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PTEN Nuclear Localization Is Regulated by Oxidative Stress and Mediates p53-Dependent Tumor Suppression▽

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The tumor suppressor gene *PTEN* (phosphatase and tensin homologue deleted on chromosome 10) is frequently mutated or deleted in various human cancers. PTEN localizes predominantly to the cytoplasm and functions as a lipid phosphatase, thereby negatively regulating the phosphatidylinositol 3-kinase–AKT signaling pathway. PTEN can also localize to the nucleus, where it binds and regulates p53 protein level and transcription activity. However, the precise function of nuclear PTEN and the factors that control PTEN nuclear localization are still largely unknown. In this study, we identified oxidative stress as one of the physiological stimuli that regulate the accumulation of nuclear PTEN. Specifically, oxidative stress inhibits PTEN nuclear export, a process depending on phosphorylation of its amino acid residue Ser-380. Nuclear PTEN, independent of its phosphatase activity, leads to p53-mediated G_1 growth arrest, cell death, and reduction of reactive oxygen species production. Using xenografts propagated from human prostate cancer cell lines, we reveal that nuclear PTEN is sufficient to reduce tumor progression in vivo in a p53-dependent manner. The data outlined in this study suggest a unique role of nuclear PTEN to arrest and protect cells upon oxidative damage and to regulate tumorigenesis. Since tumor cells are constantly exposed to oxidative stress, our study elucidates the cooperative roles of nuclear PTEN with p53 in tumor suppression.

The *PTEN* (phosphatase and tensin homologue deleted on chromosome 10) tumor suppressor gene is mutated at high frequency in many primary human cancers and several cancer predisposition disorders (2). *PTEN* encodes a dually specific phosphatase that recognizes both lipid and peptide substrates (23), including phosphatidylinositol (3,4,5)-trisphosphate (PIP3), a product of phosphatidylinositol 3-kinase (PI3K). PTEN protein contains an N-terminal catalytic phosphatase domain (18, 32), a calcium-independent C2 domain (16), two PEST motifs, and a C-terminal PDZ binding domain (1). Several critical phosphorylation sites have been found in the PTEN C2 domain, including Ser-380, Thr-382, Thr-383, and Ser-385. Importantly, phosphorylation of these residues has been implicated to increase PTEN stability but decrease PTEN catalytic activity (36, 37).

Although PTEN is localized mainly to the cytoplasm, it preferentially resides in the nucleus of differentiated or resting cells (15) as exemplified in MCF-7 cells (14), in which nuclear PTEN peaks in the G_1 phase and reaches a nadir in the S phase. Interestingly, changes in nuclear PTEN expression have also been observed in the endometrium during hormonal cycles (27). These data suggest that nuclear localization of PTEN is a dynamic process, associated with cell cycle, cell differentiation, and cellular functions. Decreased nuclear PTEN has been correlated with progressing thyroid carcinoma and melanoma (40), suggesting a functional role of nuclear PTEN in regulating tumorigenesis.

Several studies have shown that PTEN nuclear localization depends on the presence of noncanonical nuclear localization signals and major vault protein-mediated nuclear transport (5), whereas others have indicated that PTEN nuclear localization occurs by diffusion through the nuclear membrane (20). More recent results from several studies indicate that PTEN nuclear localization is regulated by collaborative events, including feedback regulation by PI3K-S6K signaling (21), an N-terminal nuclear localization domain that is modulated by a Ran-dependent mechanism, the presence of multiple exclusion motifs (13), mutations in the N-terminal cytoplasmic localization signal (8), or monoubiquitination at amino acid residues K289 and K13 (35).

Through its lipid phosphatase activity, PTEN controls AKT signaling and its downstream targets responsible for cell size, cell motility, cell cycle, and cell death (9, 28, 30). Mutations (C124S and G129R) in the PTEN catalytic domain lead to the loss of PTEN′s phosphatase activity as well as its tumor-suppressing ability. Moreover, it has been shown that catalytically inactive PTEN binds to and promotes stabilization, acetylation, and tetramerization of p53 in the nucleus through phosphatase-independent (12, 17) and MDM2-independent (4, 17) mechanisms. Thus, PTEN functions in the nucleus in both phosphatase-dependent and -independent manner.

The significance of nuclear PTEN in regulating tumorigenesis has recently been addressed. For example, forced PTEN nuclear expression can inhibit anchorage-independent growth, induce accumulation of the cells in G_1 (22), and suppress

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tumor progression by inhibiting nuclear P-AKT (35). It has also been proposed that nuclear PTEN can induce cell cycle arrest, in part, by reducing cyclin D1 levels through its protein phosphatase activity (39) or through controlling mitogen-activated protein kinase signaling (6). Furthermore, nuclear PTEN has been demonstrated to control chromosome stability and DNA repair (31). However, most of these studies were based on in vitro biochemical analyses and cannot by themselves prove or disprove the significance of nuclear PTEN in maintaining normal cellular function and modulating cancer progression.

In this study, we validate that a specific pool of PTEN (P-PTENS380) accumulates in the nucleus upon oxidative stress. Nuclear P-PTEN associates with p53 to enhance cell cycle arrest and reactive oxygen species (ROS) reduction via a p53-dependent mechanism. Moreover, in the presence of p53, xenograft studies demonstrate that nuclear PTEN, independent of its phosphatase activity, is sufficient to regulate tumorigenesis in vivo. In summary, our study suggests a unique role of nuclear PTEN to protect cells against oxidative damage and to regulate tumorigenesis.

MATERIALS AND METHODS

Cell culture and transfection. *Pten* WT (wild type) and $Pten^{\Delta/\Delta}$ mouse embryonic fibroblasts (MEFs), 293T cells, and $p53^{-/-}$; $Mdm2^{-/-}$ MEFs were cultured in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal calf serum (HyClone) and 100 U/ml penicillin and streptomycin (Invitrogen). PC3 cells were cultured in Roswell Park Memorial Institute medium supplemented with 10% fetal calf serum (HyClone) and 100 U/ml penicillin and streptomycin (Invitrogen). Cell transfection was performed using Lipofectamine (Invitrogen). Cells were examined at 24 h posttransfection for Western blotting and at 48 h posttransfection for cell cycle and apoptosis assays. For oxidative stress treatment, cells were treated with 1 mM H_2O_2 for 1 h in serum-free medium and then subjected to analyses.

Plasmid construction and retroviral infection. PTEN expression plasmids pSG5LPTEN-WT (WT), pSG5LPTEN-G129R (GR) tagged with nuclear localization signal/nucleus exclusion signal (NLS/NES) sequence, and 380A/380D mutants (gifts from W. R. Sellers) were constructed into a retroviral pMX-IRES (internal ribosome entry site) enhanced green fluorescent protein vector. Various cell lines were infected with the supernatant from 293T cells that had been transfected (as per Gary Nolan's protocol [http://www.stanford.edu/group/nolan/protocols/pro_helper_dep.html]) with the pMX-PTEN expression plasmids. Cells were examined at 72 h postinfection for xenograft experiments. Infection efficiency was determined by green fluorescence under microscopy.

Western blotting. Whole-cell extract was prepared by lysing the cells in a lysis buffer containing 50 mM Tris-HCl (pH 8.0), 120 mM NaCl, 0.5% NP-40, 0.25% sodium deoxycholate, 1 mM dithiothreitol, 2 μg/ml aprotinin, and 2 μg/ml leupeptin. Nuclear and cytoplasmic extracts were prepared with a nuclear extract kit (Active Motif). The nuclear export assay was performed as described previously (7, 39). Cell lysates from each transfection were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred onto nitrocellulose (Bio-Rad), and followed by Western blot analysis using p53 antibody (DO-1; Santa Cruz), p21, MDM2 (Ab-2; Oncogene Research), P-PTENS380 (9551; Cell Signaling), PTEN (9552; Cell Signaling), Bax (2772; Cell Signaling), histone deacetylase 1 (SC-15022; Santa Cruz), and actin (A 4700; Sigma) antibodies. Quantification was performed with ImageJ program (NIH).

Nuclear export assay. The nuclear export assay was performed according to reference 7. Briefly after digitonin permeabilization, the nuclei were incubated with fractionated cytosolic protein (75 μg), an ATP-regenerating system (5 mM ATP, 5 mM creatine phosphate, 20 U/ml creatine phosphokinase), and 2 mM GTP at room temperature for up to 60 min. Nuclear export was stopped by centrifugation, and the supernatant was removed as the exported part.

Immunoprecipitation. Five hundred micrograms of cell lysate was incubated for 16 h at 4°C with 10 μl p53 (Santa Cruz) and PTEN antibodies (Cell Signaling) plus 50 μl protein A agarose beads (Upstate). Beads were washed three times with lysis buffer and centrifuged for 5 min at 5,000 × *g* between each wash. Protein was eluted from beads with 50 μl Laemmli sample buffer (Bio-Rad) and subjected to Western blotting as described above.

Immunocytochemistry. After treatment at the indicated time(s) or 24 h posttransfection, cells were fixed with 3% formaldehyde, permeabilized with 0.1% Triton in phosphate-buffered saline (PBS), and preblocked with 1% bovine serum albumin in PBS. Cells were then incubated with anti-PTEN antibody (Cell Signaling) or monoclonal 8-oxo-dG antibody (Trevigen) at 4°C overnight, followed by the secondary antibody conjugated with Alexa Fluor 594 or 488 (Molecular Probes). The cells were mounted with mounting medium containing 1 μg/ml DAPI (4′,6′-diamidino-2-phenylindole; Vector Labs).

Real-time PCR. Total RNAs were extracted from cells by using an RNeasy kit (Qiagen). RNAs were reverse transcribed by using a Superscript II kit (Invitrogen). Results were analyzed by the iCycler (Bio-Rad) real-time PCR and relative quantification of RNA levels normalized to actin as the difference of cycle threshold (ΔC_T) = C_T (target) − C_T (control). Higher C_T values indicate relatively lower levels of RNA expression. Primers 5′-CTCACAGCTGGTCTGTG TG-3′ (forward) and 5′-CCTCCGTGTGGCAATACC-3′ (reverse) were used to detect *Sestrin* mRNA.

Cell cycle and ROS production analysis. Cells were dissociated with trypsin, washed, and resuspended in PBS as a single-cell suspension. Cells were fixed in 70% ethanol overnight, stained with propidium iodide (25 μg/ml) (Sigma), and incubated for 30 min at 37°C with RNase A (20 μg/ml). The DNA content of the cells was then evaluated by fluorescence-activated cell sorting with a FACSCalibur (BD Immunocytometry Systems). Linear red fluorescence FL2 was analyzed. For ROS production, cells were incubated with 10 μg/ml DCF reagent (Molecular Probes) for 20 min at 37°C and run on a flow cytometer with green fluorescence FL1 analyzed.

Apoptosis analysis. Apoptosis was determined with a terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) kit (Roche) with a modified protocol for immunofluorescence. The apoptotic index was determined by counting the total number of positive nuclei in 10 randomly selected fields at ×20 magnification.

Xenograft analysis. Male SCID mice (n = 3) were bilaterally inoculated subcutaneously with 1×10^6 infected PC3 and C4-2 LNCaP cells in Matrigel (BD Bioscience) at 50:50 volume for a total volume of 100 μl/injection site. Average tumor burden was calculated in millimeters with calipers every 3 days according to the formula volume = $(\pi/6) \times$ length × width × height. After 4 and 8 weeks, all of the animals were sacrificed, tumors were harvested, and wet weights were determined. All surgical procedures were performed under regulations of the Division of Laboratory Animal Medicine regulations of the University of California, Los Angeles.

Statistical analysis. All data are presented as means ± standard deviation (SD). Statistical calculations were performed with Microsoft Excel analysis tools. Differences between individual groups were analyzed by paired t test. P values of <0.05 were considered statistically significant.

RESULTS

Oxidative stress promotes PTEN nuclear accumulation and enhances p53 stabilization. To identify the potential stimuli that can modulate PTEN nuclear localization, we compared the effects of several treatments, including serum starvation, serum stimulation, UV irradiation, and oxidative stress on WT MEFs (data not shown). Treatment with hydroxyurea was used as a positive control for dominant PTEN nuclear localization as reported previously (15). Interestingly, when applying oxidative stress (1 mM H_2O_2) to WT MEF cells, we observed an increase in total PTEN level in the nucleus (Fig. 1A and B), which was concurrent with an even more pronounced increase in nuclear phospho-PTEN at amino acid residue S380 (P-PTEN S380) (Fig. 1B). Also associated with oxidative stress was increased P-PTEN–p53 complex formation (Fig. 1B and C). To further examine the interaction between p53 and PTEN upon oxidative stress, we reintroduced PTEN in *Pten*-null MEF cells and found that oxidative stress led to elevated P-PTEN and total PTEN levels in the nucleus, correlating to increased p53 level in the same compartment (Fig. 1D). This observation is also consistent with our previous reports that

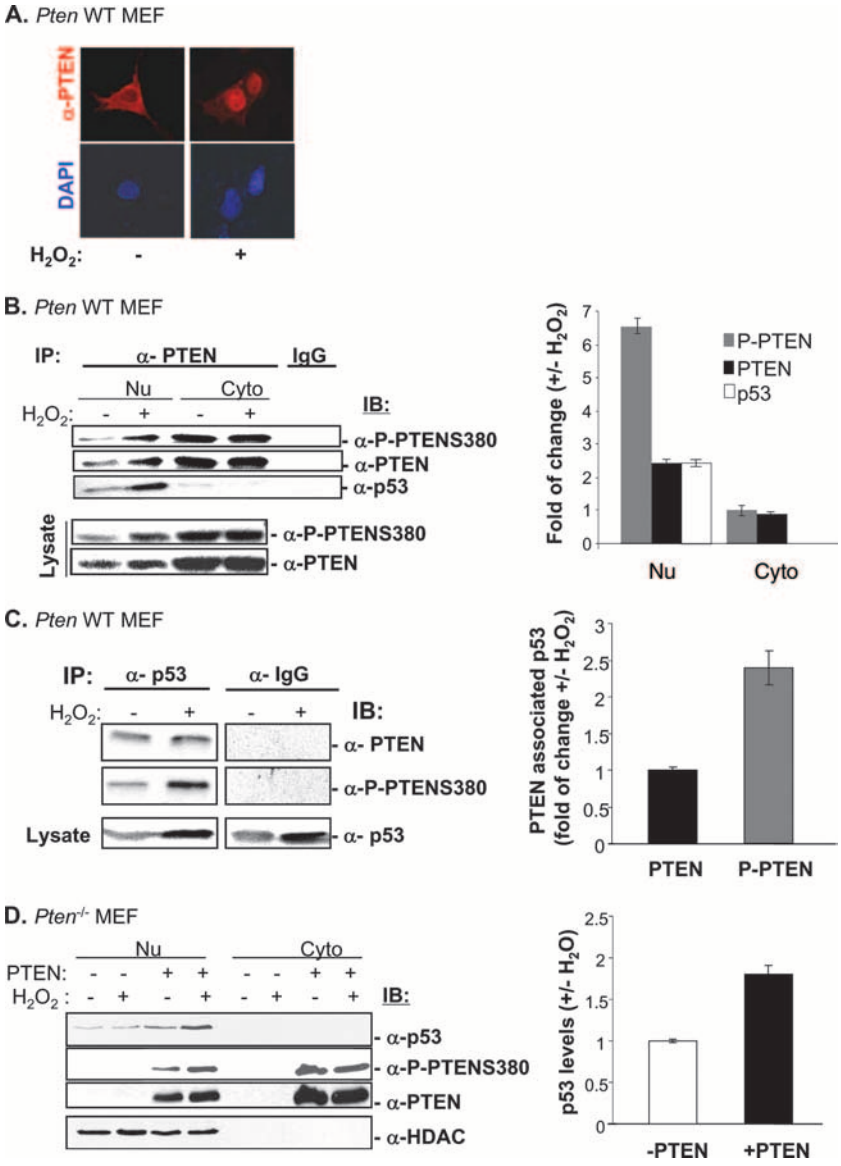

FIG. 1. Oxidative stress induces PTEN nuclear accumulation and increases p53 level and PTEN-p53 association. (A) WT MEF cells were treated without (−) or with (+) 1 mM H_2O_2 for 1 h in serum-free medium then were analyzed by immunofluorescent staining for subcellular localization of PTEN. α-, anti-. (B and C) WT MEF cell lysates, without (−) or with (+) 1 mM H_2O_2, were subjected to nuclear and cytoplasmic fractionation and immunoprecipitation (IP) with anti-PTEN (B) or anti-p53 antibody (C) and then immunoblotted (IB) with the corresponding antibodies. Immunoglobulin G (IgG) was used as a control for immunoprecipitation. (D) *Pten*$^{\Delta/\Delta}$ MEFs were transfected with PTEN and treated with H_2O_2. p53 and PTEN levels were determined in nuclear (Nu) and cytoplasmic (Cyto) fractions by immunoblotting. Changes of nuclear P-PTENS380, PTEN, and p53 levels in response to H_2O_2 were quantified by densitometry analysis and are shown as bar graphs with SD.

PTEN complexes with and stabilizes p53 in the nucleus (12, 17). Taken together, these data suggest oxidative stress as a physiological stimulus that can modulate PTEN nuclear localization. In addition, our study indicates that, in response to oxidative stress, a specific pool of P-PTEN could accumulate in the nucleus, enhance PTEN-p53 association, and increase p53 levels (see quantifications in Fig. 1C and D).

Oxidative stress specifically attenuates P-PTENS380 nuclear export and contributes to PTEN nuclear retention. Our data suggest that increased nuclear P-PTENS380 is associated with an increased pool of nuclear PTEN and p53 association upon exposure to oxidative stress. We next inquired whether PTEN nuclear localization is regulated by PTENS380 phosphorylation status. In order to test this hypothesis, we generated two PTEN phosphorylation mutants to mimic the unphosphorylated (380A) and constitutively phosphorylated (380D) PTEN at the residue serine 380, respectively. In the absence of H_2O_2, PTEN380A showed a more prominent nuclear localization than 380D (Fig. 2A), consistent with our hypothesis that PTENS380 phosphorylation may negatively regulate its nuclear localization. In response to oxidative stress, the phosphorylation-mimicking PTEN380D showed a twofold increase

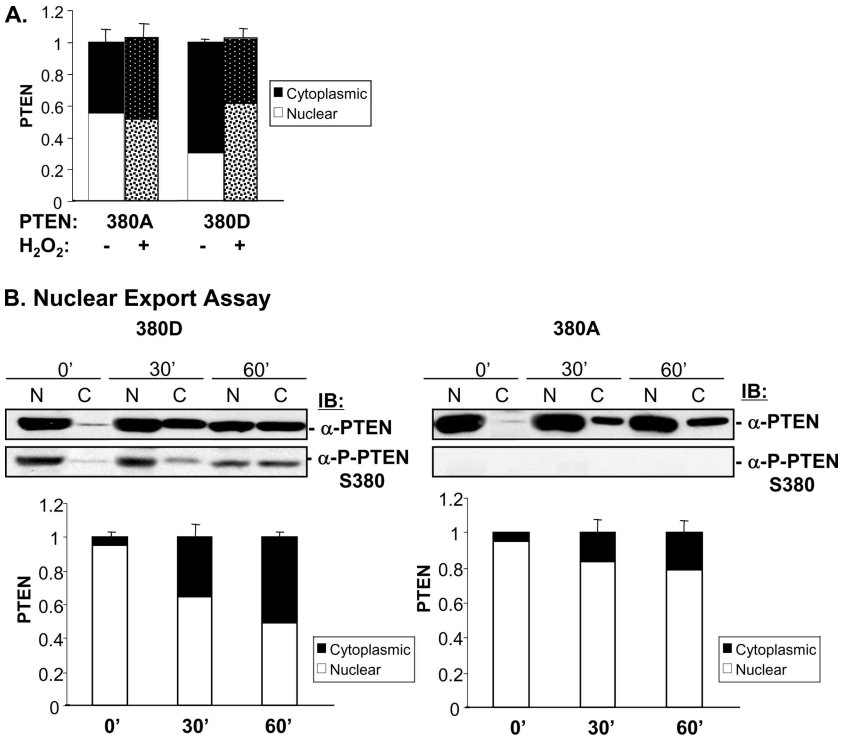

FIG. 2. Oxidative stress attenuates nuclear export of phosphorylated PTEN. (A) $Pten^{\Delta/\Delta}$ MEFs were transfected with PTEN380A or PTEN380D constructs, treated without and with H_2O_2, and subjected to nuclear and cytoplasmic fractionation. The percentages of PTEN in the cytosolic (lanes C) and nuclear (lanes N) fractions were then quantified by Western blot analysis. α-, anti-.(B) $Pten^{\Delta/\Delta}$ MEFs transfected with PTEN380A or PTEN380D constructs were subjected to nuclear export assay as described in Materials and Methods. IB, immunoblotting. Relative levels of protein expression in the nuclear and cytoplasmic compartments were quantified by densitometry analysis and are shown as bar graphs with SD.

in nuclear accumulation, as compared to PTEN380A (Fig. 2A). To ascertain whether such an accumulation is due to the effects of oxidative stress on PTEN nuclear import, export, or both, we employed a nuclear export assay (see Materials and Methods). PTEN380D was found to be rapidly exported from the nucleus to the cytoplasm (Fig. 2B, left), while PTEN380A showed retarded export within the same period of time (Fig. 2B, right), suggesting that PTEN nuclear export could be S380 phosphorylation dependent. These data indicate the potential for oxidative stress to retain the mobile portion of nuclear P-PTEN within the nucleus. The augmented pool of nuclear PTEN under oxidative stress may lead to increased chances to interact with p53 and further regulates p53 level and activity (Fig. 1).

Forced PTEN nuclear localization enhances p53-mediated cell cycle arrest independent of PTEN lipid phosphatase activity and MDM2. In order to distinguish the function of the nuclear PTEN from the cytoplasmic PTEN, we tagged an NLS or NES to a PTEN-WT expression construct to target PTEN to either the nuclear or cytoplasmic compartment, respectively (17). Also, we constructed the phosphatase-dead PTEN-GR mutant with either NLS or NES to test whether PTEN′s phosphatase activity plays a functional role in different cell compartments (Fig. 3A). To further differentiate the effects of the cytoplasmic PTEN-AKT-MDM2 axis on p53 regulation (4, 25, 26), we expressed above-mentioned PTEN constructs in $p53^{-/-}$; $Mdm2^{-/-}$ cells (17). Both NLSWT and NLSGR PTEN substantially enhanced p53 and p21 levels, whereas NESWT had more pronounced effects on inducing Bax (Fig. 3B). In $p53^{+/+}$ MEF cells, we showed that nuclear localization of PTEN, irrespective of its phosphatase activity, was sufficient for induction of G_1 cell cycle arrest. On the other hand, PTEN′s phosphatase activity was more efficient for increasing cell apoptosis (Fig. 3C). In comparison to $p53^{+/+}$ MEFs, the apoptotic response was less significant in isogenic $p53^{-/-}$ MEF cells (Fig. 3D, left panel). Furthermore, the induction of G_1 growth arrest by NLSGR PTEN expression was diminished in the p53-deficient background (Fig. 3D, right panel). These data suggest that p53 is an important factor for the function of nuclear PTEN, especially for PTEN lacking its phosphatase activity.

Nuclear PTEN promotes a reduction in p53-mediated cellular ROS production. It has been proposed that p53 can decrease intracellular ROS levels (29) and therefore protect the genome from oxidative stress-induced DNA damage. Thus, we asked whether increased PTEN nuclear localization in response to oxidative stress would mediate this process. Upon H_2O_2 treatment, a decrease in ROS level was observed in cells with PTEN380D but not with PTEN380A expression (Fig. 4A), in a manner that is p53 dependent (Fig. 4B). Furthermore, NLS PTEN expression correlated with twofold increase in the expression of p53 downstream antioxidant gene *Sestrin* (29), as compared to NES PTEN (Fig. 4D), and more significantly reduced cellular ROS (Fig. 4C). Consequently, nuclear PTEN

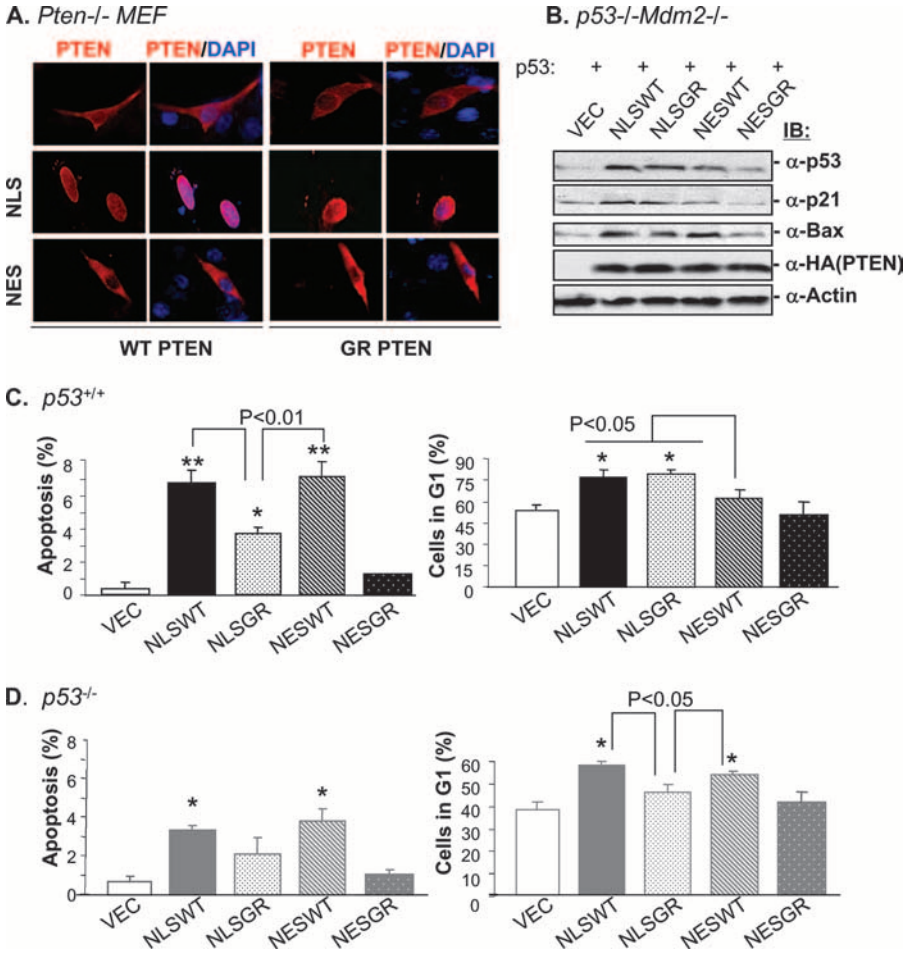

FIG. 3. Forced nuclear PTEN expression promotes p53 stabilization and apoptosis in a PTEN-phosphatase-independent manner. (A) Immunostaining of PTEN expression (red) and DAPI (blue) was shown in $Pten^{\Delta/\Delta}$ MEFs transfected with WT and GR PTEN expression constructs tagged with NLS or NES localization sequence. (B) $p53^{-/-}$; $Mdm2^{-/-}$ cells were cotransfected with p53 and NLSWT, NESWT, and NLSGR PTEN expression constructs, and immunoblotting (IB) was performed as indicated. α-, anti-. (C and D) $p53^{+/+}$ and $p53^{-/-}$ MEFs were infected with NLSWT, NESWT, and NLSGR PTEN retroviruses. Percentages of cells in G_1 cell cycle and apoptosis were examined and quantified as bar graphs with SD. * and **, $P < 0.05$ and $P < 0.01$, respectively, compared to vector control (VEC).

appeared to reduce oxidative stress-induced DNA damage, as indicated by a decrease in the percentage of 8-oxo-dG-positive cells (Fig. 4E).

Nuclear PTEN suppresses tumor progression in human prostate cancer xenografts in a p53-dependent manner. Since prostate tumors are known to be constantly exposed to oxidative stress (11), we further investigated the in vivo functions of nuclear PTEN by introducing aforementioned PTEN constructs into the human prostate cancer cell line, C4-2 LNCaP ($p53^{+/+}$; $Pten^{-/-}$). Using C4-2 prostate cancer xenografts, we found that NLSWT and NESWT PTEN acted similarly in inhibiting tumor growth (Fig. 5A), even when coexpressed with a membrane-bound constitutively activated form of AKT (Myr-AKT) (Fig. 5B). Interestingly, the NLSGR mutant had substantially greater antitumor activity than its NESGR counterpart (Fig. 5A) and such antitumor activity could be diminished but not abolished by Myr-AKT coexpression (Fig. 5B). Consistent with the antitumor activity, NLSWT, NESWT, and NLSGR PTEN significantly enhanced p53 levels and tumor apoptosis (Fig. 5C and D). These data strongly support our in vitro observations and indicate that nuclear PTEN, independent of its phosphatase activity, is able to activate p53 and suppresses tumor growth. To further address the interplay between nuclear PTEN and p53 in tumor suppression, we employed the PC3 human prostate cancer cell line that is null for both p53 and PTEN. As shown in Fig. 6A, the antitumor growth effect of NLSGR PTEN observed in C4-2 was completely abolished in PC3 xenografts (Fig. 6A). However, both NLSWT and NESWT PTEN still effectively suppressed tumor growth and induced apoptosis compared to NLSGR and NESGR (Fig. 6B), suggesting phosphatase activity could prominently contribute to the antitumor effect of nuclear PTEN in the absence of p53.

DISCUSSION

In this study, we demonstrated that oxidative stress leads to PTEN nuclear accumulation by attenuating the nuclear export of phosphorylated PTEN. Nuclear PTEN, independent of its phosphatase activity, increases p53 levels, thereby enhancing

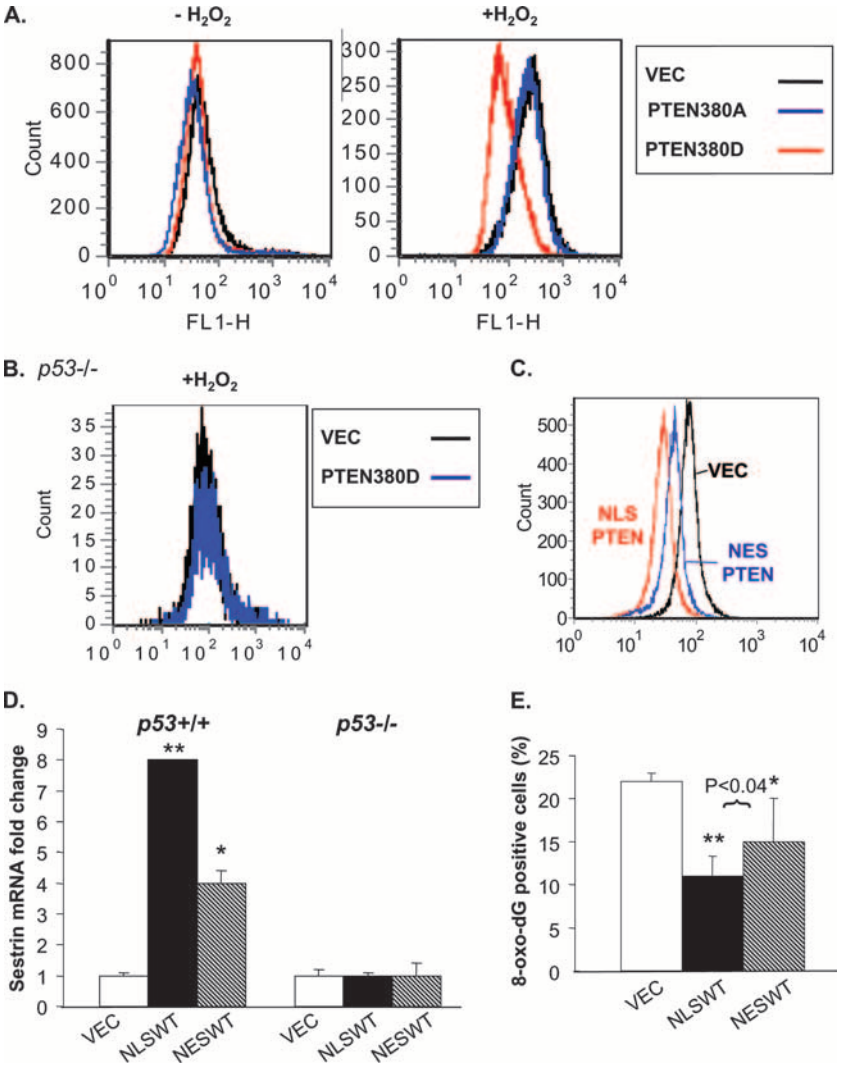

FIG. 4. Nuclear PTEN reduces cellular ROS levels in a p53-dependent manner. (A) *Pten*$^{\Delta/\Delta}$ MEFs or (B) PC3 (*p53*$^{-/-}$; *PTEN*$^{-/-}$) cells were infected with PTEN380A- or PTEN380D-expressing retroviruses, treated with H_2O_2, and examined for their cellular ROS levels as described in Materials and Methods. VEC, vector control. (C) *Pten*$^{\Delta/\Delta}$ MEFs were infected with NLS/NES PTEN-expressing retroviruses and examined for cellular ROS levels. *p53*$^{+/+}$ and *p53*$^{-/-}$ MEFs were infected with NLS/NES PTEN-expressing retroviruses. Infected cells were analyzed for *Sestrin* transcripts by real-time PCR (D) and oxidative stress-induced DNA damage with immunofluorescent staining against 8-oxo-dG (E). Representative results were quantified and are shown as bar graphs with SD. *, $P < 0.05$; **, $P < 0.01$.

p53 function. We propose that the increased PTEN nuclear localization in response to oxidative stress may protect the cells from DNA damage and tumorigenesis by modulating p53-dependent ROS reduction, cell cycle arrest, apoptosis, and possibly DNA damage repair (Fig. 7).

Oxidative stress can cause severe damage to DNA, lipids, and proteins (24). Under oxidative stress, p53 is able to suppress cellular ROS level and maintain genomic stability by inducing gene products responsible for cell cycle arrest (*p21* and *GADD45a*) and antioxidation (*Sestrins*) (29, 38). Our study suggests that nuclear PTEN can further enhance p53-mediated cell cycle arrest and ROS reduction, at least in part, independent of its phosphatase activity.

Recent studies have explored extensively the regulations and functions of PTEN subcellular localization in different cell lines and conditions. Combined data from Gil et al. (13), Denning et al. (8), and Trotman et al. (35) suggest that mutation of the N-terminal cytoplasmic anchor (PIP2 binding/adjacent cytoplasmic localization signal) and ubiquitination at K289 and K13 contribute to PTEN nuclear import. Interestingly, we also found that constitutively phosphorylated nuclear PTEN (380D) acquired high monoubiquitination, whereas the non-phosphorylated nuclear PTEN (380A) showed diminished ubiquitination (data not shown). This may elucidate an important association between phosphorylation and ubiquitination of PTEN in the nucleus. However, the regulation of different posttranslational modifications versus stability and activity of PTEN in the nucleus requires further mechanistic investigations.

Substantial evidence also suggests the correlations among PTEN phosphorylation, subcellular localization, and potential nuclear functions. First, several kinases can phosphory-

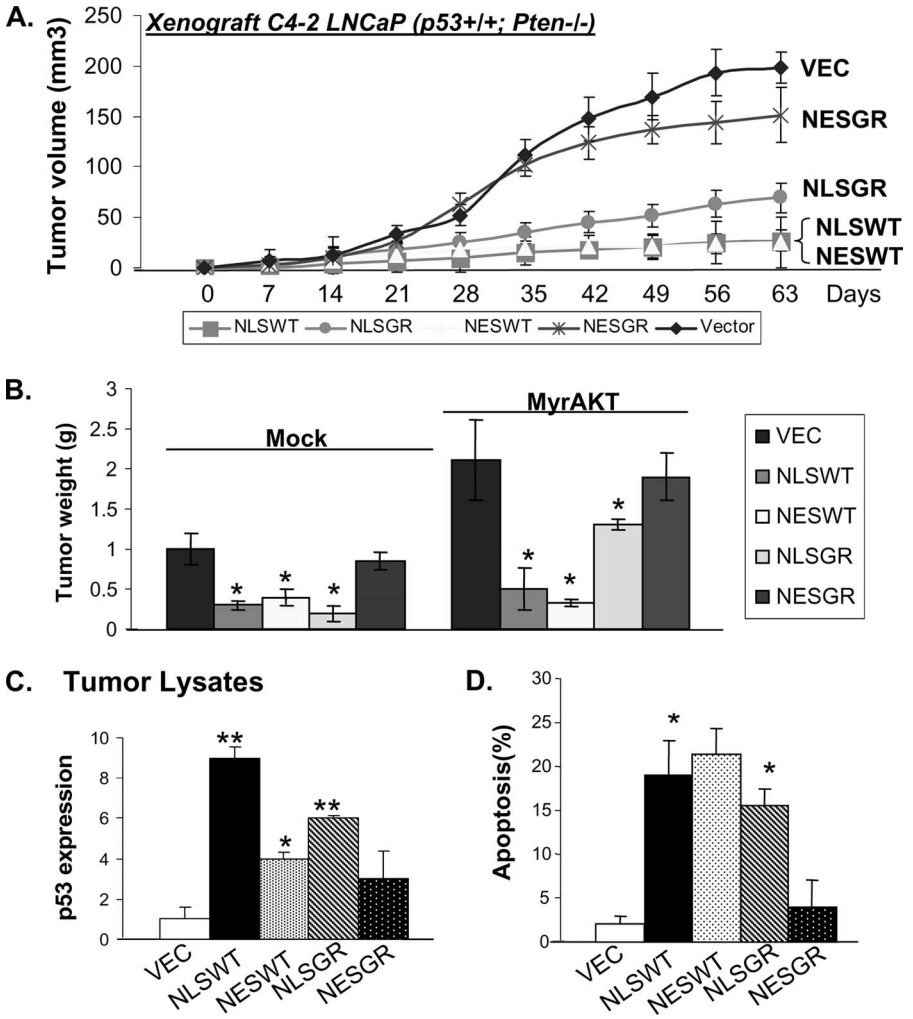

FIG. 5. Nuclear PTEN suppresses tumor progression in p53 WT human prostate cancer xenografts in a PTEN-phosphatase-independent manner. C4-2 LNCaP cells were infected with (from left to right in panel B) vector control (VEC), NLSWT, NESWT, NLSGR, and NESGR PTEN-expressing retroviruses, respectively (A), or coinfected with constitutively active AKT-expressing lentiviruses (MyrAKT in panel B). Infected cells were inoculated subcutaneously on the flank of SCID mice ($n = 3$). Tumor volume and tumor weight were calculated as described in Materials and Methods. (C) Tumors were harvested 8 weeks after inoculation. p53 levels were measured by Western blot analysis and are presented as changes (fold) relative to vector control. (D) The percentage of apoptotic cells was determined by TUNEL assay on tumor sections as described in Materials and Methods. Representative results are shown as bar graphs with SD. * and **, $P < 0.05$ and $P < 0.01$, respectively, compared to vector control.

late PTEN at its C2 domain and influence PTEN stability/activity, including CK2 (33) and glycogen synthase kinase 3β (3). In addition, Rho kinase (ROCK) phosphorylates several threonine/serine residues (S229, T223, T319, T321) in the PTEN C2 domain and delocalizes PTEN from the front edge of *Dictyostelium* during chemotaxis (19). Of note, P-PTENS380 expresses at a high level in the nucleus of quiescent hematopoietic stem cells (41). Our study suggests that PTEN nuclear accumulation is regulated by S380 phosphorylation status: P-S380 mediates rapid exportation of nuclear PTEN to the cytosol. Moreover, upon oxidative stress, more P-PTEN can accumulate in the nucleus, bind to p53, and enhance p53-mediated functions. Recently, it was demonstrated that PTEN can undergo nuclear export through a feedback regulation of the PI3K-S6K pathway (21). However, the detailed mechanisms of how oxidative stress or other signaling pathways regulate PTEN export systems remain to be further investigated.

Our study also reveals that nuclear P-PTEN associates with and enhances p53 function upon oxidative stress. Additionally, a recent study (17) provided elaborate data showing nuclear PTEN is recruited to the p53-p300 complex and maintains high p53 acetylation and activation in response to DNA damage. Interestingly, the same study showed low levels of p53 acetylation and tetramer formation are required for PTEN to interact and function within the p53-p300 complex. It is possible that the p53-p300 complex may serve as an anchor for PTEN nuclear localization under cell stress.

Using prostate cancer xenograft models, we showed that nuclear PTEN regulates cell proliferation and tumorigenesis through various signaling pathways, either dependent on or independent of its phosphatase activity. Trotman et al. (35)

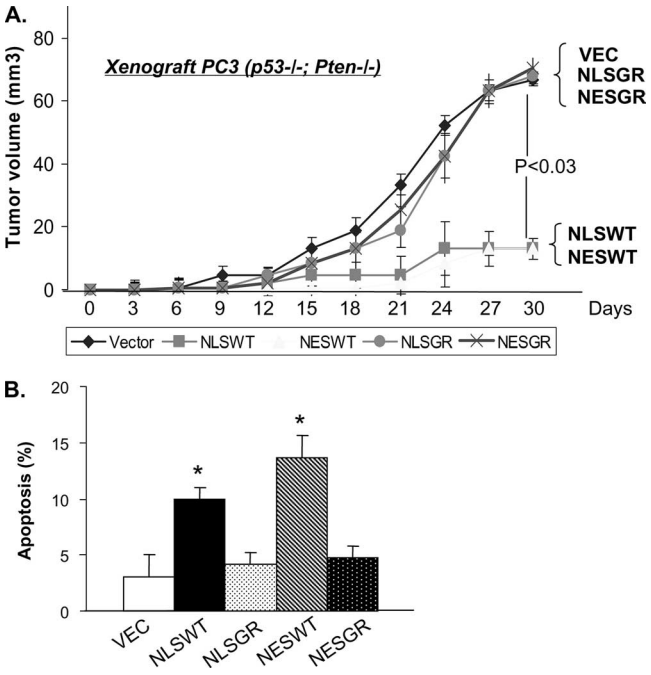

FIG. 6. Nuclear PTEN suppresses tumor progression in p53-null human prostate cancer xenografts in a phosphatase-dependent manner. (A) PC3 cells were infected with vector control (VEC), NLSWT, NESWT, NLSGR, and NESGR PTEN-expressing retroviruses, respectively. Infected cells were inoculated subcutaneously on the flank of SCID mice ($n = 3$), and tumor volume was calculated as described in Materials and Methods. (B) The percentage of apoptotic cells was determined by TUNEL assay as described in Materials and Methods. Representative results are shown as bar graphs with SD. *, $P < 0.05$.

showed that catalytically active nuclear PTEN is able to down-regulate nuclear P-AKT; nuclear P-AKT was previously known to inactivate FOXO3a and accelerate tumor progression (34). Our data indicate that nuclear PTEN′s lipid phosphatase activity can be dispensable, in the presence of p53, in suppressing the growth of human prostate cancer cell tumorigenesis. Since mutations in PTEN phosphatase domain occur in approximately 65% of PTEN mutations found in human predisposition cancers (10), our results underlie the possibility of treating PTEN phosphatase domain-mutated tumors via enhanced p53 expression or activity.

Nuclear PTEN has recently been demonstrated to control chromosome stability and DNA repair (31). Our in vitro and in vivo data provide novel findings indicating that, in addition to inducing growth arrest and apoptosis, nuclear PTEN is able to reduce oxidative damage and to sufficiently suppress tumor growth independent of its phosphatase activity. Thus, regulation of cell growth and survival through the canonical PI3K-AKT pathway may only endow partial antitumor function of nuclear PTEN. Therefore, further understanding PTEN nuclear localization and nuclear function by exploring the involved cellular partners and pathways will be important for future studies.

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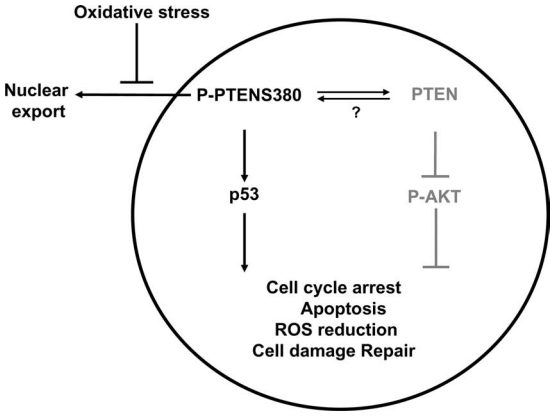

FIG. 7. Model for PTEN function as a tumor suppressor in the nucleus. Oxidative stress attenuates P-PTEN nuclear export and increases p53 level. PTEN enhances p53 function independent of its phosphatase activity while suppressing nuclear P-AKT activation through a phosphatase-dependent mechanism. Activation of p53 and reduction of P-AKT can lead to enhanced cell cycle arrest, apoptosis, ROS reduction, and possibly DNA damage repair.

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