Metastasis-associated Protein 1/Histone Deacetylase 4-Nucleosome Remodeling and Deacetylase Complex Regulates Phosphatase and Tensin Homolog Gene Expression and Function*[^5]

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**Background:** MTA1 is overexpressed in several advanced cancers, but its role in cell survival signaling is yet to be elucidated.

**Results:** MTA1 transcriptionally represses the expression of PTEN and, consequently, PTEN-dependent signaling and functions.

**Conclusion:** PTEN is a target of the MTA1/HDAC4-containing NuRD complex.

**Significance:** This study provides a novel mechanistic insight into the regulation of PTEN by MTA1.

Chromatin structure is an integral part of the mechanism by which gene transcription is controlled in eukaryotic cells. Nucleosomes are compact chromatin structures that undergo remodeling by ATP-dependent factors. Histones undergo covalent modifications, such as acetylation and deacetylation, that correlate with gene activation or gene silencing. MTA1, a component of the nucleosome remodeling and deacetylating (NuRD) complex, plays a central role in chromatin remodeling and transcriptional regulation of genes (1). MTA1 is overexpressed in a variety of human cancers, including breast, ovarian, pulmonary, gastrointestinal, and colorectal cancer (2,3). MTA1 was initially identified in a highly metastatic rat cancer cell line (4). Although a number of studies have indicated a role for MTA1 in motility and metastasis, its role in cell survival remains unknown.

Accumulating data indicate a role for PTEN[^3], a tumor suppressor gene, in the regulation of PI3K by dephosphorylating the lipid signaling intermediate phosphatidylinositol-3,4,5-triphosphate and consequently resulting functions (5). PTEN antagonizes the activity of PI3K by dephosphorylating phosphatidylinositol-3,4,5-triphosphate, leading to activation of AKT, a major substrate with a significant role in cell survival, proliferation, and invasion and angiogenesis through phosphorylation of a myriad of its cellular substrates (6,7). Recent studies indicate that PTEN can physically interact with PI3K subunits p85 and p110 (8–10) and was shown to dephosphorylate p85β (11). PTEN inactivation also leads to stimulation of MAPK and activation of Mammalian target of rapamycin (mTOR) kinase complex 1 (mTORC1) (12,13). PTEN is expressed in normal cells but is often deleted or mutated in many human cancers (14,15). In general, cellular levels of PTEN protein inversely correlate with the incidence of invasive cancer (16). Mutation with subsequent loss of function of PTEN is the most common mechanism. Also, methylation of the PTEN promoter is a common occurrence in a variety of cancers such as thyroid, melanoma, lung, and glioblastoma (17–19).

Over the years, both positive and negative regulators of PTEN have been identified (20). Positive regulators of PTEN include the early growth regulated transcription factor 1 (EGR1), peroxisome proliferator activated receptor γ (PPARγ), p53, and Human sprouty homolog 2 (SPRY2) (20). Negative regulators include mitogen-activated protein kinase kinase 4 (MKK4) that inhibits PTEN transcription by activating NF-κB.
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a transcriptional suppressor of PTEN (21). The transforming growth factor β and the transcription factor c-Jun also decrease PTEN transcription (22). PTEN levels in certain cell types are also modulated by changes in protein stability through interactions with proteins such as MAGI-2 (23). In addition, PTEN also undergoes posttranslational modifications such as phosphorylation, acetylation, and oxidation that regulate its subcellular localization (24). Although an inverse correlation between the levels of expression of MTA1 and PTEN was observed in breast cancer cell lines (25) and in publicly available datasets (this study), the nature of the mechanistic relationship between these two molecules remains unknown.

Here, an attempt is made to delineate the role of MTA1 in the regulation of PTEN expression and function. We found that MTA1 transcriptionally represses the expression of PTEN in association with HDAC4, a class II HDAC, and further identified a mechanistic link between two molecules widely implicated in human cancer.

EXPERIMENTAL PROCEDURES

Cell Culture and Reagents—MCF-7, MDA-MB231 (breast cancer) and HeLa (cervical cancer) cells were purchased from the ATCC. Mouse mammary epithelial HC11 cells were generously provided by Dr. Dan Medina (Baylor College of Medicine, Houston, TX). MCF7, MDA-MB231, HeLa, and mouse embryonic fibroblasts (MEFs) were cultured in Dulbecco’s modified Eagle’s medium-F12 (1:1) supplemented with 10% FCS. HC11 cells were cultured in RPMI 1640 medium (Invitrogen) with 10% (v/v) FCS, L-glutamine, 10 ng/ml epidermal growth factor, and 5 μg/ml insulin. Mouse EGF, insulin, and other chemicals were purchased from Sigma. Antibodies against the T7 epitope were purchased from Novagen (Milwaukee, WI). Antibody to vinculin and FLAG were purchased from Sigma. Antibody to myc was purchased from Neomarkers (Fremont CA). Antibodies to PTEN and HDAC4 were purchased from Cell Signaling Technology (Danvers, MA). Antibody to HDAC2 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody to MTA1 was purchased from Bethyl (Montgomery, TX). Alexa Fluor 488 anti-rabbit antibody, Alexa Fluor 546 anti-mouse antibody, and DAPI were purchased from Molecular Probes. Anti-mouse and anti-rabbit horseradish peroxidase-tagged antibodies were purchased from Amersham Biosciences (Piscataway, NJ). The stable clones of MCF-7 and HC11 cells expressing MTA1 or pcDNA were generated as mentioned elsewhere (26).

siRNA and Transient Transfection of Cells—siRNA against human MTA1 was purchased from Dharmaco (Lafayette, CA). Cells were cultured for 24 h and thereafter transfected with control siRNA, MTA1 siRNA using 100 nmol/liter siRNA, and 4 μl oligofectamine in 6-well plates according to the suggested protocol of the manufacturer. 48 h after transfection, the MTA1 knockdown status of transfected cells was evaluated by Western blot analysis. For transient transfection, MCF-7, HC11, or MEF cells were grown to ~50% confluence and then transfected with different plasmids as stated in the text using the FuGENE 6 transfection reagent (Roche Applied Science, Indianapolis, IN).

Luciferase Reporter Assay—Cells were seeded in 6-well culture plates. After 24 h, they were transfected with promoter luciferase constructs using FuGENE 6 transfection reagent (Roche Applied Science). Luciferase activity was measured after 48 h using a luciferase assay kit (Promega, Madison, WI) following the protocol of the manufacturer. 50 ng of MTA1 or YY1 or HDAC4 or pcDNA plasmid DNA was used along with 100 ng of PTEN-luc construct to transfect the cells. Results were presented as relative promoter luciferase activity units.

qPCR Analysis—Total RNA was extracted from cells using TRIzol reagent (Invitrogen), and cDNA synthesis was carried out with Superscript III RT (Invitrogen) using 2 μg of total RNA and oligo(dT). qPCR analysis was performed using gene-specific primers as described elsewhere (26). The human PTEN primers were used was 5’-TGGGCTGAGCAGCTTCTATT (forward) and 5’-GAGATCAGCCTTCTTTTGT (reverse). The human β-actin primer set was used was 5’-ATGGAATGATGATATCGGCTCTTGCGCGTGGACGAT (forward) and 5’-CTAGAAGCATTGCTCGGTGGACGAT (reverse).

ChIP—ChIP assay was performed according to the protocol described previously (27). Briefly, cells were cross-linked with formaldehyde (1% final concentration) and sonicated on ice to fragment the chromatin to an average length of 500 bp to 1 kb. The lysates were diluted using chromatin dilution buffer. MTA1, HDAC4, or mouse IgG antibodies were used to immunoprecipitate the respective antigens at 4 °C overnight. Protein–A-Sepharose beads saturated with bovine serum albumin was added to the lysate to isolate the antibody-bound complexes. The beads were washed to remove nonspecific binding, and the antibody-bound chromatin was eluted. The protein–DNA cross-link is reversed by heating at 65 °C for 6 h. During this step, RNase was added to digest the RNA contaminants. Samples were treated with proteinase K for 1 h at 45 °C to digest the proteins pulled down by immunoprecipitation. Finally, the DNA was extracted using the phenol chloroform method. For the double ChIP experiment, an initial ChIP assay was done with MTA1 antibody to immunoprecipitate MTA1-bound chromatin, which was eluted from the protein A-Sepharose beads and subjected to a second ChIP assay with HDAC4 antibody. With the DNA eluted at the end of ChIP analysis, PCR was performed using four sets of primers specific for each region (R) of human PTEN promoter. The region 1 primer set was 5’-TACCTTGTGTGTAGATCCTTGC-3’ (forward) and 5’-CTATCTGTGTTGCAGAACCAGTC-3’ (reverse). The region 2 primer set was 5’-ATGTAAGCAAAGGAGTAGTACGTC-3’ (forward) and 5’-GGAATCGTTACACAGACAGCTC-3’ (reverse). The region 3 primer set was 5’-TCGGCTGAGCCTTCATTT-3’ (forward) and 5’-GCATTCGCTCTTTTTC-3’ (reverse). The region 4 primer set was 5’-AATTCTAGGGGTAGGGCAACAG-3’ (forward) and 5’-GACTTGGTACGGAAGCT-3’ (reverse).

EMSA—Nuclear extracts were prepared using a Nonidet P-40 lysis method. EMSA for PTEN promoter binding was performed using the annealed [γ-32P]ATP end-labeled PCR product or oligonucleotides in a 20-μl reaction mixture for 15 min at 20 °C. Samples were run on a non-denaturing 5% polyacrylamide gel and imaged by autoradiography. Specific competi-
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FIGURE 1. PTEN is a target gene of MTA1. A, recruitment of MTA1 on to PTEN chromatin in MCF-7 cells. MCF-7 cells were treated with 1% formaldehyde to cross-link the histones to DNA and were subsequently lysed by sonication and immunoprecipitated (IP) either by anti-MTA1 antibody or IgG antibody. The immunoprecipitates were collected by adding beads. Washing was performed to elute the DNA from the beads, and purified DNA was subjected to PCR. R1-R4 indicate regions of the PTEN promoter. B, recruitment of MTA1 onto the PTEN promoter in MCF-7 cells stably expressing pcDNA and MTA1. C, qPCR ChIP analysis representing the recruitment of MTA1 onto the PTEN promoter in MCF-7 cells stably expressing pcDNA and MTA1. D, PTEN promoter luc activity in MCF-7 cells stably overexpressing pcDNA and MTA1. E, PTEN promoter luc activity in HC11 cells stably overexpressing pcDNA and MTA1. F, PTEN promoter luc activity in MTA1+/+ and MTA1−/− MEFs.

FIGURE 2. MTA1 represses PTEN expression. A, Western blot analysis of PTEN protein expression from the cell lysates isolated from MCF-7 cells stably expressing pcDNA and MTA1. B, Western blot analysis of PTEN protein expression from the cell lysates isolated from HC11 cells stably expressing pcDNA and MTA1. C, Western blot analysis of PTEN protein expression from the cell lysates isolated from MTA1 knockout mouse embryonic fibroblasts (MTA1−/−) and MTA1+/− MEFs. D, Western blot analysis for PTEN protein expression from the cell lysates isolated from MCF-7 and HC11 cells after MTA1 knockdown using MTA1-specific siRNA. E, qPCR analysis of PTEN mRNA expression in MCF-7 cells stably over expressing pcDNA and MTA1 and in MTA1−/− and MTA1+/− MEFs. F, qPCR analysis was carried out for the PTEN and β-actin mRNA levels from the RNA isolated from the above mentioned cell lines, and results were presented in terms of fold change after normalizing with β-actin mRNA levels. 2 μg of total RNA from each cell lines has been used for cDNA synthesis. Each value represents the mean of ± S.D. of three independent experiments. G, PTEN promoter luciferase activity in HC11 cells after cotransfection with pcDNA and MTA2.

| RESULTS AND DISCUSSION |

PTEN Is a Target of MTA1—ChIP-on-ChIP analysis revealed MTA1 recruitment onto the region 89611175 to 89,613,175 (Chr10) of PTEN chromatin. This led us to investigate a potential relationship between these two molecules. To confirm that PTEN is bona fide target of MTA1, we performed a ChIP-based PTEN promoter walk with an anti-MTA1 antibody in MCF-7 cells. We found the recruitment of MTA1 to one specific region, region 3 (-700 to −451), of the PTEN promoter (Fig. 1A). We also observed an increased baseline recruitment of MTA1 on to PTEN promoter in MCF-7 cells stably expressing MTA1 (Fig. 1, B and C). To understand the basis of MTA1 regulation of PTEN, we next carried out PTEN promoter luciferase activity under conditions of MTA1 overexpression in MCF-7 and HC11 cells. We found decreased PTEN promoter activity upon MTA1 overexpression (Fig. 1, D and E). We noticed consistent increased in PTEN promoter luciferase activity in MTA1−/− MEFs as compared with the wild-type MEFs (Fig. 1F). These observations suggest that MTA1 regulates PTEN transcription and that PTEN is a novel target gene of MTA1.

MTA1 Expression Inversely Correlates with PTEN Expression—To demonstrate the significance of MTA1 regulation of PTEN expression, we next analyzed the expression levels of MTA1 and PTEN in MCF-7 and HC11 cells that stably expressed MTA1. We found that MTA1 overexpression downregulated the levels of PTEN protein (Fig. 2, A and B). Further, we found that genetic deletion of MTA1 in the MTA1 knockout mouse embryonic fibroblasts (MTA1−/− MEFS) increased the expression of PTEN protein (Fig. 2C). We also knocked down the endogenous MTA1 in the MCF-7 and HC11 cells using MTA1-specific siRNA to verify that this inverse correlation is not cell type-specific. As shown in Fig. 2D, MTA1 depletion resulted in increased levels of PTEN protein as compared with control cells. These results strongly suggest that MTA1 negatively regulates PTEN expression.

itor to the incubation mixture and supershift EMSAs were performed by adding the antibodies indicated above.

Immunoblot Analysis—Cells were washed three times with PBS and incubated in lysis buffer (50 mmol/liter Tris-HCl (pH 7.5), 120 mmol/liter NaCl, 1% Triton X-100, 1× protease inhibitor mixture (Roche)) and were subsequently placed in 1 mmol/liter sodium vanadate on ice for 30 min. Cell lysates containing equal amounts of protein were resolved by 8% SDS-PAGE, transferred to nitrocellulose membranes, probed with appropriate antibodies, and detected by enhanced chemiluminescence.

Microarray Data Analysis—Publicly available cancer datasets GSE26304 (breast cancer) and GSE8511 (prostate cancer) were analyzed as described in supplemental methods.
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We next examined the effect of MTA1 on the status of PTEN mRNA. We found that MTA1 overexpression in the MCF-7 cells and MTA1−/− MEFs results in decreased expression of PTEN mRNA (Fig. 2E). We consistently noticed a decreased expression of PTEN mRNA in breast tissue samples isolated from MTA1−/− mice as compared with MTA1+/− mice (Fig. 2F), suggesting that MTA1 regulates PTEN expression, at least in part, at the transcriptional level. Interestingly, we found that PTEN repression is only manifested by MTA1 and not by MTA2 (Fig. 2G). This could be due to differential association of MTA1 and MTA2 with HDACs in regulating target gene transcription and/or the existence of biochemically distinct NuRD complexes (28).

Further, we evaluated the status of MTA1 and PTEN in various cancers from publicly available microarray datasets containing various primary tumor samples. We analyzed the levels of MTA1 and PTEN transcripts (supplemental methods). We found a significant negative correlation between the levels of expression of MTA1 and PTEN in breast cancer data sets (Fig. 3A) and in prostate cancer (B). Together, these results suggest that MTA1 negatively regulates the expression of PTEN, implying a potential role for the MTA1-PTEN pathway in regulating the signaling components that are downstream of PTEN.

Recruitment of the MTA1-HDAC4 Complex on the PTEN Promoter Inhibits PTEN Expression—To understand the molecular mechanism underlying the noted MTA1 repression of PTEN transcription, we carried out a ChIP analysis with HDAC2, a component of the MTA1 corepressor complex (29). However, we failed to detect any recruitment of HDAC2 onto the PTEN promoter (Fig. 4A). From the recent studies, it is evident that class II HDACs are involved in the regulation of PTEN expression (30, 31). Accordingly, we subsequently performed ChIP analysis using an antibody against class II HDAC4. We found the recruitment of HDAC4 onto PTEN chromatin (Fig. 4A). To validate the involvement of the MTA1-HDAC4 complex in PTEN regulation, we carried out a double ChIP analysis using the MCF-7 cells that stably expressed MTA1. We found an easily detectable recruitment of the MTA1-HDAC4 complex onto the PTEN promoter (Fig. 4B) in the MCF-7/MTA1 cells as compared with control MCF-7/pcDNA cells (B). These findings have raised the possibility of interaction between MTA1 and HDAC4 proteins.

To demonstrate the suspected binding of MTA1 and HDAC4 on to the PTEN promoter, we next performed an EMSA analysis using an end-labeled PCR product encompassing the region −700 to −451 of the PTEN promoter using nuclear extracts prepared from the MCF-7 cells. We found distinct DNA-protein complexes that were further super shifted by antibodies to MTA1 (Fig. 4C and lane 3) or HDAC4 (D and lane 5) but not IgG. These results suggest that the MTA1-HDAC4 complex participates in the regulation of PTEN expression.

To further validate the role of HDAC4 in PTEN transcriptional regulation, we next assessed PTEN promoter activity in the MTA1+/+ and MTA1−/− MEFs in the presence of HDAC4. We found that HDAC4 represses PTEN transcription in MTA1+/+ MEFs. HDAC4 alone did not repress PTEN transcription in MTA1−/− MEFs but was able to do so only in the presence of ectopically expressed MTA1 (Fig. 4E). In addition,
we observed increased repressive activity by HDAC4 on the PTEN promoter with MTA1 overexpression in MCF-7 (Fig. 4F). Besides HDAC4, no other HDACs cooperate with MTA1 to repress PTEN expression (Fig. 4G). Together, these results suggest the existence of a MTA1-HDAC4 corepressor complex in the regulation of PTEN expression.

Our laboratory, along with others, has shown that MTA1 associates with class I HDACs-HDAC1 and HDAC2 (28, 32, 33). However, MTA1 association with class II HDACs has not, as of yet, been documented. We next studied the interaction of MTA1 with class II HDACs and found that MTA1 interacts with HDAC4 but not with HDAC6 or HDAC3 in HeLa cells (Fig. 5A) or ZR-75 cells (B). We also found that HDAC4 interacts with MTA1 but not with MTA2 (Fig. 5C). Results from in vitro interaction studies indicated that the catalytic domain of HDAC4 is required for binding to MTA1 to repress PTEN transcription (Fig. 5, D and E). Together, these findings reveal an inherent role of the MTA1-HDAC4 complex in PTEN gene transcription.

The MTA1-YY1-HDAC4 Corepressor Complex Regulates PTEN Transcription—As evident in Fig. 1A, MTA1 regulates PTEN transcription by being recruited onto region 3 of the PTEN promoter. Hence, to identify the available transcriptional factors involved in suppression of PTEN, we scanned region 3 of the PTEN promoter (-451 to -700) using Allgen promo software. This analysis revealed the presence of only one consensus binding site for transcriptional factor YY1. As the YY1-HDAC4 repressor complex is involved in repressing gene transcription (34) and MTA1-HDAC4 represses PTEN expression (in this study), we next carried out single and sequential ChIP analysis with YY1 antibody on region 3 of the PTEN promoter. To our surprise, YY1 MTA1>YY1 and HDAC4>YY1 are also recruited to region 3 of the PTEN promoter (Fig. 6A). To determine whether YY1 has a mechanistic role along with MTA1 and HDAC4 in the noted transcriptional down-regulation of PTEN expression, we examined the effects of YY1 alone and in combination with MTA1 and HDAC4 on PTEN promoter activity. We found that MTA1+YY1 and YY1+HDAC4 cooperatively repress PTEN promoter activity (Fig. 6B). Collectively, these results demonstrate that, in a physiological setting, MTA1-YY1-HDAC4 may exist as a corepressor complex and repress PTEN expression by being recruited onto its promoter.

To further demonstrate a direct binding of YY1 to the PTEN promoter, we next performed EMSA analysis using oligonucleotides (wild-type and the mutant of YY1 consensus motif) in nuclear extracts of MCF-7 cells. Interestingly, anti-MTA1 and anti-HDAC4 antibodies had similar effects on binding to protein/DNA complexes, which were then significantly super-
shifted on inclusion of anti YY1 antibody (Fig. 6 C), suggesting the existence of the MTA1-HDAC4 YY1 complex and its ability to interact with the PTEN promoter. Together, these results demonstrate the involvement of MTA1-HDAC4 YY1 corepressor complex in the down-regulation of PTEN transcription.

The Role of MTA1 in the PI3K/AKT Pathway—Because of the potential role of PTEN in regulating the PI3K/AKT signaling pathway in cancer (20), we next analyzed the contribution of MTA1 repression of PTEN expression upon PI3K/AKT signaling. We found evidence of reduced activation of the PI3K/AKT pathway in MTA1−/− MEFs as compared with the levels in the MTA1+/+ MEFs, presumably because of increased PTEN expression. The status of the p85/AKT pathway was assessed by examining the phosphorylation status of p85, AKT, and FAK1 in the MTA1−/− MEFs compared with MTA1+/+ (Fig. 7A). Interestingly, the noticed defect in the PI3K/AKT signaling in MTA1−/− MEFs could be rescued with the introduction of MTA1 (Fig. 7A).

To further strengthen our findings that MTA1 affects PI3K activity by suppressing PTEN expression, we examined the effect of PTEN knockdown in MTA1−/− MEF on PI3K/AKT signaling. We found a resumed activation of the PI3K/AKT pathway after PTEN knockdown in MTA1−/− MEF because of decreased PTEN expression (Fig. 7B). We also found decreased phosphorylation of the p85 subunit of PI3K, AKT, and FAK1 in MDA-231 cells after MTA1 knockdown using MTA1-specific siRNA as compared with levels in the control siRNA-treated cells (Fig. 7C). It is clear from our data that, indeed, an observed modulation of the PTEN status does affect the status of phospho p85-PI3K in addition to AKT and FAK. This finding is clearly in contrast to the paper referenced by Sun et al. (35) but consistent with a more recent paper by He et al. (11) showing the ability of PTEN to directly dephosphorylate p85-PI3K and support the growing notion of direct regulatory interaction between the PTEN and p85 and other PI3K component proteins (8–10). Taken together, our findings suggest that MTA1 affects PI3K/AKT signaling and, thus, raise the possibility of MTA1 regulation of signaling via genomic regulation of PTEN transcription (Fig. 7D). From these findings we conclude that MTA1 overexpression results in inhibition of PTEN expression which, in turn, leads to the activation of the PI3K/AKT pathway that plays a role in cell survival.

A large body of work suggests a role for MTA1 in human cancer (2, 36, 37) as well as a critical contribution of the loss of PTEN in cell survival signaling (38–40). Earlier studies have demonstrated that MTA1 forms a corepressor complex with HDAC1/2 and is recruited to the target gene promoters, thereby repressing gene transcription (29). For example, the MTA1-HDAC2 complex was identified as a transcriptional corepressor of number of tumor suppressor genes such as BRAC1, p21WAF1, and RNF144, where the MTA1-HDAC2 complex recruits onto their promoters and down-regulates the expression of these genes, leading to accelerated tumor growth and metastasis (32, 41, 42). In this study, we identified MTA1 as a transcriptional corepressor of the tumor suppressor gene PTEN, but contrary to earlier studies, for the first time, we found that MTA1 but not MTA2 associates with class II HDAC4s and identified the first target of the MTA1-HDAC4 containing the NuRD complex, the PTEN. In addition, MTA1 also forms a coactivator complex in association with RNA polymerase II, where it is recruited to the gene promoters such as BCAS3, Pax5, and Hyaluronan-mediated motility receptor (HMMR), and thereby promotes cancer invasion and migration (43–45). Recently, Salot et al. (46) reported that the MTA1 down-regulates the PTEN protein, not mRNA, and also proposed that MTA1 regulates the AKT pathway by inhibiting PTEN in cancer cells. However, the molecular mechanism of regulation of the PTEN and AKT pathway by MTA1 remains unclear. Contrary to these findings, this study clearly demonstrates that MTA1 transcriptionally regulates PTEN by being recruited onto its promoter along with transcriptional factor YY1 and HDAC4. Also, Lu et al. (46) reported that stem cell factor SALL4 represses the transcription of PTEN by being recruited onto its promoter (regions −2629 to −2383 bp and −1706 to −1571) in association with the Mi-2/NuRD complex, importantly containing MTA1, MTA2, and HDAC2 components, and thereby promotes kidney development and leukemogenesis. In this context, this study has demonstrated for first time that MTA1 represses PTEN by recruiting HDAC4 along with the novel transcriptional factor YY1 onto the PTEN promoter region (−451 to −700 bp) in cancer cells, which is different from the regulatory region identified by Lu et al. (46).
PTEN antagonizes the PI3K pathway by functioning as a lipid phosphatase (15). In mammalian cells, localized PtdIns(3, 4, 5), P3 production, and PI3K activity control polarization and migration during chemotactic movement (47). Phosphatidylinositol-3,4,5-triphosphate also provides a docking site for regulators and effectors of the small GTPase Arf6, which regulates actin remodeling (48). AKT is the other well known effector of PI3K signaling. Although AKT is known for its paramount role in cell survival, increasing evidence suggests that it might also be involved in cell migration (49). AKT can regulate actin dynamics via phosphorylation of actin (50). Loss of PTEN in MEFs leads to accumulation of cortical actin, which contributes to increased cell migration via activation of the phosphoinositide-dependent kinase-1 (PDK-1)/AKT signaling pathway (51). PTEN controls integrin-directed migration in glioma cells in a PI3K/AKT-independent manner (52). In this context, the noted repression of PTEN and activation of the PI3K/AKT pathway under MTA1 overexpression might be one mechanism that attributes to the MTA1-induced enhanced cell survival. In conclusion, this study identifies PTEN as one of the genomic targets of MTA1, and PTEN suppression by MTA1 explains, at least in part, the mechanism of MTA1-mediated increased cell survival signaling.

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