Cellular transformation by the MSP58 oncogene is inhibited by its physical interaction with the PTEN tumor suppressor

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The PTEN (phosphatase and tensin homologue) tumor suppressor protein contains a single catalytic domain with both lipid and protein phosphatase activities. The remaining C-terminal half of the PTEN protein plays a role in its stability and is mutated in many clinical cancer samples. Here, we report that the PTEN C-terminal domain physically interacts with the forkhead-associated domain of the oncogenic MSP58 protein and that this interaction requires PTEN Thr-366. We further show that while MSP58 transforms Pten–/– mouse embryo fibroblasts (MEFs), concurrent introduction of wild-type PTEN causes a dramatic reduction in the number of MSP58-induced transformed foci. This PTEN-mediated inhibition of cellular transformation requires physical interaction as evidenced by the failure of PTEN(T366A) point mutation (residing within the MSP58 interaction domain) to suppress MSP58-driven transformation. These observations, together with the capacity of catalytically inactive PTEN mutant (G129R) to suppress MSP58 oncogenicity, support the view that the C-terminal region of PTEN directly provides a previously uncharacterized biological function in its ability to regulate cellular transformation.

PTEN (phosphatase and tensin homologue, deleted on chromosome ten) is a tumor suppressor gene that is frequently somatically deleted or mutated in a variety of human cancers including those of the brain, endometrium, prostate, and lung (1, 2). Germ-line mutations of PTEN are also the cause of Cowden’s disease, an autosomal dominant hamartoma syndrome with increased risk for the development of tumors in a variety of tissues (1, 2).

PTEN dephosphorylates the 3′ position of phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P3] and phosphatidylinositol 3,4-bisphosphate [PI(3,4)P2] and tyrosine-phosphorylated FAK and Shc. These activities inhibit cell growth and cell migration, respectively (3). The PTEN protein consists of an N-terminal phosphatase domain and a C-terminal domain, which is subdivided into C2, phosphorylation, and PDZ (PSD-95, disc-large, Zonula Occludens-1) binding domains (1). Sequencing of the PTEN gene in spontaneous tumors, and those arising in genetically predisposed individuals, has shown that ~20% of all known PTEN mutations, including a number of frameshift, nonsense, and missense mutations, target the C-terminal region (2). These data suggest that this region of the PTEN protein may have biological functions distinct from those mediated by its catalytic domain.

The C-terminal domain is phosphorylated in vivo, and this phosphorylation regulates PTEN stability and activity (4). However, the physiologically relevant function of PTEN phosphorylation, other than control of protein stability, continues to be an area of active investigation. Within the C-terminal region, the C2 domain has been shown to regulate the activity and stability of the PTEN protein by binding to the plasma membrane and contributing to the proper orientation of its phosphatase domain (5). The C2 domain can also regulate cell migration, independent of the phosphatase domain (6). However, this inhibition of cell migration can be controlled by PTEN autophosphatase activity (7). It has also been reported that the PTEN C2 domain binds p53 and regulates its transcriptional activity, independent of PTEN phosphatase activity (8). To elucidate the unique functions of the C-terminal region of PTEN, the yeast two-hybrid screen was used to identify its interacting proteins, yielding a known transforming protein, MSP58. Physical interaction with the PTEN C-terminal region functions to suppress the transformation potential of MSP58, and this inhibition does not require PTEN to be catalytically active. These findings point to a previously uncharacterized mechanism through which PTEN can regulate cellular transformation.

Methods

Cell Culture, Retrovirus Infection, and Transfection. Human embryonic kidney 293T cells and Pten–/– MEFs were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (FBS) at 37°C in 5% CO2. Cells were transfected by using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol. Pten–/– MEFs were infected with PTEN and MSP58 coding retroviruses, which were produced in 293T cells. Mammalian expression vectors for PTEN and MSP58 were constructed by PCR with Pfu Turbo DNA polymerase. Various PTEN mutants were constructed in the EGFPl and murine stem cell virus vectors by PCR with Pfu Turbo DNA polymerase.

Cell Transformation Assay. Pten–/– MEFs were infected with MSP58 and either wild-type, T336A, or G129R PTEN retroviral vectors, cells were cultured for 40 days, and the resulting foci were stained with 0.5% crystal violet in 20% ethanol and counted.

Antibodies and Immunoblotting Analysis. For whole-cell extracts, cells were washed twice in PBS and lysed in Nonidet P-40 buffer (1% Nonidet P-40/50 mM TrisHCl, pH 7.4/150 mM NaCl/1 mM EDTA), containing protease and phosphatase inhibitors. Protein samples were separated on SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. Antibodies to Flag (Sigma), GFP (Santa Cruz Biotechnology), PTEN (Santa Cruz Biotechnology), and MSP58 (9) were used for detection.

Coimmunoprecipitation. 293T cells were transfected with the appropriate combination of plasmids harvested and extracted in lysis buffer containing 50 mM TrisHCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, and 0.5% Nonidet P-40. Lysates were cleared by centrifugation.

Abbreviations: FHA, forkhead-associated; MEF, mouse embryo fibroblast. See Commentary on page 2677.

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Thr-366, Ser-370, Ser-380, Thr-382, Thr-383, and Ser-385 (4, 15). The phosphorylated form of PTEN. PTEN can be phosphorylated on the C-terminal region of PTEN, we tested whether MSP58 recognizes a consensus sequences pTxxI and the FHA domain of MSP58 binds PTEN. Because the MSP58 FHA domain binds to the C-terminal region bound MSP58, whereas the PTEN phosphatase domain alone could not (Fig. 2A). We then tested whether the FHA (forkhead-associated) or leucine zipper-like domains contained within MSP58 were required for its interaction with PTEN. The MSP58 FHA domain is necessary and sufficient for interaction with PTEN (Fig. 2B). Therefore, the C-terminal half (the C2 and phosphatase domains) of PTEN binds MSP58, and the FHA domain of MSP58 binds PTEN.

FHA domains recognize phosphothereonine residues with the consensus sequences pTxxL/L/V, pTxxD/V, and pTxxS/E (13, 14). Because the MSP58 FHA domain binds to the C-terminal domain of PTEN, we tested whether MSP58 recognizes a phosphorylated form of PTEN. PTEN can be phosphorylated on Thr-366, Ser-370, Ser-380, Thr-382, Thr-383, and Ser-385 (4, 15). We mutated these serines and threonines to alanine and assessed their interactions with MSP58 (Fig. 3A). We also mutated Thr-277 to alanine, because this site is a perfect match for an FHA domain consensus recognition sequence in the PTEN C-terminal region, although it has not been reported to be phosphorylated. A PTEN 4A mutant (S380A, T382A, T383A, and S385A) and the T277A mutant were still able to bind MSP58; however, the PTEN T366A mutant was deficient for MSP58 binding (Fig. 3B). Therefore, Thr-366 of PTEN is critical for interaction with the MSP58 FHA domain.

Because MSP58 has been shown to transform chicken embryonic fibroblast cells (12), we sought to determine whether interaction with PTEN could regulate the transformation potential of MSP58. To test this, we used Pten−/− MEFs because this allowed us to introduce PTEN or various mutant forms of it in a cellular background devoid of endogenous PTEN. Exogenous expression of MSP58 did transform Pten−/− MEFs (Fig. 4), and when such transformed foci were picked, expanded, and injected s.c. into immunodeficient mice, they formed tumors (data not shown). The coexpression of wild-type PTEN with MSP58 suppressed both the number and size of the resultant foci (Fig. 4). However, coexpression of the PTEN T366A mutant, which does not interact with MSP58, was unable to effect such suppression (Fig. 4). Interestingly, the G129R phosphatase-inactive mutant of PTEN also had suppressive effects, suggesting that PTEN’s phosphatase activity is not required for suppression of MSP58-mediated transformation.

Discussion

We report here that the C-terminal domain of the PTEN tumor suppressor protein interacts with the oncogenic MSP58/MCRS1, a 58-kDa microspherule protein (MSP58), and human microspherule protein 1 (MCRS1). MSP58/MCRS1 is a nucle-
MEFs. Wild-type PTEN can inhibit MSP58-induced transformation in normal MEFs.

Colocalization of PTEN and MSP58 in vivo of MSP58. Phosphorylation of PTEN Thr-366 has been detected in a phosphoprotein fractionation (13). We show that one PTEN action is to regulate the PI3K pathway and p53 regulation through its C2 domain (6, 7) and p53-dependent gene expression (8). We add to this evidence by showing that the phosphorylation of PTEN G129R mutant also suppresses MSP58-mediated transformation. These data are consistent with the report that v-jun-derived transformation is independent of the PI3K pathway in QEFs or CEFs (17).

Although the physiological function of MSP58 is still unclear, it might control gene expression by regulating the stability of the Daxx transcriptional repressor protein (16) and telomerase activity (9). MSP58’s ability to transform cells seems contradictory to a previous report that MSP58 and MCRS2 inhibit telomerase activity (9). However, dephosphorylation of Akt significantly reduces telomerase activity and induces apoptosis (18). Because PTEN inhibits AKT phosphorylation, it likely reduces AKT-dependent telomerase activity. PTEN may also enhance MSP58 inhibition of telomerase activity by interacting with MSP58.

PTEN is an established tumor suppressor protein that down-regulates the PI3K pathway and p53 regulation through its phosphorylation, suggesting a synergy between v-Jun activity and loss of PTEN expression in cell transformation. PTEN likely has functions other than inhibition of the PI3K pathway with its lipiddiphosphatase activity. There are several reports that the PTEN C2 domain can regulate cell migration (6, 7) and p53-dependent gene expression (8). We add to this evidence by showing that the phosphorylate defective PTEN G129R mutant also suppresses MSP58-mediated transformation. These data are consistent with the report that v-jun-derived transformation is independent of the PI3K pathway in QEFs or CEFs (17).

We show that MSP58 and PTEN interact through the FHA domain of MSP58 and the C-terminal region of PTEN. The MSP58 FHA domain is well conserved (12) and functions other than inhibition of the PI3K pathway with its lipiddiphosphatase activity. There are several reports that the PTEN C2 domain can regulate cell migration (6, 7) and p53-dependent gene expression (8). We add to this evidence by showing that the phosphorylate defective PTEN G129R mutant also suppresses MSP58-mediated transformation. These data are consistent with the report that v-jun-derived transformation is independent of the PI3K pathway in QEFs or CEFs (17).

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Expression of MSP58 can induce transformation of Pten−/− MEFs. Wild-type PTEN can inhibit MSP58-induced transformation, suggesting a synergy between v-Jun activity and loss of PTEN expression in cell transformation. PTEN likely has functions other than inhibition of the PI3K pathway with its lipiddiphosphatase activity. There are several reports that the PTEN C2 domain can regulate cell migration (6, 7) and p53-dependent gene expression (8). We add to this evidence by showing that the phosphorylate defective PTEN G129R mutant also suppresses MSP58-mediated transformation. These data are consistent with the report that v-jun-derived transformation is independent of the PI3K pathway in QEFs or CEFs (17).

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catalytic phosphatase activity. We propose that PTEN suppression of MSP58 transformation through their protein–protein interaction may represent an addition to the ability of PTEN to interfere with processes of tumorigenesis.

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