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Resveratrol regulates PTEN/Akt pathway through inhibition of MTA1/HDAC unit of the NuRD complex in prostate cancer

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ABSTRACT

Metastasis associated protein 1 (MTA1) is a component of the nucleosome remodeling and deacetylating (NuRD) complex which mediates gene silencing and is overexpressed in several cancers. We reported earlier that resveratrol, a dietary stilbene found in grapes, can down-regulate MTA1. In the present study, we show that PTEN is inactivated by MTA1 in prostate cancer cells. Further, we show that resveratrol promotes acetylation and reactivation of PTEN via inhibition of the MTA1/HDAC complex, resulting in inhibition of the Akt pathway. In addition, we show that MTA1 knockdown is sufficient to augment acetylation of PTEN indicating a crucial role of MTA1 itself in the regulation of PTEN acetylation contributing to its lipid phosphatase activity. Acetylated PTEN preferentially accumulates in the nucleus where it binds to MTA1. We also show that MTA1 interacts exclusively with PTEN acetylated on Lys^{125} and Lys^{128}, resulting in diminished p-Akt levels. Finally, using orthotopic prostate cancer xenografts, we demonstrate that both resveratrol treatment and MTA1 knockdown enhance PTEN levels leading to a decreased p-Akt expression and proliferation index. Taken together, our results indicate that MTA1/HDAC unit is a negative regulator of PTEN which facilitates survival pathways and progression of prostate cancer and that resveratrol can reverse this process through its MTA1 inhibitory function.

1. Introduction

"All men are at risk for prostate cancer. Changing the risk factors that can be controlled, such as diet, is one way to reduce prostate cancer risk". Since prostate cancer is age-related and relatively slow growing cancer, chemoprevention becomes the most promising strategy for the future management of this disease. Consequently, bio-reactive dietary compounds with anticancer properties, such as resveratrol, are of particular interest.

Resveratrol, a dietary compound found in berries, peanuts and mostly in grapes and wine, is known for its cardioprotective, anti-inflammatory, antioxidant and anticancer activities [1]. The anticancer action of resveratrol involves modulation of multiple molecular targets and pathways resulting in cell cycle arrest and apoptosis and inhibition of angiogenesis [2–7]. However, there are serious knowledge gaps regarding resveratrol's epigenetic mechanisms of action.

We reported earlier on a new epigenetic target of resveratrol, the metastasis-associated protein 1 (MTA1), which is a part of nucleosome remodeling and deacetylation (NuRD) co-repressor complex that mediates gene silencing [8,9]. We showed that resveratrol causes apoptosis in prostate cancer cells by inhibiting MTA1 and reversing MTA1-mediated deacetylation of p53 [10]. In prostate cancer xenografts, resveratrol treatment reduced mitotic activity and angiogenesis, and in co-operation with MTA1-knockdown, showed improved response with noticeable delay in tumor growth. Importantly, there was significantly increased levels of acetylated p53 (Ac-p53) and significant increase in apoptotic index upon resveratrol treatment [11]. These results clearly indicated that resveratrol alters post-translational modification of tumor suppressor p53 through targeting negative epigenetic modifier MTA1. We observed an inverse relationship between MTA1 and PTEN (phosphatase and tensin homolog deleted on chromosome 10) tumor suppressor protein levels and subsequently hypothesized that MTA1, which is abundantly accumulated in aggressive prostate cancer and metastasis [12–14], may participate in the deacetylation and inactivation of PTEN thereby activating downstream Akt cell survival and migration pathways [15–18]. We further hypothesized that resveratrol by down-regulating MTA1 may reactivate PTEN and rescue apoptotic pathways.

The tumor suppressor PTEN controls a variety of biological processes by negatively regulating the PI3K/Akt cell survival pathway [15,19]. The *Pten* gene is frequently mutated in prostate cancer [20]. At the same time, hypermethylation of the *Pten* promoter, and reduction of PTEN

Abbreviations: MTA1, metastasis-associated protein 1; PTEN, phosphatase and tensin homolog deleted on chromosome 10; Res, resveratrol

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protein levels without mutations in the gene were observed in several cancers, indicating possible epigenetic mechanisms of PTEN regulation [21]. Therefore, it is possible that in addition to a direct mutation of the *Pten* gene, deregulation of PTEN protein on post-translational level plays role in cancer initiation and progression opening therapeutically attractive approaches for PTEN reactivation. Indeed, it has been reported that PTEN can be modified by phosphorylation, oxidation, nitrosylation and ubiquitination [15], and that all these modifications exert negative regulatory effects on PTEN function. However, there is limited literature on acetylation/deacetylation of PTEN and how this modification regulates its function.

In the current study, we provide evidence that there is an inverse relationship between MTA1 and PTEN and that in aggressive prostate cancer, nuclear MTA1 interacts with acetylated PTEN and inhibits its tumor-suppressive functions. Importantly, resveratrol can reactivate PTEN by reversing the negative effect of MTA1/HDAC mediated deacetylation. Our finding offers novel chemopreventive and therapeutic potential of dietary agents for management of prostate cancer.

2. Materials and methods

2.1. Reagents

Resveratrol (Res) (3,5,4′-trihyrdoxy-trans-stilbene) and Trichostatin A (TSA) were purchased from Sigma-Aldrich, IN, USA. SAHA (SuberoylAnilide Hydroxamic acid) was purchased from Cayman Chemicals, MI, USA. Resveratrol was used at 50 μM, SAHA at 10 μM and TSA at 300 nM concentrations for all experiments unless otherwise mentioned.

2.2. Cell culture

Human prostate cancer cells, DU145 and PC3M (ATCC, VA, USA and a gift from Dr. Bergan, Northwestern University, Chicago, respectively), were cultured in RPMI 1640 containing 10% fetal bovine serum (FBS) and 1% antibiotics (Life Technologies, NY, USA) at 37 °C and 5% CO_2. *Pten*$^{+/+}$ and *Pten*$^{-/-}$ mouse prostate epithelial cell lines were a gift from Dr. Tzivion, UMMC and were maintained in DMEM supplemented with 10% FBS with 1% antibiotics at 37 °C and 5% CO_2. 293T cells (ATCC, VA, USA) were maintained in DMEM supplemented with 10% FBS with 1% antibiotics at 37 °C and 5% CO_2. For experiments involving treatments with resveratrol, phenol red-free media containing 10% charcoal-stripped serum (Life Technologies, NY, USA) was used to provide steroid-free background. Cells were transferred to phenol red-free medium one day prior to treatments. Subsequently, cells were either treated with control (Ctrl, Ethanol/DMSO) or resveratrol (Res, 50 μM, unless otherwise mentioned) or SAHA (10 μM) or TSA (300 nM) for 18–24 h.

2.3. Plasmids, transfections and mutagenesis

The 800 pSG5L HA-PTEN (plasmid 10750), [22] and HDAC1-Flag (plasmid 13820) [23] plasmids were received from Addgene, MA, USA. Myc-MTA1 construct was a kind gift from Dr. Rakesh Kumar (George Washington University) and myc-p300 plasmid was a kind gift from Dr. Tzivion, Cancer Institute, UMMC. For transient transfections, 293T and/or PC3M cells were plated in 10 cm dishes (BD Falcon, Corning Life Sciences, MA, USA) to reach 80–90% confluence and were transfected with myc-MTA1, HA-PTEN, HDAC1-Flag, myc-p300 and PTEN mutant constructs or their respective empty vectors (10 μg) in OptiMEM (LifeTechnologies, NY, USA) using Fugene HD (Promega, WI, USA). After 36 h, cells were harvested and immunoprecipitation (IP) and western blot procedures were followed as described in the following sections.

Mutant PTEN constructs were created using the Infusion HD cloning kit (Clontech, CA, USA) following the mutagenesis protocol as per manufacturer's instructions. The following primer sequences were used in inverse PCR reactions for amplifying the designated clones; K125128Q Fwd 5′-tgt**caa**gctgga**caa**ggacgaactggtgtaatgatatgtgcata-3′ and Rev 5′-c**ttg**tccagc**ttg**acagtgaattgctgcaacatgattgtcatctt-3′, K125128R Fwd 5′-ttcactgt**aga**gctgga**aga**ggacgaac-3′ and Rev 5′-gttcgtcc**tct**tccagc**tct**acagtgaaaacg-3′, K402Q Fwd 5′-tacacaaatt aca**caa**gtctgagaattccccgatatcgcctcga-3′ and Rev 5′-tcagac**ttg**tgt aatttgtgtatgctgatcttcatcaaaagg-3′, K402R Fwd 5′-acaaattaca**aga** gtctgagaattccccgatatcgccagctca-3′ and Rev 5′-tcagac**tct**tgtaatt tgtgtatgctgtctctcaaaaggttcatt-3′. The sequences in bold indicate the mutant amino acid(s) which were verified by sequencing (Davis Sequencing, CA, USA).

2.4. MTA1 silencing

Establishment of stable prostate cancer cell lines with MTA1 knockdown (MTA1shRNA) was described and characterized previously [10–12]. Briefly, the expression ArrestTMGIPZ lentiviral shRNAmir vectors expressing MTA1shRNA and non-silencing empty vector (EV) were purchased from Open Biosystems (GE Dharmacon, CO, USA) and virus particles were generated using 293T cells and packaging plasmid mixture (psPAX2 and pMD2.G, Addgene, MA, USA). Stable positive clones were selected using 1 μg/ml puromycin. The cell lines were stably transfected with luciferase (luc) and selected on G418 (neomycin, Sigma-Aldrich, IN, USA) for high luciferase expression to be used *in vivo* [11].

2.5. Western blot

Western blot analysis was performed as previously described [10–12]. Briefly, cells were harvested in chilled RIPA buffer with protease and phosphatase inhibitors (ThermoScientific, IL, USA). In the experiments with TSA/SAHA treatments we also used deacetylase inhibitors in the buffer. Protein estimation was performed using the Bradford reagent on a SmartSpec 3000 spectrophotometer (BioRad, CA, USA). 70 μg protein was loaded on 10% TGX gel, transferred to PVDF membrane, and probed with the following primary antibodies: Acetylated lysine (#9441), PTEN (#9188), p-Akt (Ser473, #9271), Akt (#4691) and Erk1/2 (#4695) (1:1000, Cell Signaling, MA, USA); MTA1 (A-11), HDAC1 (10E2) and anti-HDAC2 (B-2) (1:200, Santa Cruz Biotechnologies, CA, USA), β-actin (AC-15) and Lamin A (H-102) (1:1000, Santa Cruz Biotechnologies, CA,USA), myc (hybridoma supernatant, 1:250) and Flag (Clone M2, 1:1000, Sigma-Aldrich, IN, USA). Goat anti-mouse or goat anti-rabbit secondary antibodies (1:2500, Sigma-Aldrich, IN, USA) and goat anti-mouse light chain specific antibody (1:1000, Jackson Immunoresearch, PA, USA) were used for detection. Signals were visualized using Supersignal West Dura enhanced chemiluminescent substrate (ThermoScientific, IL, USA) on a Chemidoc Imager (BioRad, CA, USA). Images were quantified using ImageJ software (NIH).

2.6. Immunoprecipitation

Immunoprecipitation assays were performed using the Pierce Classic IP kit (ThermoScientific, IL, USA) as per manufacturer's instructions. Briefly, DU145 cells were plated in 150 mm dishes and cultured till 70–80% confluence. 293T or PC3M cells were transfected as described in Section 2.3. The cells were washed in DPBS (Life Technologies, NY, USA) and harvested in 0.5 ml of IP lysis buffer (Pierce Classic IP kit) with protease and phosphatase inhibitors (ThermoScientific, IL, USA). Lysates were prepared with 20 strokes on a Dounce homogenizer (Sartorius, Goettingen, Germany) and supernatants collected after centrifugation at 14,000 rpm for 10 min at 4 °C (Eppendorf 5430R, NY, USA). The lysates were incubated with 6 μg of primary antibodies or as per manufacturer's instructions: MTA1 (H-166), HDAC1 (10E2), HDAC2

(B-2), PTEN (A2B1), myc (hybridoma supernatant), Flag (Clone M2), acetyl-lysine (#9441,) on a rotating chamber overnight at 4 °C. 20 μl of Protein A/G beads (Pierce Classic IP kit, ThermoScientific, IL, USA) was added to the lysates after washing in spin columns and incubated for 2 h at 4 °C. The IP products were eluted in 2× sample reducing buffer with 20 mM dithiothreitol (DTT). IP elutes were loaded on 10% TGX gels (BioRad, CA, USA) and western blot was performed with indicated antibodies.

2.7. Subcellular fractionation

Fractionations were performed using the NE-PER Nuclear and Cytoplasmic extraction reagent kit (Thermo Scientific, IL, USA) according to the manufacturer's instructions. Briefly, cells were harvested with 0.25% trypsin-EDTA (Life Technologies, NY, USA), washed in DPBS and pelleted by centrifugation at 12,000 rpm for 10 min at 4 °C. 200 μl of buffer CER I was added with protease and phosphatase inhibitors to the cell pellet and vigorously vortexed for 15 s and incubated on ice for 10 min. Thereafter, buffer CER II (11 μl) was added and additionally vortexed and incubated on ice for 5 min. Cytoplasmic extracts were collected as supernatants by centrifuging at 16,000 *g* for 10 min. The cell pellet was washed thrice with 1 ml DPBS to remove cytoplasmic contamination. To the nuclear pellet, 100 μl buffer NER was added and vortexed for 15 s every 10 min for 40 min and supernatants were collected by centrifuging at 16,000 *g* for 10 min. Protein estimations and IP reactions were performed immediately or the extracts were stored at −80 °C for later analysis.

2.8. Immunoflourescence

DU145 cells were plated on sterile cover slips in 35 mm petri dishes (BD Falcon, Corning Life Sciences, MA, USA) so that they were at a confluence of 60–70% on the day of experiment. Cells were fixed in freshly prepared 2% chilled paraformaldehyde (ThermoScientific, IL, USA) for 15 min at room temperature on a rocker, washed with cold PBS and permeabilized in 0.5% TritonX-100/PBS (Fisher Scientific, PA, USA) for 15 min and washed with cold PBS. Non-specific binding was blocked by incubating cells with 10% normal mouse serum, 30 min at room temperature. For dual staining, cells were incubated first with MTA1 antibody (1:50, A-11, Santa Cruz Biotechnologies, CA, USA) followed by secondary antibody Alexa 488 (1:200, Life Technologies, NY, USA) in staining-wash buffer (5% FBS/0.5% TritonX-100/PBS) in dark for 1 h on a rocker. This was followed by washing and incubation with PTEN antibody (FL-403, 1:50, Santa Cruz Biotechnologies, CA, USA) followed by secondary antibody Alexa 633 (1:200, Life Technologies, NY, USA) for 1 h at room temperature. Finally cells were stained with Propidium Iodide (PI, 10 μg/ml, Sigma-Aldrich, IN, USA) and washed and mounted in Vectashield with DAPI (Vector Laboratories, CA, USA). Unstained cells and cells incubated with secondary antibody alone were used as negative controls. Images were acquired with a 63× objective on a Leica Brightfield Fluorescent microscope and Leica TCS SP2 confocal microscope and analyzed using the LAS AF Lite software version 2.6.0 (Leica Microsystems, IL, USA). For quantitation of co-localization, NIS elements Imaging software version 3.10, SP3, Hotfix (Build 644, LO) was used.

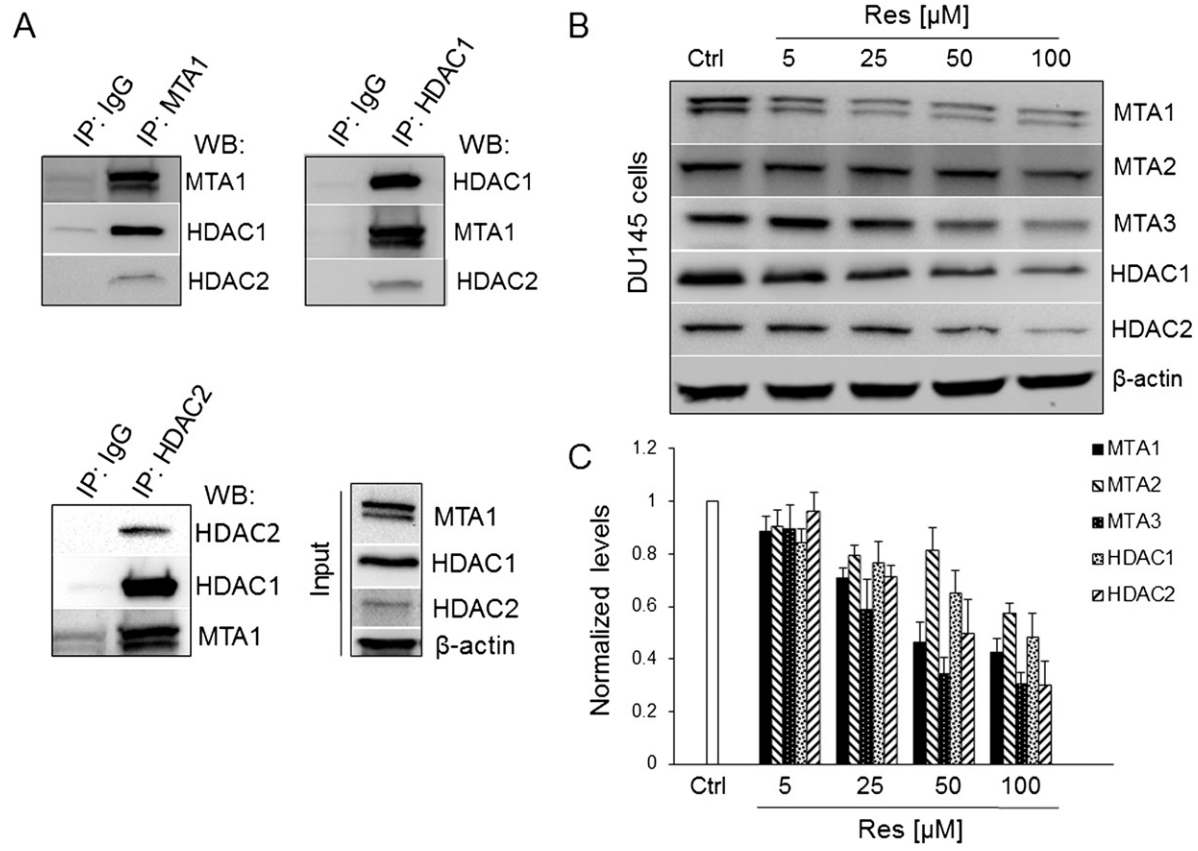

Fig. 1. A) MTA1 and HDAC1 and 2 are in complex. Reciprocal co-immunoprecipitations (co-IP) were performed with lysates from DU145 cells using anti-MTA1, anti-HDAC1 and anti-HDAC2 antibodies and western blot analysis was performed to detect MTA1, HDAC1 and HDAC2 respectively in IP elutes and in whole cell lysates (input). B) Resveratrol inhibits MTA1, MTA2, MTA3, HDAC1 and HDAC2 protein levels in DU145 cells in a dose-dependent manner. Western blot analysis of lysates from DU145 cells treated with Ethanol (Ctrl) or indicated concentrations of resveratrol (Res) for 24 h. C) Graphical representation of results. The vehicle-treated control (Ctrl) is normalized to 1 and protein level changes are expressed relative to Ctrl. The means ± SE of three independent experiments are shown.

2.9. Establishment of orthotopic prostate cancer xenografts in nude mice

Tumor tissues from the orthotopic prostate cancer xenografts described previously [11], were used for immunohistochemistry. Briefly, male nude mice were fed with phytoestrogen-free diet, injected orthotopically (intraprostatic) with DU145-luciferase-tagged MTA1-expressing (Ctrl) and MTA1-knockdown (MTA1shRNA) cells. Control group was treated with vehicle 10% DMSO and treatment group was injected 50 mg/kg bw/day of resveratrol through intraperitoneal (i.p.) administration. Bioluminescence signals were detected every week. The mice were sacrificed at week 8 after cell inoculation. At necropsy, prostates were excised and fixed in 10% neutral-buffered formalin for histopathological and immunohistochemical analysis.

2.10. Immunohistochemistry

Immunochemistry was performed with 4 μm formalin-fixed paraffin embedded tumor sections to evaluate Ki67, PTEN, MTA1 and p-Akt levels as described previously [11–13]. The VECTASTAIN ABC Elite Kit and the ImmPACT DAB kit (Vector Laboratories, CA, USA) were used to visualize staining. The following antibodies were used: anti-Ki67 (1:100, Abcam, MA, USA) and anti-PTEN (1:200, Santa Cruz Biotechnologies, CA, USA), anti-MTA1 and anti-pAkt (1:50, Cell Signaling Technologies, MA, USA). Images were recorded on a Nikon Eclipse 80i microscope and analyzed using the NIS elements BR 3.22.11 software.

2.11. Statistical analysis

Statistical significance (p values) in mean values of two-sample comparison was determined with paired two-tailed Student's *t*-test. A p-value < 0.05 was considered to be statistically significant.

3. Results

3.1. Resveratrol down-regulates components of the nucleosome remodeling and deacetylase (NuRD) complex

We have previously shown that MTA1 physically associates with HDAC1 in prostate cancer cells [10]. Others also demonstrated that MTA1 interacts with both HDAC1 and HDAC2 in breast cancer cells [24,25]. We too found that MTA1 interacts with both HDAC1 and HDAC2, which also interact with each other in DU145 cells (Fig. 1A). These results demonstrate that, in a physiological setting, MTA1/HDAC1/2 occurs as a complex in prostate cancer cells. We reported earlier that resveratrol down-regulates MTA1 and dissociates MTA1/HDAC1 complex [10]. This prompted us to investigate whether resveratrol has any effects on HDAC1 and HDAC2 protein levels directly. Interestingly, we found that resveratrol along with MTA1 inhibition decreases MTA2, MTA3, HDAC1 and HDAC2 levels in DU145 (Fig. 1B and C) and LNCaP cells in a dose-dependent manner (Supplementary Fig. 1).

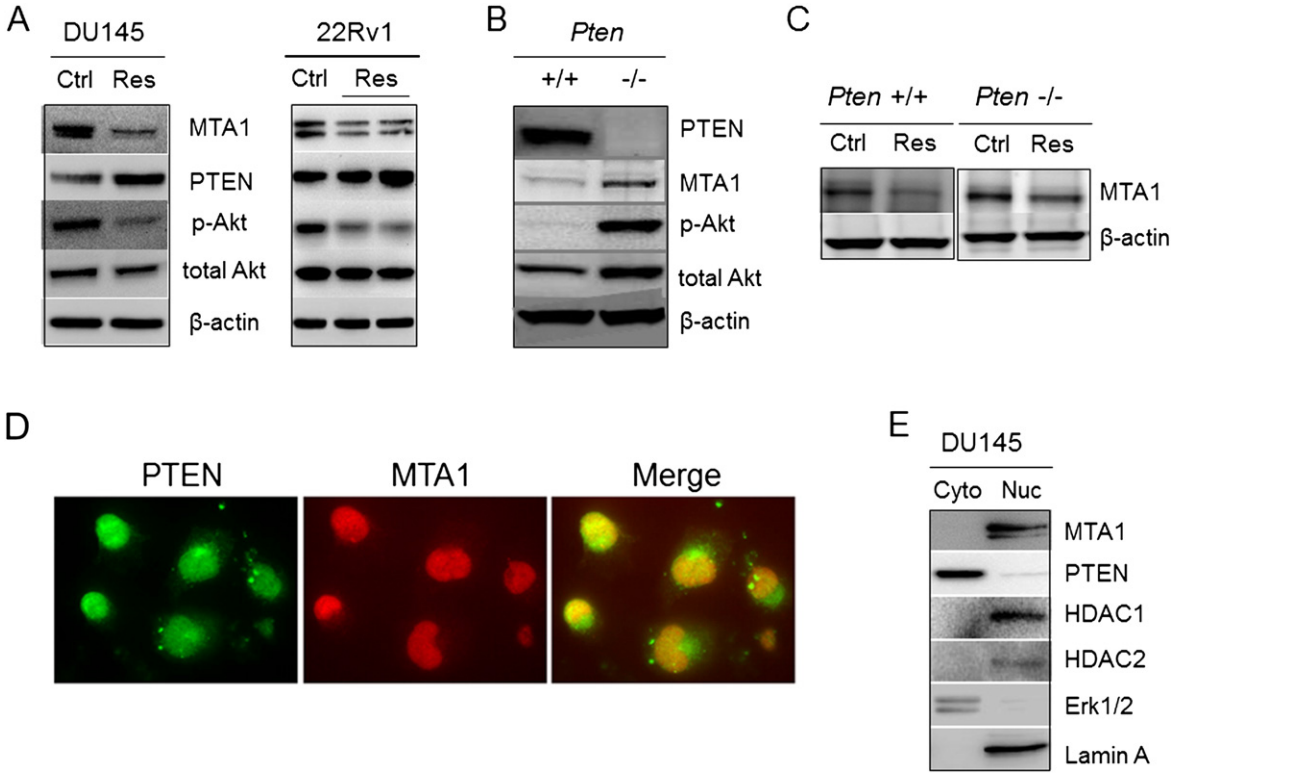

Fig. 2. MTA1 and PTEN are inversely related. A). Resveratrol inhibits MTA1 concomitantly with up-regulation of PTEN and down-regulation of pAkt (Ser 473). Western blot analysis of lysates from DU145 and 22Rv1 cells treated with Ethanol (Ctrl) or resveratrol (Res, 50 μM for Du145 and 50 μM and 100 μM for 22Rv1 cells) for 24 h. B). MTA1 is up regulated in prostate epithelial cells from *Pten* null mice. Western blot analysis of lysates from $Pten^{+/+}$ and $Pten^{-/-}$ cells for MTA1, PTEN, p-Akt (Ser 473), Akt and β-actin as loading control is shown. C) Resveratrol inhibits MTA1 in $Pten^{-/-}$ cell line. $Pten^{+/+}$ and $Pten^{-/-}$ cells were either treated with Ethanol (Ctrl) or resveratrol (Res, 50 μM) and lysates were probed for MTA1. D) Fluorescence microscopy showing co-localization of MTA1 and PTEN in DU145 cells. Cells were fixed, permeabilized and stained with anti-PTEN and anti-MTA1 primary antibody followed by visualization with secondary anti-rabbit Alexa 488 and anti-mouse Alexa 633 antibodies, respectively. Images were acquired using 63× objective on a Leica fluorescent microscope. Images were pseudo-colored in red to represent MTA1 and in green to represent PTEN. Images show PTEN (green) both in nucleus and cytoplasm, and co-localization with MTA1 in nucleus (merge, yellow). E) Subcellular localization and differential expression of MTA1 and PTEN in DU145 cells. Western blot analysis of cytoplasmic (Cyto) and nuclear (Nuc) lysates from DU145 cells is shown. Lamin A and Erk1/2 levels were used as loading controls for nuclear and cytoplasmic fractions, respectively. Subcellular localization shows exclusive nuclear distribution for MTA1, HDAC1 and HDAC2 proteins while PTEN is mostly cytoplasmic and partially nuclear.

3.2. Resveratrol inhibits MTA1 and up-regulates PTEN protein levels in prostate cancer cells: inverse correlation between MTA1 and PTEN

In conjunction with reports on transcriptional regulation of PTEN by HDAC inhibitors [26] and by the MTA1/HDAC4 complex [27], coupled with our own observation of resveratrol behaving as an HDAC inhibitor (Fig. 1B), we found a significant up-regulation of PTEN mRNA upon resveratrol treatment in DU145 and 22Rv1 cells (Supplementary Fig. 2). Importantly, resveratrol mediated down-regulation of MTA1 protein levels was concomitant with up-regulation of PTEN protein and accompanied by a decrease in phosphorylated Akt (p-Akt, S473) levels in DU145 and 22Rv1 cells suggesting an inverse relationship between MTA1 and PTEN (Fig. 2A). To confirm the inverse relationship between these two molecules, we examined MTA1 expression in murine prostate epithelial cells from conditional *Pten* knockout mice and found that $Pten^{-/-}$ cells express significantly higher levels of MTA1 compared to $Pten^{+/+}$ cells (Fig. 2B) and that resveratrol inhibits MTA1 expression in these cells (Fig. 2C) suggesting a possible negative co-relation between MTA1 expression and PTEN signaling.

In an earlier report by Salot and Gude, MTA1 and PTEN have been shown to co-localize in the nuclei of breast cancer cells [28]. Our immunofluorescence experiments revealed co-localization of MTA1 and PTEN in the nuclei of DU145 cells (Fig. 2D, Mander's overlap coefficient = 0.73, Pearson's coefficient = 0.84). Further experiments using confocal microscopy also confirmed nuclear co-localization of these two proteins in addition to evident cytoplasmic localization of PTEN (Supplementary Fig. 3). On the other hand, subcellular fractionation showed exclusive nuclear localization for MTA1, HDAC1 and HDAC2 while PTEN was mostly cytoplasmic with some amount in the nucleus (Fig. 2E). The fact that MTA1 and PTEN can be detected in the nucleus suggested that MTA1/HDACs co-repressor complex might be involved in post-translational regulation/deactivation of PTEN, which in turn, can lead to the activation of PI3K/Akt survival pathway. This finding substantiated the rationale to explore the crosstalk between MTA1 and PTEN and the role of resveratrol in regulating this signaling.

3.3. PTEN activity is regulated by resveratrol-induced reversible acetylation

To further confirm the inverse relationship between PTEN and MTA1 and to understand the role of the MTA1/HDAC deacetylase complex, we treated DU145 MTA1-expressing (EV) and MTA1 shRNA cells with HDAC inhibitor Trichostatin A (TSA) and performed western blot analysis. MTA1 knockdown enhanced PTEN expression, which was further augmented upon TSA treatment (Fig. 3A). Concomitantly, with increase in total PTEN levels upon MTA1 knockdown, substantiated by TSA treatment, there was a decrease in p-Akt (Ser 473). Interestingly, in addition to expected inhibition of HDAC 1/2 activity, TSA treatment alone reduced MTA1 levels as well (Fig. 3A).

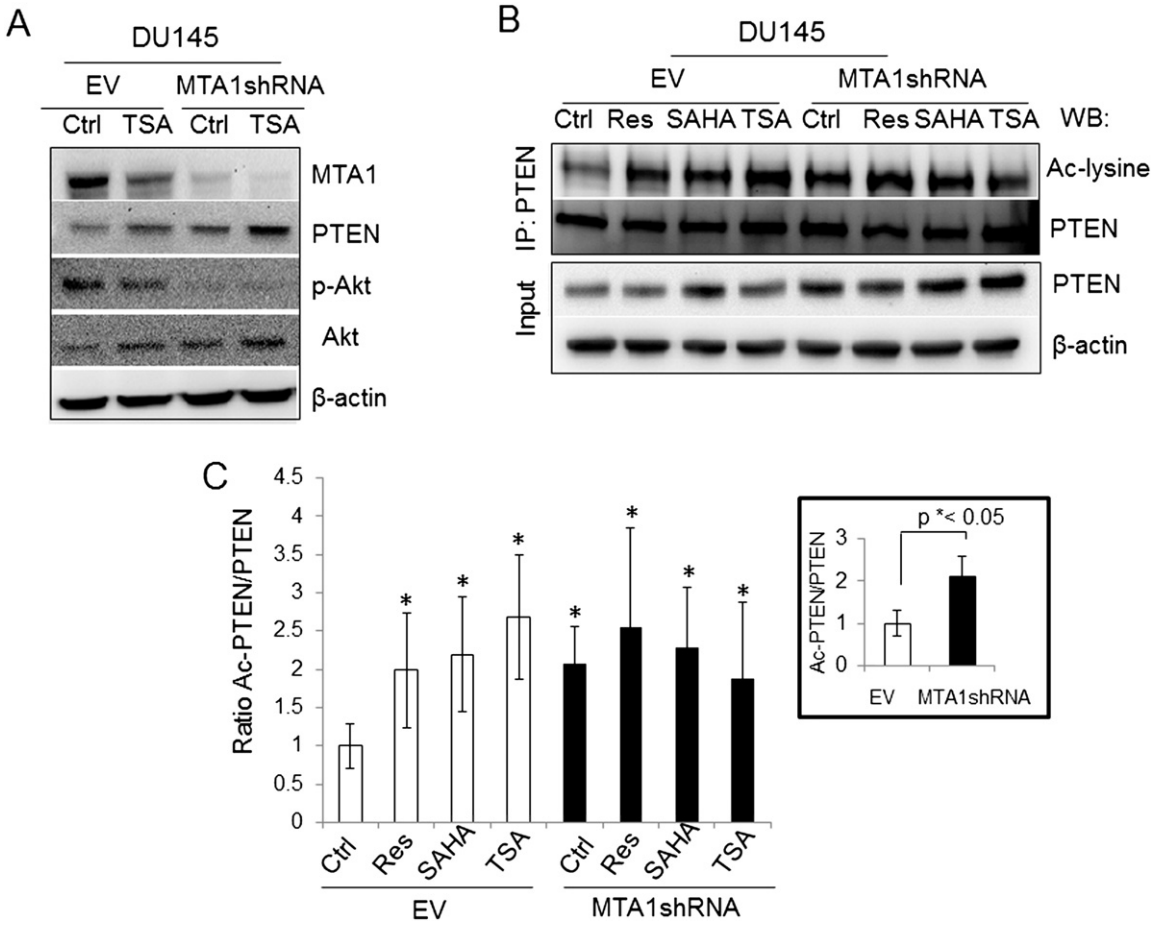

Fig. 3. MTA1 knockdown enhances PTEN while decreasing p-Akt which is augmented by HDAC inhibitor, TSA. A) Western blot analysis of lysates from DU145 empty vector (EV) or MTA1 knockdown (MTA1 shRNA) cells either treated with Ethanol (Ctrl) or Trichostatin A (TSA, 300 nM) for 18 h is shown. B) Resveratrol enhances acetylation of PTEN which is augmented upon MTA1 knockdown. DU145 EV or MTA1 shRNA cells were treated with DMSO (Ctrl), resveratrol (50 μM), Suberoylanilidehydroxamic acid (SAHA, 10 μM) and Trichostatin A (TSA, 300 nM) for 20 h. Cell lysates were immunoprecipitated with anti-PTEN antibody and western blot analysis performed with anti-acetyl-lysine and anti-PTEN antibodies. C) Quantitation for ratio of Ac-PTEN/PTEN was done using Image J software (NIH). EV (Ctrl) was normalized to 1 and all values were calculated relative to Ctrl, $^*p < 0.05$. (Inset) Quantitation for ratio of Ac-PTEN/PTEN in DU145 EV versus MTA1 shRNA cells is shown, $^*p < 0.05$. Error bars indicate means ± SEM. Representative data from three independent experiments is depicted.

To assess the mechanism of how resveratrol counteracts the negative regulatory effect of MTA1/HDACs on PTEN, we treated DU145 EV and MTA1shRNA cells with resveratrol and HDAC inhibitors, SAHA and TSA and performed IP PTEN and western blot for acetyl lysine (Ac-lysine). Resveratrol significantly increased acetylated PTEN (Ac-PTEN), almost to the same extent as SAHA and TSA (Fig. 3B and C). Interestingly, MTA1 knockdown alone was sufficient to significantly increase Ac-PTEN (Fig. 3C, inset, $p < 0.05$, Ctrl vs MTA1shRNA) suggesting that resveratrol's effect on PTEN acetylation occurs mainly through MTA1 inhibition. Since TSA and SAHA in addition to inhibiting HDACs functions also can directly inhibit MTA1 expression (Fig. 3A and data not shown), it is possible that the observed effects with SAHA and TSA are also mediated through MTA1 and not only through HDACs. Collectively, these results demonstrate that, all three agents resveratrol, SAHA and TSA, may suppress deacetylation and increase acetylation of PTEN by inhibiting each of the three proteins in the complex, although MTA1 knockdown experiments showed dominant role of MTA1 in PTEN deacetylation. These data demonstrate the negative association of MTA1/HDAC1/2 complex with acetylation-dependent activation of PTEN. Further, resveratrol and HDAC inhibitors which target the MTA1/HDACs complex, enhance acetylation of PTEN that, in turn may inhibit Akt phosphorylation.

We noticed that in all our experiments treatment of MTA1 knockdown cells with SAHA and TSA did not induce an additive effect on PTEN acetylation (Fig. 3C) although they did induce PTEN expression (Fig. 3B input). We then asked whether HDAC1 or HDAC2 or both are needed to mediate the effect on PTEN deacetylation. As shown in Fig. 3B, HDAC class I inhibitors, which can inhibit both HDAC1 and 2, increased PTEN acetylation. We then used HDAC1-specific inhibitor (4-(dimethylamino)N-[6-(hydroxyamino)-6-oxohexyl]-benzamide) and found that PTEN acetylation was increased but to a lesser extent than when treated with TSA and SAHA suggesting involvement of HDAC2 in addition to HDAC1 in PTEN deacetylation (data not shown).

3.4. MTA1 knockdown induces accumulation of acetylated PTEN in the nucleus of prostate cancer cells

Since we already observed co-localization of MTA1 and PTEN in the nucleus of DU145 cells (Fig. 2D and E), we further hypothesized that for MTA1 a nuclear protein, to deacetylate PTEN which is predominantly cytoplasmic, would require an event most likely occurring in the nucleus. To answer this possibility, we fractionated cytoplasmic and nuclear extracts from DU145 EV and MTA1shRNA cells treated with TSA and subsequently performed IP PTEN and western blot for Ac-lysine. We found that Ac-PTEN expression was increased in both cytoplasm and nuclear compartments; however, the increase in the nuclear compartment was more dramatic (Fig. 4A and B, *Nuc* vs *Cyto*, $p < 0.05$). Importantly, this effect was prominent in MTA1 knockdown cells, which was further augmented upon TSA treatment (Fig. 4A and B). This data clearly indicated that MTA1 knockdown favorably enhances acetylation of PTEN largely in the nuclear compartment. Further, it indicated that for such a mechanism to be functional a physical interaction between MTA1 and PTEN itself maybe important. Therefore, we next raised the question: does MTA1 interact with PTEN?

3.5. MTA1 does not interact with PTEN in prostate cancer cells

We and others have shown that MTA1 can be localized in both nucleus and cytoplasm with predominance in nuclei in cultured cancer cells and tissues [13,29–33]. In our studies with tissue microarrays of 291 human prostate cancer specimens, MTA1 nuclear localization was directly correlated with aggressiveness of prostate cancer and metastasis [13]. HDAC1 and HDAC2 are mostly nuclear proteins and in prostate cancer, nuclear HDAC1 overexpression has been reported to be correlated with poor prognosis [34] and HDAC2 expression

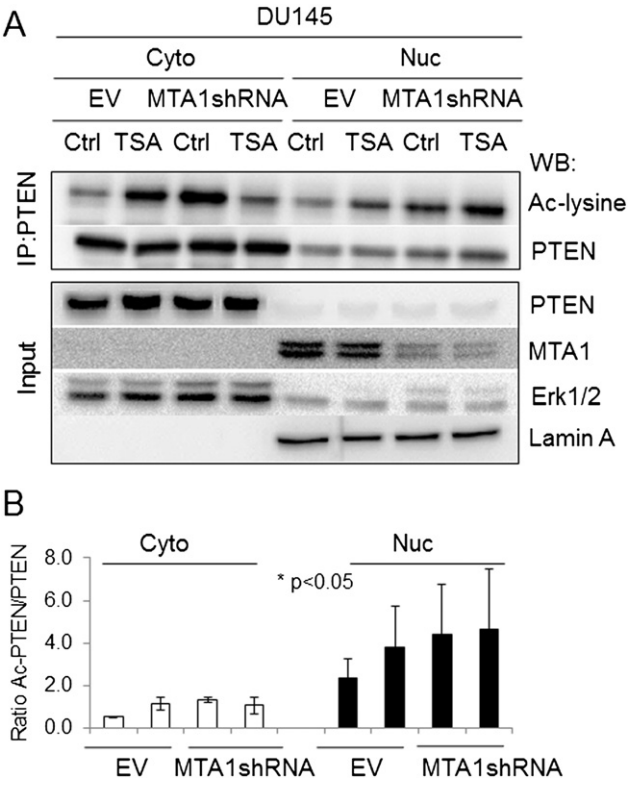

Fig. 4. MTA1 knockdown-induced acetylation of PTEN predominantly occurs in the nucleus of prostate cancer cells. A) DU145 EV and MTA1 shRNA cells were treated with Ethanol (Ctrl) or Trichostatin A (TSA, 300 nM) for 20 h. Cell lysates were fractionated to obtain cytoplasmic and nuclear fractions as detailed in *materials and methods*. Cytoplasmic and nuclear extracts were immunoprecipitated using anti-PTEN antibody and analyzed by western blotting using antibodies to acetyl lysine and PTEN. Erk1/2 and Lamin A were used as loading controls for cytoplasmic and nuclear fractions respectively, *Cyto* Cytoplasm, *Nuc* Nuclear. Representative data from four independent experiments is shown. B) Quantitation for ratio of Ac-PTEN/PTEN, *$p < 0.05$ indicates significance of nuclear ratio of Ac-PTEN/PTEN versus cytoplasmic determined by paired Student's *T* test. Histogram prepared by calculating raw values obtained using Image J software (NIH). Error bars indicate means ± SEM.

had highly significant prognostic value [35]. This means that deacetylation of PTEN by MTA1/HDACs complex most likely occurs in the nucleus. Since by immunofluorescence and subcellular fractionation experiments we found that MTA1 and PTEN can co-localize in the nucleus, we hypothesized that they could physically interact with each other. However, reciprocal co-immunoprecipitation (Co-IP) analysis for interaction between endogenous MTA1 and PTEN revealed association between MTA1 and HDAC1 and HDAC2 but not PTEN (Fig. 5A). Ectopic overexpression of MTA1, PTEN and HDAC1 by co-transfections of myc-MTA1, HDAC1-Flag and HA-PTEN in 293T cells revealed no interaction between MTA1 and PTEN, consistent with our results in DU145 cells (Fig. 5B). In addition, co-IP experiments with ectopic overexpression of PTEN in PC3M cells that express very high endogenous MTA1 revealed no detectable interaction between MTA1 and PTEN (not even under stringent conditions when MG132, a proteasome inhibitor, was applied to block degradation of MTA1 and increase the probability of interaction, data not shown).

3.6. MTA1 interacts with acetylated PTEN in the nucleus

Since we could not detect an interaction between MTA1 and PTEN, we surmised over the possibility that MTA1/HDACs complex could have more affinity to interact with the acetylated form of PTEN and in turn deacetylate it. To prove this hypothesis, we devised two experiments. First, we boosted acetylation by co expressing HA-PTEN

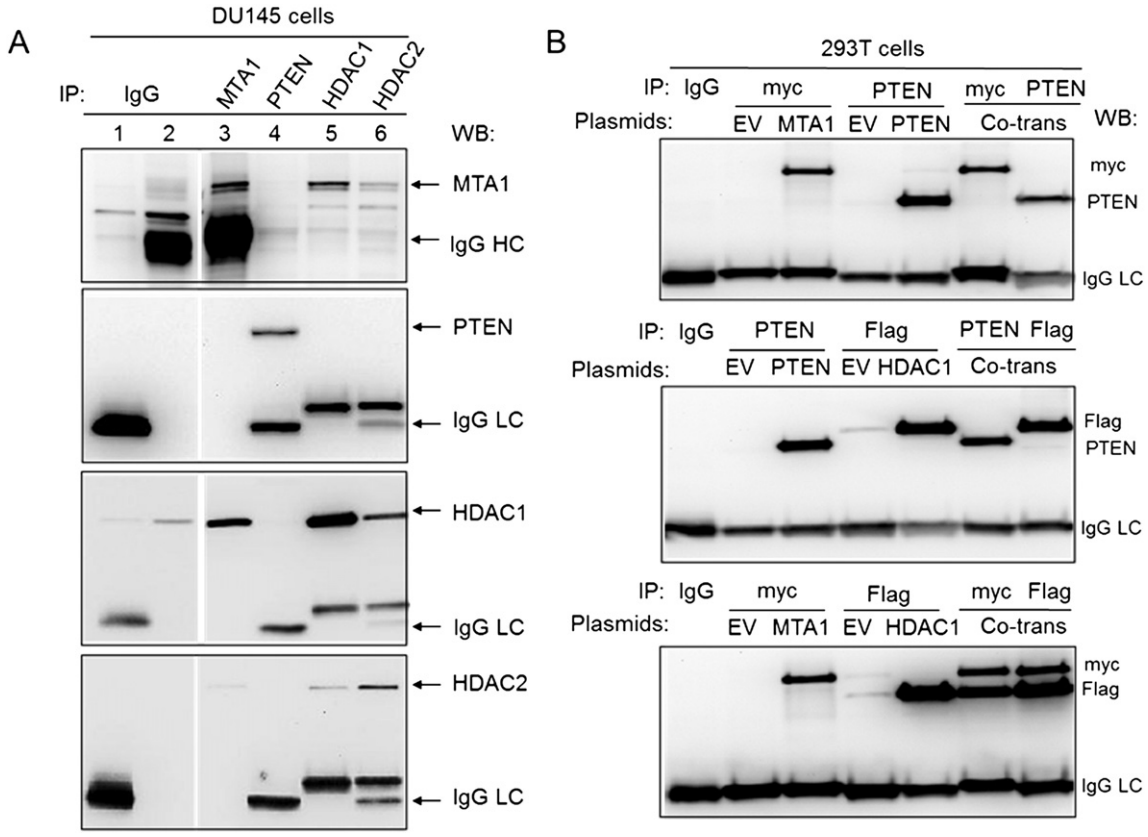

Fig. 5. MTA1 does not interact with PTEN. A) Cell lysates from DU145 cells were immunoprecipitated with antibodies to MTA1, PTEN, HDAC1 and HDAC2 or IgG controls and analyzed by western blotting with corresponding antibodies. B) 293T cells were co-transfected with myc-MTA1, HA-PTEN and HDAC1-Flag and cell lysates were immunoprecipitated with antibodies to myc, PTEN and Flag followed by western blotting. Myc and Flag antibodies were used for detection of MTA1 and HDAC1, respectively. PTEN was detected using anti-PTEN antibody.

plasmid with myc-p300 or with Flag-PCAF constructs, two known acetyltransferases reported for PTEN acetylation [36,37], in PC3M cells and performed IP-western blot to detect MTA1 and PTEN in the IP elutes. We were able to detect an interaction of PTEN with MTA1 and vice versa in cells where acetylation of PTEN was enhanced exclusively using myc-p300 construct (Fig. 6A). The acetylated status of PTEN in these cells was concomitantly confirmed in IP reactions with Ac-lysine antibody and immunoblotting for PTEN (Fig. 6A). Interestingly, co-expression of PCAF did not induce acetylation of PTEN and subsequently did not show an interaction between PTEN and MTA1. These results indicated that MTA1 interacts with the acetylated form of PTEN. Moreover, co-expression of myc-p300 and HA-PTEN leads to a further inhibition of p-Akt levels as compared to expression of HA-PTEN alone, suggesting that acetylation of PTEN leads to an increase in its lipid phosphatase activity (Fig. 6A, input).

In our second strategy, we sought to determine the acetylation site (s) in PTEN that can be involved in the interaction between PTEN and MTA1/HDAC complex. Lysine residues that have been reported to be involved in PTEN acetylation (K125/128 and K402) [36,37] were replaced by either arginine (R) or glutamine (Q) to create acetylation resistant and acetylation mimetic mutants, respectively. PC3M cells were transfected with either wild type PTEN or mutant constructs and IP-western blots were performed to detect PTEN in the lysates. Our data showed that the K125/128Q acetylation mimetic PTEN mutant construct exclusively interacted with MTA1 (Fig. 6B). There was no interaction of MTA1 either with the acetylation resistant K125/128R and K402R mutants or with the acetylation mimetic K402Q mutant. In addition, the K125/128Q acetylation mimetic showed decreased p-Akt (Ser 473) expression, confirming that acetylation of PTEN on these residues enhances its lipid phosphatase activity (Fig. 6B, input). We conclude from the above data that MTA1 interacts exclusively with PTEN which is acetylated at lysines 125 and 128 and that acetylation on these lysines renders PTEN active, as reflected by a reduction in p-Akt.

3.7. Resveratrol-induced tumor regression in orthotopic prostate cancer xenografts is mediated through MTA1 inhibition and PTEN activation

In our previous study, in which we examined MTA1-dependent tumor development and progression in PCa using orthotopic mouse model, we found that resveratrol treatment and MTA1 knockdown significantly inhibited tumor growth, progression, local invasion and spontaneous metastasis [11]. Xenografts expressing MTA1shRNA exhibited significantly reduced tumor growth at week 5 post-transplantation of cancer cells [11]. Moreover, MTA1 knockdown sensitized cells to resveratrol resulting in additional reduction of tumor progression. Using prostate tissues from the same experiment, we stained tumors for MTA1, PTEN and p-Akt and found that treatment with resveratrol simultaneously with decreasing MTA1 levels increased levels of PTEN in control tumors (Fig. 7). Further, MTA1-knockdown xenografts express much higher levels of PTEN compared to MTA1-expressing tumors, and that combination of MTA1-knockdown with resveratrol treatment keeps PTEN levels high. Unfortunately, since acetyl PTEN antibody is not commercially available we were unable to examine levels of acetylated PTEN in these tissues. However, as a readout of PTEN activity we examined p-Akt levels and found that MTA1-knockdown and resveratrol-treated tumors express lower levels of p-Akt compared to untreated controls (Fig. 7). Consistent with the potent antitumor effects, resveratrol-treated and MTA1-knockdown tumors showed reduced

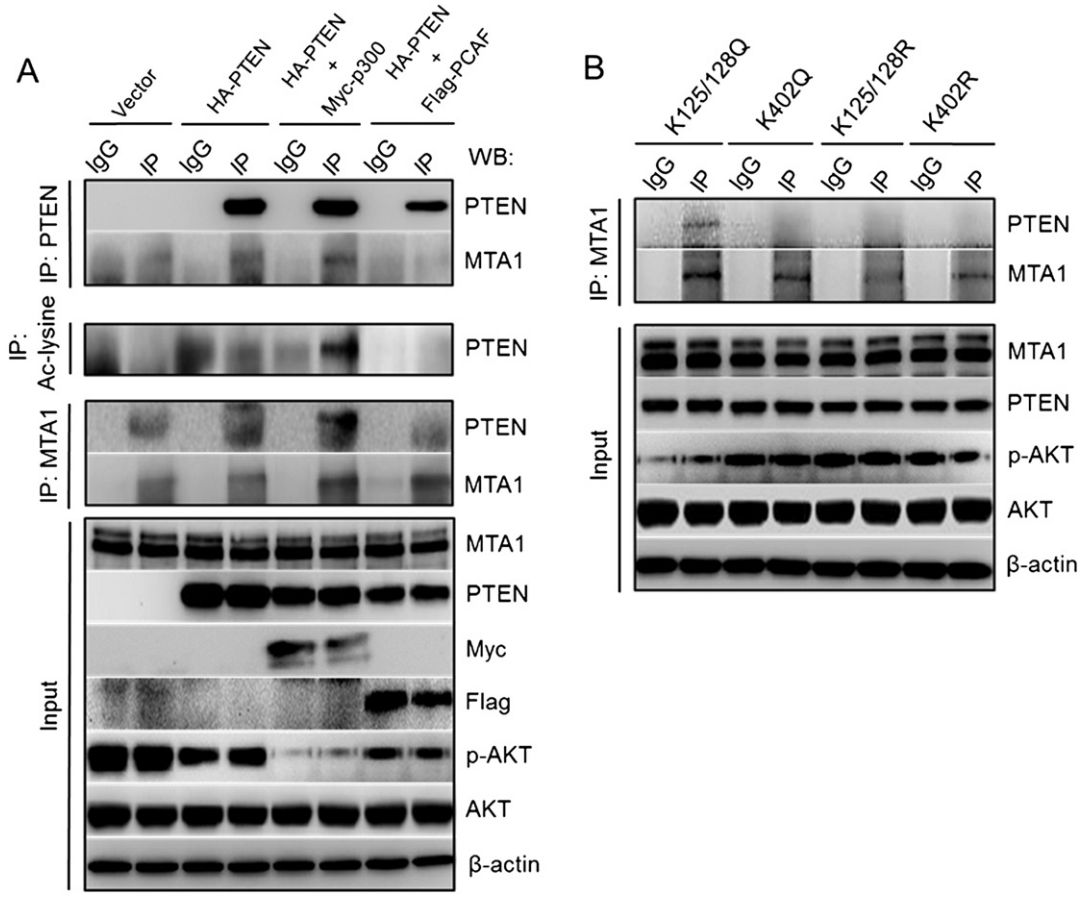

Fig. 6. MTA1 interacts with acetylated PTEN. A) PC3M cells were transfected with empty vector (HA-pSG5L), HA-PTEN, HA-PTEN and myc-p300 or HA-PTEN and Flag-PCAF plasmids. Lysates were immunoprecipitated with anti-PTEN, anti-acetyl lysine and anti-MTA1 antibodies followed by western blot. B) MTA1 interacts with PTEN when it is exclusively acetylated at lysines 125 and 128. PTEN mutant constructs K125/128R, K125/128Q, K402R and K402Q were prepared by site directed mutagenesis using the InFusion HD Cloning kit (Clontech) and confirmed by sequencing as detailed in *materials and methods* section. PC3M cells were transfected with mutant constructs, lysates were immunoprecipitated with anti-MTA1 antibody and analyzed by western blot using anti-PTEN antibody.

mitotic activity (Ki67, Fig. 7) and increased apoptosis compared to control mice as described earlier [11].

4. Discussion

Pharmacologically safe dietary compounds with epigenetic mechanisms of action are of great interest for cancer chemoprevention and therapy [38–40]. We identified resveratrol as a potent inhibitor of MTA1/HDAC1 and re-activator of p53 acetylation in prostate cancer cells [10]. Moreover, combined treatment of LNCaP prostate cancer cells with resveratrol and HDAC inhibitor SAHA resulted in a profound decrease of MTA1 and synergistic increase in acetylated p53 and apoptosis [10]. Since resveratrol had little effect on MTA1 mRNA, we sought the posttranslational regulation of MTA1. Our unpublished results imply that resveratrol causes MTA1 degradation by ubiquitin–proteasome system.

The aim of the present study was to further investigate resveratrol's epigenetic properties, particularly, the MTA1-mediated reversal of acetylation/activation of another tumor suppressor, PTEN, the loss of expression of which or inactivation is frequently found in prostate cancer [41]. We report here on resveratrol regulated rescue of PTEN expression, acetylation, and activity through MTA1 inhibition, which corresponds to its increased ability to inhibit Akt phosphorylation and cancer cell survival.

It has been shown that 30% primary and 60% metastatic tumors contain some kind of PTEN alteration [42,43]. Low PTEN expression is associated with an increased risk of lethal prostate cancer [44]. Consistently increased Akt activation (p-Akt) is associated with high Gleason grade, advanced disease and poor prognosis of prostate cancer [45,46]. Accordingly, mice with prostate-specific loss of *Pten* develop stage-defined prostate cancer with high levels of p-Akt, which progresses to invasive adenocarcinoma and occasionally lymph node metastasis [47]. However, homozygous *Pten* deletion is not common in human prostate cancer, suggesting that additional attenuation of the remaining allele caused by its posttranslational regulation could be a significant contributor for cancer development and progression. Therefore, activation of the remaining allele by reversible epigenetic therapy can restore PTEN activation.

Our original observation of inverse relationship between MTA1 and PTEN in addition to resveratrol's ability to regulate both proteins posed two major questions: First, since MTA1 is part of the deacetylating NuRD complex, does it negatively regulate PTEN by deacetylating it? Second, what role does resveratrol play in MTA1-PTEN signaling?

We and others reported an up-regulation of PTEN protein levels by resveratrol in prostate cancer [48,49]. In this study we have presented evidence that not only up-regulation but activation of PTEN occurs, at least in part, through resveratrol inhibition of components of NuRD deacetylation complex. It has already been reported that TSA promotes PTEN transcriptional activation by increasing acetylation of histones at the PTEN promoter [26] and TSA treatment induced PTEN-mediated apoptosis in oral squamous cell carcinoma [50]. In our experiments, resveratrol increased acetylation of PTEN in MTA1 knockdown cells similar to the effects of HDAC inhibitors, SAHA and TSA, indicating the involvement of MTA1/HDAC complex in PTEN deacetylation.

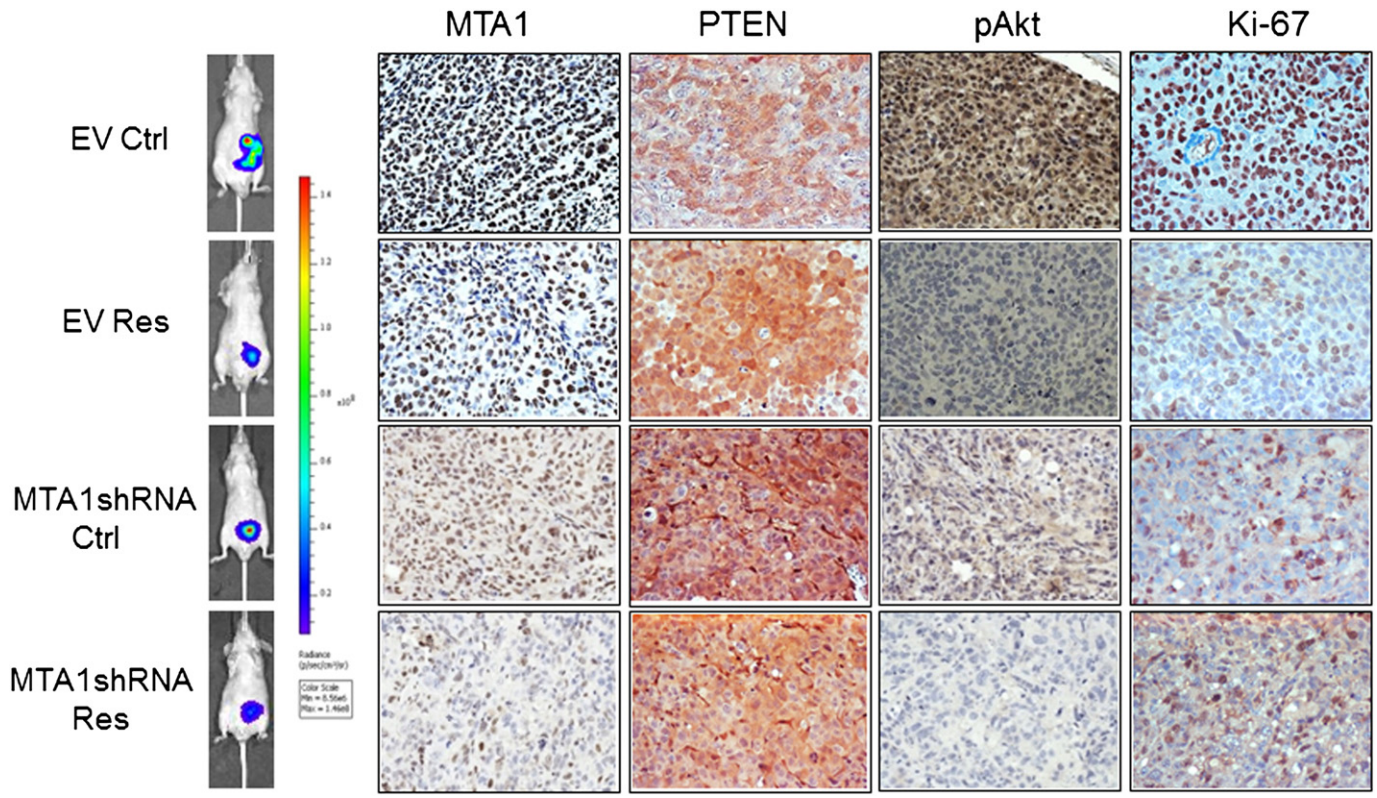

Fig. 7. Resveratrol induces tumor regression in orthotopic xenografts accompanied by decreased MTA1 and pAkt expression which is enhanced upon MTA1 knockdown and mediated via PTEN activation. Male nude mice were injected orthotopically with DU145-luciferase-tagged EV (Ctrl) or MTA1 knockdown (MTA1shRNA) cells and treated with 10% DMSO (Ctrl) or 50 mg/kg bw/day resveratrol (Res). Upon sacrifice, 4 μm sections were prepared from formalin-fixed paraffin embedded prostate tumors and stained using antibodies for MTA1, PTEN, pAkt and Ki-67. *Left*, bioluminescent images of prostate tumors at week 5 are shown. *Right*, representative immunohistochemistry images of MTA1, PTEN, pAkt and Ki67 in Ctrl EV, Res-treated EV, Ctrl MTA1shRNA and Res-treated MTA1shRNA tumors are shown, magnification (×200).

Based on the known preferential nuclear localization of MTA1 and HDACs 1 and 2 and our findings on MTA1 accumulation as a characteristic of aggressive prostate cancer [13], MTA1-mediated deacetylation of PTEN most likely occurs in the nucleus. On the other hand, "classic" lipid phosphatase activity of cytoplasmic PTEN is attributed to its regulation of Akt signaling and apoptosis, while nuclear PTEN plays other important non-canonical roles such as chromosomal stability, DNA repair, and cell cycle arrest [51–54]. Nuclear PTEN positively regulates DNA repair through up-regulation of RAD51, independent of its phosphatase activity [55]. Importantly, nuclear PTEN plays an important role in regulating p53 acetylation and activity [56] as well as p53-mediated G1 growth arrest, cell death, and reduction of reactive oxygen species production [57].

Interestingly, Salot & Gude, reported a physical association of MTA1 with PTEN in breast cancer cells and a possible post-translational regulation of PTEN by MTA1 [28]. However, our vigorous attempts to detect physical interaction between MTA1 and PTEN in prostate cancer, breast cancer and other cells did not yield any positive results.

Therefore, we envisioned a scenario in which MTA1 interacts with acetylated subpopulation of PTEN in prostate cancer cells. Indeed, we visualized an interaction of PTEN with MTA1 and vice versa only upon enhancing acetylation of PTEN, induced specifically by overexpression of p300 acetyltransferase (Fig. 6A). To our knowledge, this is the first report on PTEN acetylation by p300 acetyltransferase while an indirect transcriptional regulation of PTEN by p300 was demonstrated through acetylation of histone H4 by Pan et al. [26]. However, we did not see this effect with PCAF overexpression which counteracts the work by Okumura et al., where they showed that PCAF is responsible for PTEN acetylation at Lys^{125} and Lys^{128}, leading to inhibition of its lipid phosphatase activity [36].

There are only two reports on PTEN acetylation which have identified three acetylation sites (Lys 125 and 128 and Lys 402) but there is a disagreement on the potential role of acetylation in regulating PTEN function. Okumura *et al.* reported that the lipid phosphatase function of PTEN is inhibited when Lys^{125} and Lys^{128} are acetylated by histone acetyltransferase PCAF [36], whereas Ikenoue *et al.* showed that Lys 402 acetylation modulates PTEN interaction with PDZ domain-containing proteins [42]. In our experiments, acetylation mimetic PTEN mutant K125/128Q revealed an exclusive interaction with endogenous MTA1 in PC3M cells (Fig. 6B).

We repeatedly observed an inhibition in p-Akt levels (Ser 473) linked to an increase in PTEN acetylation upon MTA1-knockdown, treatment with TSA and resveratrol, and overexpression of p300 acetyltransferase.

In retrospect, in the study by Okumura et al., acetylation mimetic mutants K125Q and K128Q showed increased p-Akt thus suggesting that acetylation on these lysine residues renders PTEN inactive. However, intriguingly, the double acetylation resistant mutant K125/128R and the double acetylation mimetic mutant K125/128Q, both showed similar enhanced levels of p-Akt which is difficult to comprehend. We, on the other hand, believe that since Lys^{125} and Lys^{128} residues are located in the phosphatase domain of PTEN protein, acetylation of these residues in turn activates lipid phosphatase activity of PTEN protein as indicated by decreased p-Akt levels (Fig. 6B, input). We further believe that in addition to induction of lipid phosphatase activity, acetylation of PTEN on these two residues is a prerequisite for its non-canonical nuclear functions. Moreover, it is conceivable that MTA1/HDAC deacetylating complex will have more affinity towards an acetylated pool of PTEN that is active and present in the nucleus as shown by our data, which following an interaction with MTA1 gets deacetylated and subsequently loses

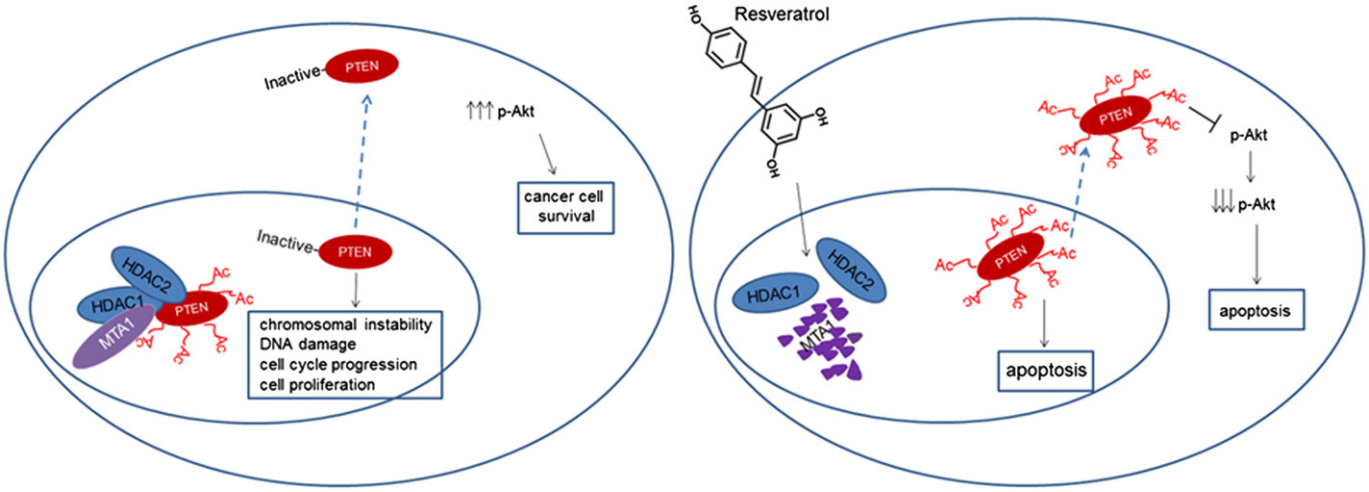

Fig. 8. Our results suggest a model of MTA1-mediated resveratrol regulation of PTEN/Akt pathway. In the absence of resveratrol, MTA1/HDAC complex is intact and localized in the nucleus where it can bind to acetylated PTEN. As a result, PTEN becomes de-acetylated, inactive and either stays in the nucleus, however without tumor suppressive functions, or diffuses to the cytoplasm without any negative consequence to Akt allowing amplification in p-Akt activity which leads to cancer cell survival. In contrast, when treated with resveratrol, owing to down-regulation of MTA1 and HDAC1/2 and as a result of MTA1/HDAC non-functionality, the acetylation of PTEN increases, which leads to enhanced lipid phosphatase activity of PTEN and its nuclear function culminating in apoptosis and inhibition of Akt survival pathway.

its activity. Taken together, we describe a novel association of MTA1 with acetylated PTEN and demonstrate acetylated PTEN as the active form that inhibits p-Akt-associated survival pathways.

Finally we substantiated the functional relevance of enhanced PTEN expression upon resveratrol treatment in orthotopic xenografts with DU145 MTA1 knockdown cells in nude mice. Immunohistochemistry analysis clearly revealed that both resveratrol treatment and MTA1 knockdown enhanced PTEN expression while concomitantly enhancing Ki-67 proliferative index (Fig. 7) and apoptosis as reported earlier [36] and simultaneously lowering p-Akt expression (Fig. 7). Unfortunately, specific acetylated PTEN antibodies are not commercially available restricting our ability to examine acetylation/deacetylation status of PTEN in prostate tissues.

Our results provide a plausible mechanism by which natural compound resveratrol acts on reversing pathological epigenetic changes mediated by co-repressor MTA1/HDAC complex in prostate cancer (Fig. 8). In aggressive prostate cancer, when MTA1 and HDAC1 and 2 are overexpressed and co-localized in the nucleus forming active suppressor unit and causing deacetylation of PTEN protein, tumor-suppressive functions of nuclear PTEN are inhibited. Deacetylated enzymatically inactive PTEN can also translocate to the cytoplasm however without negative consequences on Akt signaling, allowing accumulation of p-Akt and ultimately cancer cell survival. In the presence of resveratrol, which inhibits MTA1 and HDACs and dissociates the complex, interaction between MTA1/HDAC and acetylated PTEN is interrupted leading to accumulation of "free" acetylated PTEN with tumor-suppressive functions such as chromosomal stability, DNA repair and cell cycle arrest. Acetylated PTEN also can translocate to the cytoplasm, resulting in repression of its downstream Akt signaling pathway. In less aggressive prostate tissues, the proportion of acetylated PTEN is higher in cytoplasm and p-Akt signaling is inhibited, partly, due to low and inactive presence of MTA1/HDACs which does not interfere or sequester PTEN by deacetylation. Therefore, we believe that in aggressive prostate cancer, MTA1/HDAC complex associates with nuclear acetylated subpopulation of PTEN reducing its tumor-suppressive functions, although the mechanisms that regulate accumulation of acetylated PTEN in the nucleus and its interaction with MTA1/HDACs are not yet understood. Thus, we conclude that there is an inverse relationship between MTA1 and PTEN levels and propose that under resveratrol treatment which deactivates MTA1/HDACs complex, PTEN regains its activity, possibly by reversed acetylation, which leads to apoptosis & activation of p53 in the nucleus and inhibition of PI3K/Akt survival pathway.

5. Conclusions

We report herein that resveratrol, a dietary compound found in grapes and wine, exerts its anticancer activity in prostate cancer, at least in part, through epigenetic mechanisms, including posttranslational modification and reactivation of PTEN tumor suppressor. Our data indicate that PTEN is inactivated in prostate cancer by a novel negative regulator, MTA1/HDACs: the co-repressor complex deacetylates and inactivates PTEN resulting in inhibition of its nuclear tumor suppressive functions, such as apoptosis, cell cycle arrest, and p53 activation and its cytoplasmic lipid phosphatase function such as down-regulation of p-Akt. We discovered that resveratrol by targeting the MTA1/HDAC complex is able to reverse this negative epigenetic effect and reactivate tumor suppressor PTEN. Together, these findings underscore the importance of dietary compounds with epigenetic capacity and provide valuable awareness for future development of a combinatorial strategy for prostate cancer chemoprevention and treatment involving resveratrol and other MTA1/HDAC inhibitors.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamcr.2014.11.004.

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References

[1] B.B. Aggarwal, A. Bhardwaj, R.S. Aggarwal, N.P. Seeram, S. Shishodia, Y. Takada, Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies, Anticancer Res. 24 (2004) 2783–2840.

[2] M. Jang, L. Cai, G.O. Udeani, K.V. Slowing, C.F. Thomas, C.W. Beecher, H.H. Fong, N.R. Farnsworth, A.D. Kinghorn, R.G. Mehta, R.C. Moon, J.M. Pezzuto, Cancer chemopreventive activity of resveratrol, a natural product derived from grapes, Science 275 (1997) 218–220.

[3] N. Kuwajerwala, E. Cifuentes, S. Gautam, M. Menon, E.R. Barrack, G.P. Reddy, Resveratrol induces prostate cancer cell entry into s phase and inhibits DNA synthesis, Cancer Res. 62 (2002) 2488–2492.

[4] E. Pozo-Guisado, M.J. Lorenzo-Benayas, P.M. Fernandez-Salguero, Resveratrol modulates the phosphoinositide 3-kinase pathway through an estrogen receptor alpha-dependent mechanism: relevance in cell proliferation, Int. J. Cancer 109 (2004) 167–173.

[5] E. Brakenhielm, R. Cao, Y. Cao, Suppression of angiogenesis, tumor growth, and wound healing by resveratrol, a natural compound in red wine and grapes, FASEB J. 15 (2001) 1798–1800.

[6] S.H. Tseng, S.M. Lin, J.C. Chen, Y.H. Su, H.Y. Huang, C.K. Chen, P.Y. Lin, Y. Chen, Resveratrol suppresses the angiogenesis and tumor growth of gliomas in rats, Clin. Cancer Res. 10 (2004) 2190–2202.

[7] Y. Kimura, H. Okuda, Resveratrol isolated from *Polygonum cuspidatum* root prevents tumor growth and metastasis to lung and tumor-induced neovascularization in Lewis lung carcinoma-bearing mice, J. Nutr. 131 (2001) 1844–1849.

[8] Y. Toh, S.D. Pencil, G.L. Nicolson, A novel candidate metastasis-associated gene, mta1, differentially expressed in highly metastatic mammary adenocarcinoma cell lines. cDNA cloning, expression, and protein analyses, J. Biol. Chem. 269 (1994) 22958–22963.

[9] Y. Xue, J. Wong, G.T. Moreno, M.K. Young, J. Cote, W. Wang, NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities, Mol. Cell 2 (1998) 851–861.

[10] L. Kai, S.K. Samuel, A.S. Levenson, Resveratrol enhances p53 acetylation and apoptosis in prostate cancer by inhibiting MTA1/NuRD complex, Int. J. Cancer 126 (2010) 1538–1548.

[11] K. Li, S.J. Dias, A.M. Rimando, S. Dhar, C.S. Mizuno, A.D. Penman, J.R. Lewin, A.S. Levenson, Pterostilbene acts through metastasis-associated protein 1 to inhibit tumor growth, progression and metastasis in prostate cancer, PLoS One 8 (2013) e57542.

[12] L. Kai, J. Wang, M. Ivanovic, Y.T. Chung, W.B. Laskin, F. Schulze-Hoepfner, Y. Mirochnik, R.L. Satcher Jr., A.S. Levenson, Targeting prostate cancer angiogenesis through metastasis-associated protein 1 (MTA1), Prostate 71 (2011) 268–280.

[13] S.J. Dias, X. Zhou, M. Ivanovic, M.P. Gailey, S. Dhar, L. Zhang, Z. He, A.D. Penman, S. Vijayakumar, A.S. Levenson, Nuclear MTA1 overexpression is associated with aggressive prostate cancer, recurrence and metastasis in African Americans, Sci. Rep. 3 (2013) 2331.

[14] M.D. Hofer, R. Kuefer, S. Varambally, H. Li, J. Ma, G.I. Shapiro, J.E. Gschwend, R.E. Hautmann, M.G. Sanda, K. Giehl, A. Menke, A.M. Chinnaiyan, M.A. Rubin, The role of metastasis-associated protein 1 in prostate cancer progression, Cancer Res. 64 (2004) 825–829.

[15] M.S. Song, L. Salmena, P.P. Pandolfi, The functions and regulation of the PTEN tumour suppressor, Nat. Rev. Mol. Cell Biol. 13 (2012) 283–296.

[16] V. Stambolic, A. Suzuki, J.L. de la Pompa, G.M. Brothers, C. Mirtsos, T. Sasaki, J. Ruland, J.M. Penninger, D.P. Siderovski, T.W. Mak, Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN, Cell 95 (1998) 29–39.

[17] M.A. Lim, L. Yang, Y. Zheng, H. Wu, L.Q. Dong, F. Liu, Roles of PDK-1 and PKN in regulating cell migration and cortical actin formation of PTEN-knockout cells, Oncogene 23 (2004) 9348–9358.

[18] N. Dey, H.E. Crosswell, P. De, R. Parsons, Q. Peng, J.D. Su, D.L. Durden, The protein phosphatase activity of PTEN regulates SRC family kinases and controls glioma migration, Cancer Res. 68 (2008) 1862–1871.

[19] M.P. Myers, I. Pass, I.H. Batty, J. Van der Kaay, J.P. Stolarov, B.A. Hemmings, M.H. Wigler, C.P. Downes, N.K. Tonks, The lipid phosphatase activity of PTEN is critical for its tumor supressor function, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 13513–13518.

[20] J. Li, C. Yen, D. Liaw, K. Podsypanina, S. Bose, S.I. Wang, J. Puc, C. Miliaresis, L. Rodgers, R. McCombie, S.H. Bigner, B.C. Giovanella, M. Ittmann, B. Tycko, H. Hibshoosh, M.H. Wigler, R. Parsons, PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer, Science 275 (1997) 1943–1947.

[21] A. Carracedo, A. Alimonti, P.P. Pandolfi, PTEN level in tumor suppression: how much is too little? Cancer Res. 71 (2011) 629–633.

[22] S. Ramaswamy, N. Nakamura, F. Vazquez, D.B. Batt, S. Perera, T.M. Roberts, W.R. Sellers, Regulation of G1 progression by the PTEN tumor suppressor protein is linked to inhibition of the phosphatidylinositol 3-kinase/Akt pathway, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 2110–2115.

[23] S. Emiliani, W. Fischle, C. Van Lint, Y. Al-Abed, E. Verdin, Characterization of a human RPD3 ortholog, HDAC3, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 2795–2800.

[24] P.R. Molli, R.R. Singh, S.W. Lee, R. Kumar, MTA1-mediated transcriptional repression of BRCA1 tumor suppressor gene, Oncogene 27 (2008) 1971–1980.

[25] A. Mazumdar, R.A. Wang, S.K. Mishra, L. Adam, R. Bagheri-Yarmand, M. Mandal, R.K. Vadlamudi, R. Kumar, Transcriptional repression of oestrogen receptor by metastasis-associated protein 1 corepressor, Nat. Cell Biol. 3 (2001) 30–37.

[26] L. Pan, J. Lu, X. Wang, L. Han, Y. Zhang, S. Han, B. Huang, Histone deacetylase inhibitor trichostatin a potentiates doxorubicin-induced apoptosis by up-regulating PTEN expression, Cancer 109 (2007) 1676–1688.

[27] S.D. Reddy, S.B. Pakala, P.R. Molli, N. Sahni, N.K. Karanam, P. Mudvari, R. Kumar, Metastasis-associated protein 1/histone deacetylase 4-nucleosome remodeling and deacetylase complex regulates phosphatase and tensin homolog gene expression and function, J. Biol. Chem. 287 (2012) 27843–27850.

[28] S. Salot, R.P. Gude, MTA1 aids the Akt pathway by inhibiting expression of a key regulator, PTEN, J. Cancer Sci. Ther. 2 (2010) 114–119.

[29] G.L. Nicolson, A. Nawa, Y. Toh, S. Taniguchi, K. Nishimori, A. Moustafa, Tumor metastasis-associated human MTA1 gene and its MTA1 protein product: role in epithelial cancer cell invasion, proliferation and nuclear regulation, Clin. Exp. Metastasis 20 (2003) 19–24.

[30] W.S. Moon, K. Chang, A.S. Tarnawski, Overexpression of metastatic tumor antigen 1 in hepatocellular carcinoma: Relationship to vascular invasion and estrogen receptor-alpha, Hum. Pathol. 35 (2004) 424–429.

[31] M.D. Martin, S.G. Hilsenbeck, S.K. Mohsin, T.A. Hopp, G.M. Clark, C.K. Osborne, D.C. Allred, P. O'Connell, Breast tumors that overexpress nuclear metastasis-associated 1 (MTA1) protein have high recurrence risks but enhanced responses to systemic therapies, Breast Cancer Res. Treat. 95 (2006) 7–12.

[32] X. Zhu, Y. Guo, X. Li, Y. Ding, L. Chen, Metastasis-associated protein 1 nuclear expression is associated with tumor progression and clinical outcome in patients with non-small cell lung cancer, J. Thoracic Oncol. 5 (2010) 1159–1166.

[33] S.H. Li, H. Tian, W.M. Yue, L. Li, C. Gao, W.J. Li, W.S. Hu, B. Hao, Metastasis-associated protein 1 nuclear expression is closely associated with tumor progression and angiogenesis in patients with esophageal squamous cell cancer, World J. Surg. 36 (2012) 623–631.

[34] K. Halkidou, L. Gaughan, S. Cook, H.Y. Leung, D.E. Neal, C.N. Robson, Upregulation and nuclear recruitment of HDAC1 in hormone refractory prostate cancer, Prostate 59 (2004) 177–189.

[35] W. Weichert, A. Roske, V. Gekeler, T. Beckers, C. Stephan, K. Jung, F.R. Fritzsche, S. Niesporek, C. Denkert, M. Dietel, G. Kristiansen, Histone deacetylases 1, 2 and 3 are highly expressed in prostate cancer and HDAC2 expression is associated with shorter PSA relapse time after radical prostatectomy, Br. J. Cancer 98 (2008) 604–610.

[36] K. Okumura, M. Mendoza, R.M. Bachoo, R.A. DePinho, W.K. Cavenee, F.B. Furnari, PCAF modulates PTEN activity, J. Biol. Chem. 281 (2006) 26562–26568.

[37] T. Ikenoue, K. Inoki, B. Zhao, K.L. Guan, PTEN acetylation modulates its interaction with PDZ domain, Cancer Res. 68 (2008) 6908–6912.

[38] T.M. Hardy, T.O. Tollefsbol, Epigenetic diet: impact on the epigenome and cancer, Epigenomics 3 (2011) 503–518.

[39] J. Chen, X. Xu, Diet, epigenetic, and cancer prevention, Adv. Genet. 71 (2010) 237–255.

[40] A. Link, F. Balaguer, A. Goel, Cancer chemoprevention by dietary polyphenols: promising role for epigenetics, Biochem. Pharmacol. 80 (2010) 1771–1792.

[41] M.M. Shen, C. Abate-Shen, Molecular genetics of prostate cancer: new prospects for old challenges, Genes Dev. 24 (2010) 1967–2000.

[42] Y.E. Whang, X. Wu, H. Suzuki, R.E. Reiter, C. Tran, R.L. Vessella, J.W. Said, W.B. Isaacs, C.L. Sawyers, Inactivation of the tumor suppressor PTEN/MMAC1 in advanced human prostate cancer through loss of expression, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 5246–5250.

[43] I.C. Gray, L.M. Stewart, S.M. Phillips, J.A. Hamilton, N.E. Gray, G.J. Watson, N.K. Spurr, D. Snary, Mutation and expression analysis of the putative prostate tumour-suppressor gene PTEN, Br. J. Cancer 78 (1998) 1296–1300.

[44] K. Zu, N.E. Martin, M. Fiorentino, R. Flavin, R.T. Lis, J.A. Sinnott, S. Finn, K.L. Penney, J. Ma, L. Fazli, M.E. Gleave, T.A. Bismar, M.J. Stampfer, M.N. Pollak, M. Loda, L.A. Mucci, E. Giovannucci, Protein expression of PTEN, insulin-like growth factor I receptor (IGF-IR), and lethal prostate cancer: a prospective study, Cancer Epidemiol. Biomark. Prev. 22 (2013) 1984–1993.

[45] G.E. Ayala, H. Dai, M. Ittmann, R. Li, M. Powell, A. Frolov, T.M. Wheeler, T.C. Thompson, D. Rowley, Growth and survival mechanisms associated with perineural invasion in prostate cancer, Cancer Res. 64 (2004) 6082–6090.

[46] J.A. Engelman, Targeting PI3K signalling in cancer: opportunities, challenges and limitations, Nat. Rev. Cancer 9 (2009) 550–562.

[47] S. Wang, J. Gao, Q. Lei, N. Rozengurt, C. Pritchard, J. Jiao, G.V. Thomas, G. Li, P. Roy-Burman, P.S. Nelson, X. Liu, H. Wu, Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer, Cancer Cell 4 (2003) 209–221.

[48] Y. Wang, T. Romigh, X. He, M.S. Orloff, R.H. Silverman, W.D. Heston, C. Eng, Resveratrol regulates the PTEN/AKT pathway through androgen receptor-dependent and -independent mechanisms in prostate cancer cell lines, Hum. Mol. Genet. 19 (2010) 4319–4329.

[49] S. Dhar, C. Hicks, A.S. Levenson, Resveratrol and prostate cancer: promising role for microRNAs, Mol. Nutr. Food Res. 55 (2011) 1219–1229.

[50] Y.H. Gan, S. Zhang, PTEN/AKT pathway involved in histone deacetylases inhibitor induced cell growth inhibition and apoptosis of oral squamous cell carcinoma cells, Oral Oncol. 45 (2009) e150–e154.

[51] S.M. Planchon, K.A. Waite, C. Eng, The nuclear affairs of PTEN, J. Cell Sci. 121 (2008) 249–253.

[52] L. Salmena, A. Carracedo, P.P. Pandolfi, Tenets of PTEN tumor suppression, Cell 133 (2008) 403–414.

[53] A. Gericke, M. Munson, A.H. Ross, Regulation of the PTEN phosphatase, Gene 374 (2006) 1–9.

[54] J.H. Chung, C. Eng, Nuclear-cytoplasmic partitioning of phosphatase and tensin homologue deleted on chromosome 10 (PTEN) differentially regulates the cell cycle and apoptosis, Cancer Res. 65 (2005) 8096–8100.

[55] W.H. Shen, A.S. Balajee, J. Wang, H. Wu, C. Eng, P.P. Pandolfi, Y. Yin, Essential role for nuclear PTEN in maintaining chromosomal integrity, Cell 128 (2007) 157–170.

[56] A.G. Li, L.G. Piluso, X. Cai, G. Wei, W.R. Sellers, X. Liu, Mechanistic insights into maintenance of high p53 acetylation by PTEN, Mol. Cell 23 (2006) 575–587.

[57] C.J. Chang, D.J. Mulholland, B. Valamehr, S. Mosessian, W.R. Sellers, H. Wu, PTEN nuclear localization is regulated by oxidative stress and mediates p53-dependent tumor suppression, Mol. Cell. Biol. 28 (2008) 3281–3289.