Published in final edited form as:

J Invest Dermatol. 2011 December ; 131(12): 2497–2500. doi:10.1038/jid.2011.232.

Deletion of *PTENP1* pseudogene in human melanoma

Laura Poliseno[1], **Adele Haimovic**[1], **Paul J. Christos**[2], **Eleazar C. Vega y Saenz de Miera**[1], **Richard Shapiro**[1], **Anna Pavlick**[1], **Russell S. Berman**[1], **Farbod Darvishian**[1], and **Iman Osman**[1]

[1]The Interdisciplinary Melanoma Cooperative Group of the New York University School of Medicine, New York, NY, USA

[2]Division of Biostatistics and Epidemiology, Weill Medical College of Cornell University, New York, NY, USA

Dear Editor,

The aberrant activation of the PI3K/AKT pathway in melanoma is known to be caused by genomic deletion, promoter methylation or loss-of-function mutations of phosphatase and tensin homolog on chromosome 10 (PTEN) (see ref. (Madhunapantula and Robertson, 2009) for a review).

More recently, PTEN protein abundance was shown to be decreased at the post-transcriptional level by a complex microRNA network (He, 2010).

The processed pseudogene of *PTEN*, *PTENP1*, is a modulator of the interaction between *PTEN* mRNA and *PTEN*-targeting microRNAs (Poliseno et al., 2010). *PTENP1* shows extensive sequence similarity with *PTEN*. The high level of conservation in the most upstream region of the 3'UTR allows *PTENP1* mRNA to be bound by many of the microRNA families that bind *PTEN* mRNA (Figure 1 and Supplemental Text S1). Therefore, *PTENP1* can protect *PTEN* from microRNA-mediated downregulation. Consistent with its activity as a decoy for *PTEN*-targeting microRNAs, *PTENP1* is a *bona fide* tumor suppressor gene that causes growth inhibition in prostate cancer cells and undergoes deletion in various human malignancies (Poliseno et al, 2010).

As recently pointed out (Chen, 2010), the post-transcriptional regulation of PTEN levels in melanoma has not been studied yet and the status of *PTENP1* on 9p13 is unknown. Here, we report the analysis of *PTENP1* locus in human melanoma cell lines and tissues (Table S1). We also analyze the relationship between: 1) *PTENP1* deletion and *CDKN2A* deletion, as they are located approximately 20cM apart on chromosome 9p (Bennett, 2008); 2) *PTENP1* deletion and *PTEN* deletion, as 9p and 10q losses often coexist in melanoma (Indsto et al.,

Corresponding Author: Iman Osman, MD, New York University School of Medicine, 522 First Avenue, Smilow 405, New York, NY 10016, Phone: (212) 263-9076, Fax: (212) 263-9090, iman.osman@nyumc.org.

CONFLICT OF INTEREST

The authors declare no conflict of interest

1998); 3) *PTENP1* deletion and BRAF/NRAS mutation, as the combination of mutated BRAF (but not NRAS) and loss of *PTEN* expression is a common event in human melanoma (Tsao et al., 2004).

Our genomic analysis revealed partial deletion of *PTENP1* locus in 14.3% of the cell lines tested (Figure 2a). In addition, *PTENP1* was found to be deleted in 20.9% of melanoma tissues (8 partial and 1 complete deletion) (Figure 2b). These results illustrate that *PTENP1* is under selective pressure to undergo copy number losses (Poliseno et al., 2010). The average PTEN expression level in the 9 samples with either partial or complete deletion of *PTENP1* (P5, P9, P11, P12, P20, M1, M9, M14 and M16) was lower than in the remaining 34 samples without any *PTENP1* deletion (5.22 ± 2.30 N=9 vs. 20.59 ± 3.98 N=34, p = 0.06), confirming the protective effect of *PTENP1* on PTEN levels (Poliseno et al., 2010).

The details of the analyses of *CDKN2A* deletion, *PTEN* deletion, and BRAF and NRAS mutation in the human melanoma cell lines and tissues are provided in Supplemental Text S2.

We observed that the partial or complete deletion of *PTENP1* is always concomitant with the partial or complete deletion of *CDKN2A* in human melanoma cell lines, but not in human melanoma tissues. The “focal” deletion of *PTENP1* (5/9 cases) has already been reported in other cancer types, such as breast and colon cancer (Poliseno et al., 2010), but is particularly interesting in melanoma, because it happens in spite of the fact that *CDKN2A* deletions are the most common alterations (Bennett, 2008).

As far as the relationship between the partial or complete deletion of *PTENP1* and that of *PTEN* is concerned, we observed that *PTEN* is deleted in 7/9 melanoma tissues that show *PTENP1* deletion (Figure 2b). This result is supported by previous loss-of-heterozygosity analyses (Herbst et al., 1999) and is in agreement with the observation that 9p and 10q losses often coexist in melanoma (Indsto et al., 1998).

The co-deletion of *PTEN* and *PTENP1* can be reconciled with the function of *PTENP1* as decoy for *PTEN*-targeting microRNAs. *PTEN* deletion, either partial or complete, was found in 23 melanoma tissues. Excluding the cases in which *PTEN* deletion is complete and therefore *PTENP1* deletion cannot affect PTEN levels, on average we observed lower PTEN protein expression in the samples showing both *PTEN* partial deletion and *PTENP1* deletion compared to those showing *PTEN* partial deletion only, as exemplified by P5 and M5 tissues in Figure 2b. In the context of partial *PTEN* deletion, *PTENP1* deletion may cause a further decrease in PTEN levels, possibly due to less efficient sheltering of *PTEN*-targeting microRNAs. Of note, *PTENP1* deletion may also affect PTEN levels when PTEN is downregulated by mechanisms other than genomic deletion, such as inactivating mutations.

The biological effects of *PTENP1* deletion are likely to be particularly strong on PTEN because of the intrinsic nature of this haploinsufficient tumor suppressor whose variations of even 20% can have profound consequences on cancer onset and progression (Berger and Pandolfi, 2011). Nonetheless, the concomitant deletion of *PTENP1* and *PTEN* points towards *PTEN*-independent functions of *PTENP1* as well. Two possible explanations can be

invoked. First, *PTENP1* is likely to function as decoy for additional microRNA families which are not *PTEN*-targeting. These microRNAs should preferentially bind to the R2 region of the 3'UTR that shows low homology with the corresponding one on *PTEN* (Figure 1a) and might affect targets that belong to other signaling cascades, so that the concomitant deletion of *PTENP1* and *PTEN* causes the activation of independent pathways that possibly cooperate in tumorigenesis. Second, the *PTEN*-targeting microRNAs for which *PTENP1* acts as decoy have additional targets. Therefore, the deletion of *PTENP1* might be advantageous for the tumor because of the decrease of other oncosuppressor genes besides PTEN (Poliseno et al., 2010).

Examples of these genes are BIM, p21 and Sprouty2. The pro-apoptotic factor BIM is a miR-17 family-target (Fontana et al., 2008). Mutant BRAF and NRAS induce the MAPK-dependent phosphorylation and consequent proteosomal degradation of BIM in order to provide melanoma cells with resistance to anoikis (Akiyama et al., 2009). The CDK inhibitor p21, another target of miR-17 family (Fontana et al., 2008), is repressed transcriptionally by TBX2 and TBX3 transcription factors which are frequently amplified in melanoma and are downstream effectors of the MAPK pathway (Bennett, 2008). Sprouty2 is a *wt* BRAF inhibitor that is targeted by miR-21. The downregulation of Sprouty2 observed in melanoma has been hypothesized as an alternative mechanism responsible for the aberrant activation of MAPK pathway besides BRAF mutation (Tsavachidou et al., 2004). Of note, the three genes described above share a common feature as antagonists of the MAPK pathway. Since the PI3K/AKT and the MAPK pathways have been shown to cooperate in melanomagenesis both *in vitro* and in mouse models (Dankort et al., 2009; Meier et al., 2007), it is tempting to hypothesize that, in the context of *PTEN* deletion, the deletion of *PTENP1* might be an alternative mechanism evolved by the tumor to activate the MAPK pathway. In this respect, it is worth noticing that most of the cases showing both *PTENP1* and *PTEN* deletion (71.4%) harbor *wt* BRAF.

In conclusion, our data indicate that the recently identified tumor suppressor gene *PTENP1* undergoes genomic deletion in human melanoma. Our data also suggest that *PTENP1* deletion might be advantageous for the tumor not only because of its *PTEN*-related function, but also for *PTEN*-unrelated ones.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We thank Ting Tu, Daniel Lackaye and Holly Greenwald for the management of the clinical data of the IMCG patients. Dr. Paul J. Christos was partially supported by the Clinical Translational Science Center (CTSC) grant UL1-RR024996. This work was supported by the National Cancer Institute Cancer Center Support Grant (Grant No. 5 P30 CA 016087-27) and the Marc Jacobs Campaign to support the Interdisciplinary Melanoma Cooperative Group.

REFERENCES

Akiyama T, Dass CR, Choong PF. Bim-targeted cancer therapy: a link between drug action and underlying molecular changes. Mol Cancer Ther. 2009; 8:3173–3180. [PubMed: 19934277]

Bennett DC. How to make a melanoma: what do we know of the primary clonal events? Pigment Cell Melanoma Res. 2008; 21:27–38. [PubMed: 18353141]

Berger AH, Pandolfi PP. Haplo-insufficiency: a driving force in cancer. J Pathol. 2011; 223:137–146. [PubMed: 21125671]

Chen M. Smoke and miRrors: pseudogenes tricking miRNAs. Pigment Cell Melanoma Res. 2010; 23:583–584. [PubMed: 20701690]

Dankort D, Curley DP, Cartlidge RA, Nelson B, Karnezis AN, Damsky WE Jr. et al. Braf(V600E) cooperates with Pten loss to induce metastatic melanoma. Nat Genet. 2009; 41:544–552. [PubMed: 19282848]

Fontana L, Fiori ME, Albini S, Cifaldi L, Giovinazzi S, Forloni M, et al. Antagomir-17-5p abolishes the growth of therapy-resistant neuroblastoma through p21 and BIM. PLoS ONE. 2008; 3:e2236. [PubMed: 18493594]

He L. Posttranscriptional regulation of PTEN dosage by noncoding RNAs. Sci Signal. 2010; 3:pe39. [PubMed: 21045203]

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. [PubMed: 10552217]

Indsto JO, Holland EA, Kefford RF, Mann GJ. 10q deletions in metastatic cutaneous melanoma. Cancer Genet Cytogenet. 1998; 100:68–71. [PubMed: 9406584]

Madhunapantula SV, Robertson GP. The PTEN-AKT3 signaling cascade as a therapeutic target in melanoma. Pigment Cell Melanoma Res. 2009; 22:400–419. [PubMed: 19493313]

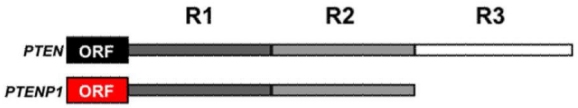
Meier F, Busch S, Lasithiotakis K, Kulms D, Garbe C, Maczey E, et al. Combined targeting of MAPK and AKT signalling pathways is a promising strategy for melanoma treatment. Br J Dermatol. 2007; 156:1204–1213. [PubMed: 17388918]

Poliseno L, Salmena L, Zhang J, Carver B, Haveman WJ, Pandolfi PP. A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. Nature. 2010; 465:1033–1038. [PubMed: 20577206]

Tsao H, Goel V, Wu H, Yang G, Haluska FG. Genetic interaction between NRAS and BRAF mutations and PTEN/MMAC1 inactivation in melanoma. J Invest Dermatol. 2004; 122:337–341. [PubMed: 15009714]

Tsavachidou D, Coleman ML, Athanasiadis G, Li S, Licht JD, Olson MF, et al. SPRY2 is an inhibitor of the ras/extracellular signal-regulated kinase pathway in melanocytes and melanoma cells with wild-type BRAF but not with the V599E mutant. Cancer Res. 2004; 64:5556–5559. [PubMed: 15313890]

a



b

miRNA family	R1	R2	R3	Conserved in *PTENP1*?	Ref
miR-17 miR-302	◆			yes	1
miR-19	◆	◆	◆	yes (R1)	1-5
miR-21	◆			yes	6
miR-22	◆			yes	1, 7, 8
miR-23		◆	◆	no	9
miR-25			◆	no	1
miR-26	◆	◆	◆	yes (R1)	10
miR-136	◆			yes	11
miR-141		◆		no	12
miR-144	◆			yes	11
miR-193	◆			yes	9
miR-214	◆			yes	13
miR-216		◆		no	14
miR-217		◆		no	14
miR-221	◆			yes	15
miR-486	◆		◆	yes (R1)	16
miR-494			◆◆	no	17

Figure 1. *PTEN*- and *PTENP1*-targeting microRNAs

a. Schematic representation of *PTEN* and *PTENP1* 3'UTR. The most upstream region of the 3'UTR (R1, dark grey) is highly conserved between *PTEN* and *PTENP1*. In the middle R2 region (light grey), the conservation drops dramatically. The downstream R3 region (white) is present only in *PTEN* (modified from Poliseno et al., 2010). **b.** List of the *PTEN*-targeting microRNA families validated so far. The region of the 3'UTR where the microRNA binding sites are located and the conservation of these sites in *PTENP1* 3'UTR are indicated. Out of a total of 18 *PTEN*-targeting microRNA families, 12 are *PTENP1*-targeting as well (grey). All these families have at least 1 binding site in the high homology R1 region.

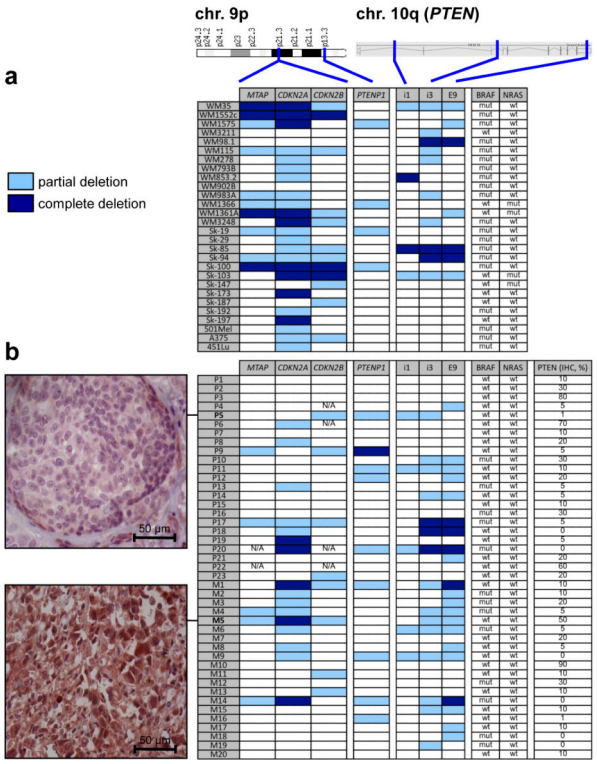


Figure. 2. Analysis of *CDKN2A, CDKN2B, MTAP, PTENP1, PTEN*, BRAF and NRAS status in human melanoma cell lines (a) and tissues (b)

a. 14 primary (WM series) and 14 metastatic (Sk-Mel series, 501Mel, A375 and 451Lu) melanoma cell lines and **b.** 23 primary (P1-P23) and 20 metastatic (M1-M20) melanoma tissues were analyzed by qPCR on genomic DNA to identify deletions at 9p21 (*CDKN2A*, *CDKN2B* and *MTAP* loci), 9p13 (*PTENP1* locus) and 10q (*PTEN* locus). Mutations identified in exon 11 and 15 of BRAF and exon 2 and 3 of NRAS are reported. PTEN protein levels were analyzed in melanoma tissues by immunohistochemistry (IHC) and the percentage of positive cells is indicated. N/A: genomic qPCR not performed due to constraints on DNA availability. On the left, IHC shows lower PTEN levels in P5 tissue harboring partial *PTEN* and *PTENP1* deletion compared to M5 tissue harboring partial *PTEN* deletion only.