G	ND	[69]
HT144	3	56–70	Del 45 bp	Premature stop	[87]
UACC903	4	76	Y76X	ND	[66–68]
Ma-Mel-11	5	90–95	Del 90–95	ND	[147]
D32	5	101	Del 1 bp	NP	[149]
30966M	5	105	C105R/Del314	Stop at 112	[148]
Ma-Mel-134	5	108	L108P	ND	[147]
Ma-Mel-19	5	118	H118L	ND	[147]
FM88	5	127	G127E	ND	[5, 69]
Ma-Mel-05	5	128	L128N	ND	[147]
Ma-Mel-38	5	130	R130Q	ND	[147]
FM62	5	139	L139X	ND	[5, 69]
MM622	5	139	L139X	NP	[87, 149]
MM455	6	165–211	Del 142 bp	No transcription	[87]
Sk-Mel-28	6	167	T167A	PP	[67, 68, 87, 149]
Sk-Mel-39	6	183	546 Ins A	Premature stop	[66–68]
MM455	6	–	Del E6	Premature stop	[66–68]
FM2	6	–	Del E6	ND	[69]
WM 1026.7	6	–	Del nt1297-1438	NP	[66]
FM9	7	214	C640T	ND	[69]
FM39	7	225	675 Ins TA	Premature stop	[5]
2211M	7	246	P246S	PP	[148]
Ma-Mel-KNUD	7	247	L247X	ND	[147]
BL	8	298	Q298X	NP	[87, 149]
FM49	8	317	Del 18 bp	VLTLTKN → D	[5]
C32	1–2	1–55	Del 164 bp	Initiation in I2	[87]

Table 1 continued

Cell line	Exon	Codon	Mutations	Effect on PTEN expression	References
TCL11D7	1–2	–	Del E1–2	ND	[86]
FM72	1–2	–	Del E1–2	NP	[5]
FM95	1–2	–	Del E1–2	ND	[69]
FM95	1–5	–	Del E1–5	NP	[5]
FM2	1–8	–	Del E1–8	NP	[5]
FM60	1–9	–	Del E1–9	NP	[5]
FM70	1–9	–	Del E1–9	NP	[5]
Sk-Mel-23	1–9	–	Del E1–9	NP	[66–68]
WK	1–9	–	Del E1–9	NP	[66, 67]
ML	1–9	–	Del E1–9	NP	[66–68]
WM 168.26	1–9	–	Del E1–9	NP	[66]
MGH-LA-1	1–9	–	Del E1–9	NP	[67]
BA-1	2–5	–	Del E2–5	Premature stop	[66]
MGH-BA-1	2–6	–	Del E2–6	Premature stop	[67]
FM92	2–9	–	Del E2–9	NP	[5, 69]
MM386	3/4	55–84	Del 89 bp	Premature stop	[87]
FM3	3–9	–	Del E3–9	NP	[5]
FM82	4–9	–	Del E4–9	NP	[5]
EST71	4–9	–	Del E4–9	ND	[69]
FM82	4–9	–	Del E4–9	ND	[69]
A2058	5,6	112/186	L112Q/L186M	NP	[87, 149]
Sk-Mel-31	6–9	–	Del E6–9	NP	[5, 69]
TCL11D9	6–9	–	Del E6–9	ND	[86]
A03	8–9	268–405	Del 411 bp	No transcription	[87]
Ma-Mel-08a	–	157/162	E157X/D162V	ND	[147]
5810P	–	–	Del	NP	[148]
26258M	–	–	Del	NP	[148]
3962M	–	–	Del	NP	[148]
C32	–	–	Del	NP	[149]
D08	–	–	Del	PP	[149]
D14	–	–	Del	NP	[149]
D20	–	–	Del	NP	[149]
HT144	–	–	Del	NP	[149]
MM386	–	–	Del	NP	[149]
A06	–	–	Del	NP	[149]
A13	–	–	Del	NP	[149]
D36	–	–	Del	NP	[149]
MM488	–	–	Del	NP	[149]
MM604	–	–	Del	NP	[149]
Ma-Mel-21	–	–	Del	ND	[147]
Ma-Mel-80a	–	–	Del	ND	[147]
Ma-Mel-99	–	–	Del	ND	[147]
Ma-Mel-101	–	–	Del	ND	[147]
Ma-Mel-02	–	–	Del	ND	[147]
Ma-Mel-76	–	–	Del	ND	[147]
FM45	–	–	I2, T18A	Skipping of E3	[5]
RU	–	–	I3, del (nt1–4)	Possible splice variant	[67, 68]

Table 1 continued

Cell line	Exon	Codon	Mutations	Effect on PTEN expression	References
Sk-Mel-131	–	–	I5, T2A	Possible splice variant	[66–68]
J6	–	–	I6, G5T	Possible splice variant	[66, 67]
EST73	–	–	I6, G5T	ND	[69]
AF6	–	–	Splice mutation	NP	[149]
WM1799	–	–	Failed PCR	NP	[68]

Alterations are defined as amino acid substitutions, coding nucleotide change; *HD* homozygously deleted, *Del* deletion, *E* exon, *I* intron, *Ins* insertion, *ND* not determined, *NP* no PTEN, *PP* PTEN present

These findings are in contrast with the abundant evidence implicating *PTEN* as an important tumor suppressor in melanoma and other cancers. However, it must be taken into consideration that although an overall mutational rate of *PTEN* in cultured melanoma cell lines is around 27.6%, only seven cases with *PTEN* mutations has been detected in over 96 primary melanomas. There are several possible explanations for this observation: (1) although *PTEN* loss is important in melanoma, it occurs late in melanoma tumorigenesis since mutation is rarely detected in primary melanomas; (2) *PTEN* loss may in fact be relatively rare in melanoma, and the establishment of cell lines selects for melanomas with *PTEN* alterations; (3) the biology of *PTEN* alteration in early melanomas makes detection of alteration difficult (e.g., from dosage reduction, epigenetic downregulation of expression or homozygous deletion); (4) the number of primary melanoma samples examined is small and the subtypes of tumors have not been examined; or (5) PTEN loss following the epigenetic mechanisms, such as DNA methylation.

The negative cases in *PTEN* alteration studies could be explained by the homozygous deletion rate observed by several laboratories. As homozygous deletion makes LOH of chromosome 9p21 (at *CDKN2A*) difficult to detect in tumor samples [94], it is possible that chromosome 10q23 *PTEN* deletions in melanomas may have been underdetected too. Moreover, epigenetic studies recently suggested that the involvement of PTEN function loss in melanoma might have been in fact underestimated. Zhou et al. [95] analyzed *PTEN* protein expression, instead of analyzing *PTEN* gene mutations in melanomas. Using immunohistochemistry, they found no *PTEN* protein expression in 15% (5/34) and low expression in 50% (17/34) of melanoma samples (four primary and 30 metastatic). Surprisingly, among the five melanomas with no *PTEN* protein expression, four showed no deletion or mutation of *PTEN* gene, indicating the presence of an epigenetic mechanism of biallelic functional inactivation of *PTEN* [95].

The timing of *PTEN* alterations in melanoma development is also not understood. Cytogenetic studies, cited above, suggested an early involvement of PTEN. However, Birck et al. [13] examined primary melanoma samples and detected no *PTEN* mutations. However, they also found allelic loss of *PTEN* gene in 37.5% (3/8) primary melanomas, indicating a decreased *PTEN* dosage possibly occurring early in melanoma development [13]. Thus, different types of genetic changes may lead to higher frequency of *PTEN* alterations in melanoma cell lines, providing some explanation for the discrepancies in the literature. Additional work will be needed to accumulate data to allow for reconciliation of divergent mutation rates from different studies.

Sporadic melanomas frequently have a loss of *PTEN* through LOH, deletion, and mutation [5]. *PTEN* can also be epigenetically silenced in melanoma, as decreased *PTEN* transcript levels were associated with *PTEN* promoter methylation [96]. *PTEN* methylation also correlated with decreased survival [97]. In another study, low PTEN expression was associated with melanoma ulceration, which is characteristic of aggressive tumors, but did not significantly correlate with overall survival [98]. A link between DNA damage and *PTEN* mutation in melanoma has been suggested by Wang et al. [99] who showed that more than 50% of the melanomas from patients with xeroderma pigmentosum have *PTEN* mutations that are typically associated with ultraviolet radiation exposure.

In mice, *PTEN* deletion in pigmented mouse cells does not lead to the development of spontaneous melanoma, despite an increase in the number of dermal melanocytes. However, in this model, topical carcinogen treatment led to melanoma formation in nearly 50% of the mice within 20 weeks [100]. In conjunction with *CDKN2A* (encoding p14ARF) deletion, nearly 10% of $PTEN^{+/-}$ mice developed spontaneous melanoma [101].

In all the melanoma biopsies analyzed, 12.0% (41/342) contained *PTEN* alterations. Three mutations were found twice in different tumors, giving a total of 38 different alterations (Tables 2, 4). The huge proportion of these alterations was found in metastatic melanoma biopsies (34/224, 15.2%). Only 7.3% (7/96) of primary melanoma specimens were found to carry *PTEN* alterations.

Table 2 *PTEN* alterations in melanoma tissues

Sample ID	Exon	Codon	Mutation	Predicted effect	Tumor	References
46, sub.	1	7	AA del	Stop at 9	MM	[13]
S14126	1	19	D19N	Missense	MM	[14]
S23807	2	25	D25N	Missense	MM	[14]
MT-1	2	38	P38F	Missense	PM	[86]
M15	3	–	Ins of 45 bp	Dupl E3	PM	[89]
M16	3	–	Ins of 45 bp	Dupl E3	MM	[89]
MM15	4	79	A79T	Missense	MM	[90]
M122	5	88	T262C	Missense	MM	[91]
M138	5	95	C283T	Missense	MM	[91]
FM88	5	127	G127E	Missense	MM	[5]
FM62	5	139	L139X	Nonsense	MM	[5]
1, LN	5	139	L139X	Nonsense	MM	[13]
M062	5	160	C479T	Missense	MM	[91]
13, LN	5	164	Ins of A	Stop at 179	MM	[13]
S14456	6	211	C211X	Nonsense	MM	[14]
S20855	7	217	V217I	Missense	MM	[14]
FM39	7	225	Ins of TA	Frameshift	MM	[5]
M54	7	244	P244S	Missense	PM	[89]
40816-01	7	243–246	Del	Stop at 255	MM	[87]
KM-17	7	263	Dupl	Stop at 271	MM	[66]
4, sub.	8	276	Del 11 bp	Stop at 297	MM	[13]
M202	8	308	C922T	Missense	MM	[91]
M24	8	335	R335X	Nonsense	PM	[89]
M25	8	335	R335X	Nonsense	MM	[89]
MM11	8	339	P339L	Missense	MM	[90]
M36	8	–	Del	Frameshift	MM	[89]
KM-16	–	–	ROH	ND	MM	[66]
SSM4	–	–	I6, G11A	Silent	PM	[90]
NM2	–	–	I1, A152G	Silent	PM	[90]
NM7	–	–	UTR/E1-22C → T	–	PM	[90]
MM7	–	–	I3, G79A	Silent	MM	[90]
MM11	–	–	UTR/E1–101C → T	–	MM	[90]
MM14	–	–	I3, G53A	Silent	MM	[90]
MM15	–	–	UTR/E1–22C → T	–	MM	[90]
MM21	–	–	I1, A63T	Silent	MM	[90]
S5914	–	–	I1, G14A	Putat. splice site	MM	[14]
S4890	–	–	I2, G13A	Putat. splice site	MM	[14]
M103	–	–	I5, T14C	ND	MM	[91]
M150	–	–	I4, A3G	ND	MM	[91]
M124	–	–	I5, T14C	ND	MM	[91]
M149	–	–	I3, C47T	ND	MM	[91]

Del deletion, *Dupl* duplication, *E* exon, *HD* homozygously deleted, *I* intron, *Ins* insertion, *LN* lymph node metastasis, *MM* metastatic melanoma, *ND* not determined, *PM* primary melanoma, *Putat* putative, *ROH* retention of heterozygosity, *Sub* subcutaneous metastasis, *UTR* untranslated region

PTEN mutations and other genes

In addition to *PTEN* alterations, mutations of *CDKN2A*, as well as *NRAS* mutations have been frequently observed in melanoma [102, 103]. *CDKN2A* is the most often mutated tumor suppressor gene in melanoma, with 60% homozygous deletions and additional 15–20% point mutations [104–106]. The two proteins encoded by *CDKN2A*, p16,

Table 3 Frequency of *PTEN* alterations in melanoma cell lines

No. of cell lines	Mutation (%)	Deletion (%)	Total alterations (%)	References
7	0 (0)	4 (57.1)	4 (57.1)	[86]
35	6 (17.1)	9 (25.7)	15 (42.9)	[5]
45	4 (8.9)	9 (20.0)	13 (28.9)	[66]
53	6 (11.3)	10 (18.9)	16 (30.2)	[67]
57	8 (14.0)	5 (8.8)	13 (22.8)	[87]
47	5 (10.6)	7 (14.9)	12 (25.5)	[68]
41	3 (7.3)	3 (7.3)	6 (14.6)	[148]
71	8 (11.3)	11 (15.5)	19 (26.8)	[149]
47	5 (10.6)	7 (14.9)	12 (25.5)	[69]
60	12 (20.0)	6 (10.0)	18 (30.0)	[147]
463	**57 (12.3)**	**71 (15.3)**	**128 (27.6)**	

The numbers in bold indicate total cases

Table 4 Frequency of *PTEN* alterations in melanoma tissues

No. of biopsies	PM (%)	MM (%)	Total alterations (%)	References
10	1/10 (10.0)	ND	1/10 (10.0)	[86]
8	0/4 (0)	3/4 (75.0)	3/8 (37.5)	[5]
17	ND	2/17 (11.8)	2/17 (11.8)	[66]
40	0/23 (0)	0/17 (0)	0/40 (0)	[88]
77	0/16 (0)	4/61 (6.6)	4/77 (5.2)	[13]
40	3/15 (20.0)	3/25 (12.0)	6/40 (15.0)	[89]
21	ND	6/21 (28.6)	6/21 (28.6)	[14]
50	3/25 (12.0)	7/25 (28.0)	10/50 (20.0)	[90]
49	0/3 (0)	1/46 (2.2)	1/49 (2.0)	[87]
30	ND	8/30 (26.7)	8/30 (26.7)	[91]
342	**7/96 (7.3)**	**34/224 (15.2)**	**41/342 (12.0)**	

The numbers in bold indicate total cases

PM primary melanoma, *MM* metastatic melanoma, *ND* not determined

and p14ARF, function in the pRB and p53 pathway, respectively. p16 is a cyclin-dependent kinase inhibitor. It binds to and inhibits cyclin D/CDK4, which in turn blocks pRB phosphorylation, leading to G1 cell-cycle arrest. p14, on the other hand, binds mdm2 and relieves p53 from mdm2-mediated p53 degradation. p53 is known to block cell proliferation by inducing cell-cycle arrest or apoptosis.

As RAS is a prominent oncogene involved in melanoma tumorigenesis, like *PTEN*, it has several biological functions. *RAS* gene family members include *HRAS*, *NRAS*, and *KRAS*. They encode 21-kDa proteins with GTPase activity. RAS is involved in regulating receptor tyrosine kinase-induced MAPK activation. RAS activates MEK and MAPK through RAF. RAS also binds and activates lipid kinase PI3K, and therefore activates Akt pathway. Finally, RAS interacts with p53 and p16. In primary mouse embryonic fibroblasts, for example, HRAS was shown to induce premature cell senescence, which was associated with the accumulation of p16 and p53 [107]. Pathways controlled by these three elements, RAS, p53, and p16, therefore appear to be central to control of the malignant phenotype.

PTEN functions as a lipid and protein phosphatase that downregulates Akt and MAPK, potentially suggesting that RAS and *PTEN* have opposite functions in both protein and lipid kinase signaling pathways. Is it possible that *PTEN* loss and RAS oncogenic activation are redundant in tumor development? Tsao et al. [67] reported a reciprocal mutational status for *PTEN* and *NRAS* in human melanoma cells. Among 53 cutaneous melanoma cell lines, 16 cell lines (30%) harbored *PTEN* mutations and 11 lines (20.7%) had oncogenic *NRAS* mutations. Only one cell line showed mutations in both genes, so a total of 50% cell lines had mutations in either *PTEN* or *NRAS* (Table 2; [67]). Similar reciprocal findings have been reported in endometrial cancer [108]. Furthermore, Davies et al. [109] showed that loss of PTEN expression was not detected in melanoma cases harboring NRAS mutations. However, Nogueira et al. [110] recently found that PTEN loss cooperates with RAS activation to drive melanoma cell invasion and promote metastasis.

In mouse melanoma models, RAS and CDKN2A loss cooperate to lead to melanoma development [111]. It has been shown that CDKN2A loss coupled with PTEN loss

lead to melanoma; however, it is not clear that PTEN loss confers greater susceptibility to melanoma development than CDKN2A loss alone [101]. Recently, Dankort et al. [112] showed that a simultaneous activation of BRAF and deletion of *PTEN* in melanocytes leads to early onset spontaneous melanomas, with metastasis to the lymph nodes and lung. Notably, the mTOR inhibitor rapamycin increased survival in these mice by more than twofold [112]. These mouse studies indicate that *PTEN* is probably not a driving mutation in melanoma, but can contribute to a malignant phenotype in the presence of other genetic alterations. Further studies are needed to elucidate the details of PTEN, RAS, and CDKN2A interaction in murine models.

A direct downstream target of *RAS*, *BRAF*, has been shown to exhibit a higher mutation frequency in melanoma [113]. In all, 806 of 2,346 (34.3%) uncultured melanomas, 568 of 1,336 (42.4%) cutaneous melanomas, and 251 of 389 (64.5%) melanoma cell lines harbored mutated *BRAF* gene. In that study, *NRAS* mutations were also detected in 21.2% (379/1,790) uncultured melanomas, 26.4% (282/1,067) cutaneous melanomas, and 13.6% (58/426) of the melanoma cell lines. Furthermore, cell lines were detected with oncogenic-activated RAS–RAF–MAPK pathway. Thus, *BRAF* is a second gene whose mutations are reciprocally distributed with regard to RAS. Like RAS, RAF can activate PI3K, and PI3K and Akt can directly alter RAF kinase activity [114, 115]. Thus, understanding the relation of *PTEN*, *RAS*, and *RAF*, in the context of PI3K-Akt and RAS–MAPK pathways will be crucial to understanding melanoma tumorigenesis.

The PTEN expression in pre-malignant lesions was conducted by two different groups. Tsao et al. [116] found uniformly strong PTEN expression in the cytoplasm of almost all benign nevi, while Singh et al. [117] showed strong cytoplasmic staining of PTEN for eleven of 17 (64%) benign nevi. In accordance of these findings, PTEN is present in pre-malignant melanoma as opposed to its notable absence in a significant proportion of primary melanomas. These findings support the role of PTEN loss in the pathogenesis of melanoma.

PTEN genetic alterations in melanoma result in the activation of critical signaling pathways promoting growth and survival of tumors cells. Alterations in the RAS–RAF–MAP kinase and PI3-kinase signaling pathways are commonly altered in melanoma. Mutations in BRAF and NRAS occur in a mutually exclusive pattern and lead to MAP-kinase activation. The most common known genetic alteration in the PI3-kinase cascade is the loss of PTEN function, and was commonly associated with *BRAF* mutations [67, 68, 118].

Alterations in membrane receptors or mutations in downstream effectors such as RAS or RAF can initiate aberrant MAPK signaling in melanoma [119]. About 20% of melanoma patients harbor *NRAS* mutations, but it is BRAF (the RAS substrate) that harbors the most frequent mutations in melanoma ($\sim$40%) [113]. About 80% of these mutations display a valine to glutamic acid substitution (V600E), causing constitutive kinase activation, and about 16% harbor a valine to lysine substitution (V600K) [120, 121]. MAPK signaling is required for proliferation of both RAS and RAF-transformed melanocytes, as it was shown that RAF and MEK inhibitors decreased ERK activity and blocked their cell cycle progression. Provided the large number of melanomas that harbor activating mutations in the *BRAF* oncogene and their reliance on BRAF activity, targeted inhibition of this protein became of high interest.

The treatment of human melanoma at advanced stage using biotherapeutics or chemotherapeutics has rarely provided response rates higher than 20% [122]. This clinical aspect is changing with the advent small molecule inhibitors to treat metastatic melanoma [123, 124]. The *BRAF* mutation in melanoma provided an opportunity to target a cancer-specific oncogene and develop compounds to curb its aberrant activity. Recently, through structure-guided approaches, the specific BRAF(V600E) inhibitor PLX4032 was developed, which provided increasing proof that targeting BRAF in melanoma is a real therapeutic approach [123, 124]. PLX4032 is a well-tolerated small-molecule inhibitor inducing $\sim$80% partial or complete tumor regression for melanomas containing *BRAF(V600E)* mutations and has received FDA approval for the treatment of late-stage human melanoma.

In melanoma, *ERK* mutations have not been identified and *MEK* mutations are not frequent. However, as the MAPK pathway is constantly active in the tumor cells, these effectors can also be targeted. From preclinical models, MEK inhibitors induce significant reduction in melanoma growth [125, 126]. However, these inhibitors have not shown significant clinical efficacy in melanoma clinical trials [127]. Interestingly, inhibitors of BRAF and MEK were reported to have similar transcriptional targets; therefore, MEK inhibitors could be useful in patients with acquired BRAF inhibitor resistance if toxicities can be controlled [128].

Interestingly, *BRAF V600E* mutations are also observed in benign nevi; suggesting that *BRAF* mutations alone are insufficient for tumorigenesis and that additional factors are needed for cancer progression [129]. In fact, a mouse genetic model of $BRAF^{V600E}/PTEN^{-/-}$ that mimics melanoma progression indicates that the PI3K pathway also plays an important role in the development of aggressive tumors [112]. PI3K pathway activity was shown to be increased in melanoma through loss of activity of the tumor suppressor PTEN. This loss occurs through *PTEN*

mutation, deletion, or methylation, which can also coincide with *BRAF* mutations but not *NRAS* [68]. PTEN loss is found in 5–20% of noninherited melanomas, and similar to other neoplasia, may regulate inhibition of the MAPK pathway, cell-cycle arrest, and survival via effects on Bcl-2 and caspases [130]. Thus, as melanomas favor the deregulation of both the MAPK and PI3K pathways, their combined targeting has therapeutic merit [131]. Although multiple other signaling pathways may be involved in melanoma oncogenesis, finding which ones are essential for melanoma survival and progression will determine their therapeutic value.

Recently, several studies have identified PTEN loss in multiple mechanisms of BRAF inhibitor resistance [132–135], suggesting that PTEN inactivation can affect sensitivity to BRAF inhibition. These findings are useful in developing a new generation of BRAF inhibitors. In fact, the growth advantage conveyed by the constitutive activation of these pathways leads to positive selection of cells that have acquired the mutations and in many instances leads to critical dependency of the cancer cells on their activation. This creates opportunities for therapeutic interventions targeted at signaling components within these pathways that are amenable for pharmacological inhibition.

Relationship between PTEN alterations and functions

To date, 111 different alterations of the *PTEN* gene have been reported (Tables 1, 2). Three mutations have been found in both melanoma cell lines and tissues. Some alterations have been reported twice or more. These mutations, scattering along the whole gene, include point-stop mutations, point missense mutations, insertions, duplications, splice site mutations, and small and gross deletions of the gene. A great number of the mutations are found in exon 5 coding for the phosphatase domain, and likely alter the phosphatase activity of PTEN. The majority of mutations occurring in PTEN result either in abnormal RNA splicing, truncation, or gross deletion of the gene, thus predicting inactivation of the protein and supporting loss of various functions assigned to PTEN.

A review of the literature (considering only the mutations found in tumoral specimens and not those found in cell lines) provides 38 *PTEN* mutations occurring in various types of primary tumors or metastases (Table 2). These mutations affecting PTEN in melanoma patients have been found predominantly in metastatic tissues. It is mainly nonsense, frameshift, or splicing mutations resulting in a truncation of the protein. In addition, bi-allelic inactivation of PTEN has been evidenced with both point mutations in one allele and deletions of the other allele resulting in loss of heterozygosity (LOH). Similarly to cell lines, a great number of missense mutations occur in the exon 5 (hot spots of *PTEN* mutations). Interestingly, two of the three mutations found in both cell lines and biopsies were located in the exon 5.

PTEN alterations in different functional domains will lead to the loss of expression, thereby affecting its tumor suppressor functions. In fact, PTEN contains two key domains required for its tumor suppressor function: the lipid membrane-binding (C2) domain (amino acids 190–350), and the catalytic (phosphatase) domain (amino acids 14–185) with an active site constituted by the residues 123–130 (Fig. 1b). Many mutations affect these domains leading to a loss of function (Tables 1, 2). There are other domains such as the PDZ-binding domain (amino acids 401–403), which binds to proteins containing PDZ domains (an acronym of three proteins: Psd95, Dlg1, and Zo-1), and the carboxy-terminal region (amino acids 351–400), which contains PEST (rich in amino-acids P, E, S, T) sequences and may contribute to protein stability and activity. Only deletions were reported for these two domains and their importance in the tumor suppressor function of PTEN is less well defined.

PTEN alterations occur in both the N-terminal catalytic core motif and the C-terminal non-catalytic regulatory domain. Five phosphorylation sites (S370, S380, T382, T383, and S385) were reported for the latter [136]. When phosphorylated at these residues, PTEN is targeted for degradation through the ubiquitin/proteasome system [137]. In addition, phosphorylation may protect the carboxyl terminus from caspase 3-mediated cleavage during apoptosis [138]. Interestingly, it has been reported that phosphorylation at T383 requires the protein phosphatase activity, but not the lipid phosphatase activity [136]. Consequently, each alteration affecting these areas could disturb the functional activity of PTEN.

As reported PTEN has many roles, including: (i) lipid phosphatase activity removing the phosphate on either PtdIns(3,4,5)P_3 or (PtdIns(3,4)P_2 [40, 136, 139]; (ii) protein phosphatase activity [3, 40, 136, 139]; and (iii) as a substrate for phosphorylation by kinases [1, 3, 39]. These different roles result in reduced activation of the (PI3K)/Akt and other pathways leading to an anti-invasive, anti-proliferative and tumor suppressive effects. PTEN is also involved in mediating growth arrest and other cellular functions of the MAPK pathway [140–143]. Each of reported alterations could affect one or several functions. The biological significance of the protein phosphatase effects of PTEN is less well characterized than the lipid phosphatase effects. The relationships between genetic alterations and PTEN functions are outlined in Fig. 1b.

Although the lipid phosphatase activity of PTEN is important for its tumor suppressor functions, other functions of PTEN may also prove to be important. Indeed, several studies have demonstrated that PTEN protein

phosphatase activity is important for its functions in cell cycle arrest and inhibition of cell invasion in vitro [24–28]. The lipid phosphatase activity of PTEN is thought to mostly occur at the cell membrane, but PTEN has also been demonstrated to exert nuclear functions. The binding of PTEN to centromere protein C1 (CENP-C1) is required for centrosome stability, and its nuclear localization is required for DNA double-strand break (DSB) repair that is mediated by DNA repair protein RAD51 [144]. PTEN also regulates the tumor suppressor function of anaphase-promoting complex (APC) and its regulator E-cadherin (encoded by *CDH1*) in the nucleus, independently of its lipid phosphatase activity [145]. Altered APC–CDH1 activity has been implicated in multiple tumor types [146].

Conclusions and perspectives

The analysis of *PTEN* alteration in cell lines and tissues provides evidence that the development of many melanoma cases seems to be driven by the loss of PTEN expression and function. In this work, we have discussed function and signaling of PTEN as a lipid phosphatase as well as a protein phosphatase. The consequences of PTEN loss are alterations in the control of cell-cycle progression, apoptosis, cell contact, and migration. Together, these aberrations contribute to the malignant cell phenotype (Fig. 1b).

We have discussed several lines of evidence implicating *PTEN* in the development of melanoma. However, one key question that remains to be answered is whether tumors that develop as a consequence of *PTEN* attenuation are attributed to which biological function of this tumor suppressor. The importance of 10q23 loss in melanoma is clear, and studies of *PTEN* in tumors and cultured melanoma lines suggest strongly that mutated *PTEN* lead to a loss of function, although much remains to be learned about the precise role of PTEN in melanoma tumorigenesis. The exact frequency of PTEN loss in primary tumors, in metastases and the relation of these observations to the findings in cell lines require further confirmation. The inter-relation of *PTEN* mutation and other genes important in melanomagenesis needs to be studied. Modeling of these genetic discoveries in mouse models could be used to show whether re-expression of *PTEN* in *PTEN*-deficient melanoma could lead to tumor regression. Finally, the discovery of reagents to stimulate PTEN activity in cells that lack functional PTEN could be an important advance in cancer therapies.

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