Cooperative Phosphorylation of the Tumor Suppressor Phosphatase and Tensin Homologue (PTEN) by Casein Kinases and Glycogen Synthase Kinase 3β*

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The phosphatase and tensin homologue (PTEN) tumor suppressor is a phosphatidylinositol D3-phosphatase that counteracts the effects of phosphatidylinositol 3-kinase and negatively regulates cell growth and survival. PTEN is itself regulated by phosphorylation on multiple serine and threonine residues in its C terminus. Previous work has implicated casein kinase 2 (CK2) as the kinase responsible for this phosphorylation. Here we showed that CK2 does not phosphorylate all sites in PTEN and that glycogen synthase kinase 3β (GSK3β) also participates in PTEN phosphorylation. Although CK2 mainly phosphorylated PTEN at Ser-370 and Ser-385, GSK3β phosphorylated Ser-362 and Thr-366. More importantly, prior phosphorylation of PTEN at Ser-370 by CK2 strongly increased its phosphorylation at Thr-366 by GSK3β, suggesting that the two may synergize. Using RNA interference, we showed that GSK3 phosphorylates PTEN in intact cells. Finally, PTEN phosphorylation was affected by insulin-like growth factor in intact cells. We concluded that multiple kinases, including CK2 and GSK3β, participate in PTEN phosphorylation and that GSK3β may provide feedback regulation of PTEN.

Phosphatase and tensin homologue (PTEN) is a tumor suppressor that is frequently mutated in human cancers (4–8). The 55-kDa PTEN protein was originally described as a dual-specificity protein phosphatase, but biochemical studies soon showed that PTEN was a poor protein phosphatase but an efficient phosphoinositide D3-phosphatase, but biochemical studies soon showed that PTEN was a poor protein phosphatase but an efficient phosphoinositide D3-phosphatase (9). In cells, PTEN acts as a tumor suppressor by antagonizing phosphoinositide 3-kinase (PI3K), which activates the Akt Ser/Thr kinase, which in turn activates proliferative and antiapoptotic signaling pathways (10–14).

Posttranslationally, PTEN is regulated through phosphorylation of a cluster of serine and threonine residues in its C terminus (15–21). Although not required for the activity of the catalytic domain, phosphorylation of the C-terminal region plays an important role in stabilizing the PTEN protein. In its phosphorylated form, the tail is thought to wrap around the C2 and catalytic domains of PTEN and thereby block the translocation of PTEN to the cytoplasmic face of the plasma membrane (16, 22), thus effectively inhibiting the dephosphorylation of the substrates of PTEN. Tail mutants of PTEN tend to have increased catalytic activity but are rapidly degraded in cells.

Using a mutagenesis approach, Torres and Pulido (17) showed that the C-terminal region of PTEN is constitutively phosphorylated in vivo between residues 369 and 386, mostly on Ser-370 and Ser-385. They also found that the Ser/Thr protein kinase casein kinase 2 (CK2) can phosphorylate these residues in vitro as well as, to a lower extent, Ser-380, Thr-382, and Thr-383 (17). Vazquez et al. (16) also found that all phosphorylation of PTEN occurred in the C-terminal region (residues 354–403) and identified Ser-370 plus at least one other residue (Ser-380, Thr-382, Thr-383, or Ser-385) as the sites in vivo. They also reported that mutation of Ser-380, Thr-382, or Thr-383 to alanine reduced the half-life and increased the catalytic activity of PTEN (16). Finally, Miller et al. (21) identified Ser-370 and Ser-385 as the major phosphorylation sites in vivo and also detected phosphate on Thr-366. They also found that CK2 readily phosphorylated Ser-370 and Ser-385 in vitro.

Our own group (20) studied PTEN in a different cell type, the T lymphocyte, and we found that PTEN is heavily phosphorylated at Ser-380 and Ser-385 in these cells and that both residues can affect the half-life of PTEN. Other residues were not examined. We have now refined this study using mass spectrometry, phosphospecific antibodies, tryptic peptide mapping, phosphoamino acid analysis, site-directed mutagenesis, and RNA interference of kinases. These studies revealed a more complex regulation of PTEN by several kinases, which may act in concert or in response to different conditions or in different cell types. The participation of glycogen synthase kinase 3β (GSK3β) in PTEN phosphorylation in vivo suggested the possibility of PTEN regulation in a negative feedback loop or by stimuli that activate the PI3K/Akt pathway.

EXPERIMENTAL PROCEDURES

Antibodies—Antibodies to PTEN were from Santa Cruz Biotechnology (Santa Cruz, CA) and Upstate Biotechnology (Lake Placid, NY), anti-CK2α and anti-GSK3α were from Santa Cruz Biotechnology (Santa Cruz, CA), anti-PTEN-phospho-Ser-380, anti-phospho-Thr-Pro, anti-Akt, anti-Akt-phospho-S473, and anti-GSK3α/β-phospho-21/9 were from Cell Signaling Technology (Beverly, MA), anti-actin was from Sigma, and anti-CK2α was from StressGen Biotechnologies Corp. (Victoria, BC, Canada), anti-PTEN-phospho-Ser-370 was from Novus Biologicals (Littleton, CO), anti-PTEN-phospho-Ser-385 was from Ana-Spec, Inc. (San Jose, CA), and anti-GSK3β was from BD Transduction Laboratories and from Cell Signaling Technology (Beverly, MA). Recombinant insulin-like growth factor-1 was from PeproTech Inc. (Rocky Hill, NJ).

Plasmids and Proteins—Constructs encoding glutathione S-transferase (GST)-fused PTEN and its phosphorylation site mutants were cloned by standard PCR and recombinant DNA methods. Briefly, frag-
FIGURE 1. CK2 does not phosphorylate PTEN Ser-380 in vivo, but phosphorylates Ser-370 and Ser-385.

a, blots: anti-CK2α blot (upper left), anti-CK2α’ blot (lower left), anti-PTEN-phospho-Ser-380 (anti-pS380) blot (upper right), and anti-actin blot (lower right) of lysates of 293T cells 2 days after transfection with 25 nM each of the siRNAs against CK2α and CK2α’.

b, autoradiogram of GST-PTEN incubated with buffer (lane 1), CK2 (lane 2), or c-Akt (lane 3) and [γ-32P]ATP.

c, tryptic peptide map of the band in lane 2 in panel b. TLE, thin layer electrophoresis.

d, phosphoamino acid analysis of spots 1 and 2 from panel c. PSer, phosphorylated serine; PThr, phosphorylated threonine; PTyr, phosphorylated tyrosine. PTEN-SA/SA, PTEN-S380A/S385A.

e, autoradiogram of GST-PTEN mutants phosphorylated by CK2.

f, LC-MS/MS spectra of the m/z 1076.443 ion (left-hand panels). The 69-Da mass difference...
ments containing the entire open reading frame of PTEN were subcloned into the pGEX-2T vector (Amersham Biosciences), in-frame with the (GST) polypeptide using EcoRI and Xhol restriction sites flanking the 5' and 3' ends, respectively. The phosphorylation site mutants of PTEN (S362A/T366A, S370A, S380A, S385A, S380A/385A) were generated using the QuikChange™ site-directed mutagenesis kit (Stratagene, La Jolla, CA). All constructs were verified by sequencing. Proteins were expressed and purified according to standard techniques.

Cells, Transfection, and RNA Interference—Human embryonic kidney 293T cells were grown in Dulbecco’s modified Eagle’s medium containing 10% heat-inactivated fetal bovine serum, 1-glutamine, and antibiotics. These cells were transfected with siRNAs using Lipofectamine 2000 (Invitrogen) following the recommendations of the manufacturer.

The siRNAs used for transfections were: CK2α (Dharmacon, M-003480-00-05) and CK2α’ (Qiagen, 5'-AGC UGC GAC UGA UAG AUU G-3'), GSK3β (Dharmacon, M-003009-00), and GSK3α (Dharmacon, M-003010-00-05). Cells were lysed 48 h after transfections. In initial experiments, we co-transfected a fluorescein-labeled luciferase GL2 duplex (Dharmacon, D-001120-01-20) and then sorted out the fluorescent cells. However, this did not have any significant impact on the experimental results, and this step was therefore omitted in later experiments. Experiments were also performed with Jurkat cells, as before (12, 20).

Cell Lysis, Immunoprecipitation, and Immunoblotting—Cells were rinsed twice with ice-cold phosphate-buffered saline and lysed in 20 mM Tris/HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, and 1% Nonidet P-40, 1 mM Na3VO4, 2 mM NaF, 10 μM/32P]ATP. The reactions
to nitrocellulose, and incubated with antibodies. The blots were developed by the enhanced chemiluminescence technique (ECL kit, Amersham Biosciences) according to the manufacturer’s instructions.

In Vitro Phosphorylation, Phosphoamino Acid Analysis, and Tryptic Peptide Mapping—For in vitro kinase assays, GST-PTEN fusion proteins were incubated with GST3B (Upstate Biotechnology), CK1 (Pro-mega, Madison, WI), or/and CK2α (Upstate Biotechnology) for 30 min at 30 °C in 20 mM Heps pH 7.4, 0.05 mM ATP, 10 mM MgCl2, 1 mM dithiothreitol, 2 mM sodium orthovanadate, and 5 μCi of [γ-32P]ATP. The reactions were terminated by adding SDS sample buffer and heating to 95 °C for 5 min followed by SDS-PAGE and autoradiography.

Tryptic peptide mapping was performed as before (23–25) with the protocol of Luo et al. (26). Phosphoamino acid analysis was performed by complete acid hydrolysis in 1 M HCl, 110 °C, and separation in two dimensions in the presence of unlabeled standards.

GST Pull-down Assays—For GST pull-down assays, 293T cell lysates were incubated with GST or GST-PTEN prebound to glutathione-Sepharose beads. The beads were then washed three times with lysis buffer, suspended in SDS sample buffer, and analyzed by immunoblotting.

Mass Spectrometry—Identification of phosphorylated residues by LC-MS/MS was performed on in vitro phosphorylated GST-PTEN and digested with highest grade trypsin. The digest was injected into a high-pressure liquid chromatography instrument (LC Packings Inc.), which first separates the peptides on a reverse-phase column from which they elute directly into a quadrupole time-of-flight mass spectrometer (Q-TOF API-US) equipped with a nanoelectrospray ionization source (Waters-Micromass).

RESULTS

Loss of CK2 Has No Impact on Phosphorylation of PTEN at Ser-380 in Cells—Using a phospho-specific antibody, we recently showed that PTEN is phosphorylated at Ser-380 in Jurkat T cells and in normal human T lymphocytes (20). To determine whether CK2 is responsible for this phosphorylation in cells, we used RNA interference to reduce the cellular levels of the two catalytic subunits of this kinase, CK2α and CK2α’. 293T cells (which can be transfected to >80%) were first transfected with siRNAs for each kinase separately to verify efficacy and to establish the required concentrations (not shown) and then with both siRNAs together (Fig. 1A). Two days after transfection, these cells showed an ~90% reduction in CK2α and ~70% reduction in CK2α’, whereas actin levels were unchanged. Cell viability and morphology also remained normal. Immunoblotting with the anti-phospho-Ser-380 antibody revealed that phosphorylation of PTEN at this site was not affected at all by this dramatic reduction in CK2 levels.

CK2 Phosphorylates PTEN at Ser-370 and Ser-385 but Not Ser-380—The lack of effects of the loss of CK2 on PTEN phosphorylation at Ser-380 must mean either that very low levels of CK2 are enough to carry out a normal phosphorylation at this site or that another kinase is responsible. To first address the former possibility, we phosphorylated PTEN in vitro with CK2 and analyzed the phosphorylation by autoradiography, tryptic peptide mapping, phosphoamino acid analysis, and tandem mass spectrometry. These experiments showed that CK2 phosphorylated PTEN (Fig. 1, lane 2), whereas recombinant Akt did not (Fig. 1B, lane 3), and that this phosphorylation occurred on two distinct peptides (Fig. 1C), both of which contained only phosphoserine (Fig. 1D). The two peptides migrated a very short distance on the thin layer plates, particularly in the second dimension (ascending chromatography), indicating that they are both acidic and very hydrophilic. These properties are found only in the two long C-terminal tryptic peptides corresponding to residues 350–378 and 379–402 of PTEN, the latter containing the major phosphorylation sites in PTEN in T cells, Ser-380 and Ser-385 (20). Using PTEN proteins with either or both of these two residues mutated to alanine as substrates showed that CK2 readily phosphorylated all these proteins with some decrease observed only for PTEN proteins with Ser-385 mutated (Fig. 1E). Finally, tandem mass spectrometry (Fig. 1F) detected phosphorylation of two distinct tryptic peptides at residues Ser-370 and Ser-385, respectively (Fig. 1F). In contrast, no peptide containing phosphate at Ser-380 was found. Since the same peptide with phosphate at Ser-385 was readily detected, it seemed that Ser-380 was not phosphorylated at all by CK2.

Together, all these results indicated that CK2 indeed can phosphorylate PTEN very well but that it is not required for phosphorylation of PTEN in intact cells. Indeed, CK2 did not phosphorylate this
Cooperative Phosphorylation of PTEN by CKs and GSK3β

FIGURE 2. Phosphorylation of PTEN by GSK3β. a, autoradiogram of PTEN incubated with GSK3β (lane 1), CK2 (lane 2), or CK1 (lane 3) in kinase buffer with [γ³²P]ATP. b, tryptic peptide map of the band in lane 1 of panel a. TLE, thin layer electrophoresis. c, phosphoamino acid analysis (PAA) of the spot in panel b. PSer, phosphorylated serine; PThr, phosphorylated threonine; PTyr, phosphorylated tyrosine. d, LC-MS/MS spectra showing the y ion series of the peptide containing Ser-362 and Thr-366. The upper spectrum shows the unphosphorylated peptide, and the lower spectrum is the doubly phosphorylated peptide. Note that the sequence is in the C- to N-terminal direction. e, autoradiogram of GST-PTEN (lane 1) or GST-PTEN-S362A/T366A (lane 2) phosphorylated by GSK3β. wt, wild type.
residue even \textit{in vitro} but instead phosphorylates serine residues 370 and 385. We also found that CK2 phosphorylates only serine residues, without any trace of phosphate on threonine residues. We concluded that it is likely that additional kinases participate in the phosphorylation of PTEN at serine and threonine residues in intact cells.

\textbf{Phosphorylation of PTEN by GSK3/\beta and CK1 but Not by c-Akt—} Next, we tested a number of other Ser/Thr kinases for their ability to phosphorylate PTEN. As shown in Fig. 1b, recombinant Akt (which readily phosphorylated other proteins \textit{in vitro}; data not shown) was unable to phosphorylate GST-PTEN. Similarly, cAMP-dependent protein kinase, protein kinase C, and the protein kinases Mek1, Erk2, and PDK1 were all unable to phosphorylate GST-PTEN (not shown). In contrast, GSK3\beta and CK1 incorporated substantial amounts of \textsuperscript{32}P into PTEN (Fig. 2a), albeit less than CK2. Tryptic peptide maps of PTEN phosphorylated by GSK3\beta showed a single spot (Fig. 2b), which was both acidic and hydrophilic, suggesting that it corresponds to either of the two C-terminal tryptic peptides of PTEN. Phosphoamino acid analysis revealed that GSK3\beta phosphorylated PTEN on both threonine and serine (Fig. 2c). Tandem mass spectrometry identified the phosphorylated residues as Ser-362 and Thr-366 (Fig. 2d). None of the several other serine or threonine residues contained any phosphate. Indeed, the PTEN-S362A/T366A mutant of PTEN was not phosphorylated at all by GSK3\beta \textit{in vitro} (Fig. 2e).

The phosphorylation of PTEN by CK1, CK2, or GSK3\beta \textit{in vitro} was further analyzed with phospho-specific antibodies against PTEN-phospho-Ser-370 and PTEN-phospho-Ser-385 (Fig. 3a, first and second panels), which confirmed that CK2 readily phosphorylated both sites (lanes 3), whereas CK1 phosphorylated Ser-370 much less than CK2 but Ser-385 a bit better than CK2 (lanes 2). In contrast, GSK3\beta did not phosphorylate either site (Fig. 3a, first and second panels, lane 4). Phospho-specific antibodies against Ser-362 or Thr-366 are not available, but since Thr-366 is followed by a proline

\begin{figure}
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\caption{Phosphorylation of PTEN by CK1, CK2, and GSK3\beta and synergism between CK2 and GSK3\beta. a, anti-phospho-Ser-370 (anti-pS370) (top panel), anti-phospho-Ser-385 (anti-pS385) (second panel), anti-phospho-Thr-Pro (anti-pThr-Pro) (third panel), and anti-PTEN (bottom panel) immunoblot of GST-PTEN incubated with kinase buffer alone (lane 1) or with CK1 (lane 2), CK2 (lane 3), or GSK3\beta (lane 4) and ATP. b, upper panel, autoradiogram of GST-PTEN incubated first with kinase buffer alone (lanes 1–2) or CK2 (lanes 3 and 4) in the presence of unlabeled ATP (pre) for 40 min, washed extensively, and then incubated with kinase buffer (lanes 1 and 2) or CK3\beta (lanes 2 and 4) in the presence of \textsuperscript{[\gamma-\textsuperscript{32}P]}ATP. Lower panel, the same experiment with GST-PTEN-S370A. c, upper panel, anti-phospho-Thr-Pro immunoblot of GST-PTEN (lanes 1–5), GST-PTEN-S362A/T366A (lane 6), or GST-PTEN-S370A (lane 7) incubated with kinase buffer alone (lane 1), CK1 (lane 2), CK2 (lane 3), GSK3\beta (lane 4), or CK2 plus GSK3\beta (lanes 5–7) in kinase buffer with 1 mM ATP. Lower panel, anti-PTEN blot of the same filter.}
\end{figure}
residue, we tested whether this site would react with an anti-phos-
pho-Thr-Pro antibody. Indeed, GSK3 treatment made PTEN readily
reactive with this antibody (Fig. 3a, third panel, lane 4). This reac-
tivity was abrogated by mutation of Thr-366 (see below). CK1 was
very inefficient in phosphorylating PTEN at Thr-366, whereas CK2
was not able to cause any phosphorylation at this site.

Synergistic Phosphorylation of PTEN by GSK3β and CK2—GSK3β
typically phosphorylates serines or threonines that are located four resi-
dues amino-terminal of an already phosphorylated residue, a pattern
that fits the notion that Ser-362 becomes a good substrate only after
Thr-366 has been phosphorylated. Furthermore, phosphorylation of Ser-
370 by CK2 could have the same stimulatory effect on phosphorylation
of Thr-366 by GSK3β. To directly test this possibility, we pretreated
GSK-PTEN with CK2, or kinase buffer alone, in the presence of unla-
abeled ATP, pulled down and washed the GST-PTEN protein, and then
treated it with GSK3β in kinase buffer with [γ-32P]ATP or with kinase
buffer and [γ-32P]ATP alone. The latter also served as a control to show
that residual CK2 did not affect the assay. As shown in Fig. 3b, upper
panel, PTEN pretreated with CK2 was severalfold more phosphorylated
by GSK3β (lane 4) than PTEN pretreated with kinase buffer and ATP
without CK2 (lane 2). When this experiment was repeated with GST-
PTEN-S370A, the effect of CK2 pretreatment was completely lost (Fig.
3b, lower panel).

Very similar results were obtained with phospho-specific antibodies;
although only GSK3β was able to make PTEN reactive with the phos-
pho-Thr-Pro antibody (i.e. phosphorylate Thr-366) (Fig. 3c, lane 4),
PTEN treated with both CK2 and GSK3β became much more strongly
reactive (lane 5). In contrast, PTEN-T366A/S362A did not react at all
with the antibody (lane 6), and the effect of CK2 was completely lost in
the PTEN-S370A mutant. Together, these experiments showed that
phosphorylation of PTEN by CK2 at Ser-370 promotes phosphorylation at Thr-366 by GSK3β.

GSK3β Phosphorylates PTEN at Thr-366 in Intact Cells—To deter-
mine whether GSK3α participates in the phosphorylation of PTEN in
intact cells, we reduced the cellular levels of GSK3β, as well as the closely
related GSK3α, by RNA interference, immunoprecipitated PTEN, and
probed it with the anti-phosphothreonine-proline antibody (Fig. 4a). In
cells with reduced levels of both GSK3 isoforms, phosphorylation of
PTEN at Thr-366 was much reduced, indicating that GSK3 is needed for
this phosphorylation in intact cells. In contrast, reactivity with the phos-
pho-Ser-370 and phospho-Ser-385 specific antibodies was not changed,
and PTEN and actin levels were unaltered. We concluded that GSK3
indeed phosphorylates at least Thr-366 in intact cells. This notion was
further supported by the detection of a small amount of GSK3β (as well
as CK2) bound to GST-PTEN incubated with cell lysates followed by
extensive washing and immunoblotting (data not shown).

Inactivation of GSK3α/β by Insulin-like Growth Factor 1 Stimulation
Reduces PTEN Phosphorylation at Thr-366 in Cells—Next, we wanted
to learn whether extracellular stimuli that affect the activity of GSK3α
and β would alter the phosphorylation of PTEN by this kinase in intact
cells. Insulin-like growth factor 1 is known to cause a robust activation
of c-Akt, which, in turn, phosphorylates GSK3 and inactivates it. Indeed,
the addition of this growth factor to our cells caused a sharp increase in
phospho-Akt levels (Fig. 5a, third panel). Concomitantly, there was a clear
decrease in the reactivity of PTEN with the phospho-
pho-Thr antibody. The total levels of Akt and PTEN remained unchanged
in these experiments, whereas the levels of GSK3 tended to change, at
least in part due to nucleus-to-cytosol translocation of this kinase
(27). Nevertheless, these data demonstrated that extracellular stim-
uli that activate the PI3K-Akt-GSK3 pathway directly influence PTEN phosphorylation at Thr-366, providing a physiological regulation of this phosphorylation of PTEN.

**Phosphorylation of PTEN at Thr-366 Reduces the Activity of PTEN in Cells**—Finally, to evaluate whether phosphorylation at Thr-366 has any functional impact on PTEN, we expressed PTEN or the PTEN-T366A mutant in Jurkat T cells, which lack endogenous PTEN, and measured their effects on the phosphorylation of Akt. In these experiments, PTEN-T366A consistently reduced Akt phosphorylation to a higher extent than unmutated PTEN (Fig. 5B). Thus, it appeared that phosphorylation of PTEN at Thr-366 by GSK3 reduces its biological activity.

**DISCUSSION**

Although our data agreed with the conclusion from previous studies that CK2 plays a role in PTEN phosphorylation (17), we found that CK2 is not the only kinase involved in this important task. Like several other groups (16, 17, 21), we found that Ser-370 and Ser-385 indeed are phosphorylated in vivo and by CK2 in vitro. However, we saw little phosphorylation of other residues by CK2, and notably, no phosphorylation of threonine residues at all. We also found that CK2α and α′ are not required in intact cells for phosphorylation of Ser-380, although overall phosphorylation of PTEN was decreased by CK2 knock-down by RNA interference (data not shown). Thus, CK2 could not be the only kinase

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**FIGURE 5. Regulation and role of phosphorylation of PTEN at Thr-366 in intact cells.**

*a,* insulin-like growth factor-1 (IGF-1) reduces PTEN phosphorylation at Thr-366 in 293T cells starved from serum for 2 h. The cells were treated with medium alone (lane 1) or with 50 ng/ml insulin-like growth factor-1 from 25 min (lane 2) and total lysates blotted with anti-phospho-Akt (anti-pAkt) (top left panel), anti-Akt (second panel), anti-phospho-GSK3β (anti-pGSK3) (third panel), and anti-GSK3β (bottom left panel). Anti-PTEN immunoprecipitates from the same cells were blotted with anti-phospho-Thr-Pro (anti-pThr-Pro) (top right) and anti-PTEN (lower right panel), pSer, phospho-serine. *b,* anti-phospho-Akt blot (upper panel), anti-Akt blot (middle panel), and anti-HA tag immunoblot for PTEN (bottom panel) of Jurkat T cells transfected with empty vector (lane 1), HA-tagged PTEN (lane 2), or HA-tagged PTEN-S362A/T366A (lane 3). The cells were starved from serum for 1 h prior to lysis. Note that the PTEN mutant reduces Akt phosphorylation more than wild-type PTEN does despite similar levels of expression. *c,* schematic model of the proposed negative feedback loop. Activation of the PI3K-Akt pathway by insulin-like growth factor leads to phosphorylation and inhibition of GSK3β, which therefore reduces its phosphorylation of PTEN, leading to increased activity of PTEN to counteract PI3K.
involved in PTEN phosphorylation in cells. Indeed, we found that GSK3β also phosphorylates PTEN at two sites, Ser-362 and Thr-366, the latter detected by Miller et al. (21) as a site in intact cells. We also detected phosphate at Thr-366 in vivo, and this phosphate decreased upon knock-down of GSK3 or the addition of insulin-like growth factor 1 to the cells. Importantly, GSK3β and CK2 phosphorylated non-overlapping sites, and we found that phosphorylation of Ser-370 by CK2 strongly enhances subsequent phosphorylation of Thr-366 by GSK3β. Thus, these two kinases presumably synergized and could potentially cause rapid changes in the phosphorylation state of PTEN in cells. It also appeared that CK1 can phosphorylate PTEN, particularly at Ser-385, suggesting that this kinase may also play a role in intact cells. Fig. 4b shows a schematic view of the kinases involved in PTEN tail phosphorylation.

We previously reported that PTEN phosphorylation is influenced by D3 phosphorylated inositol lipids (the substrates for PTEN) in what appears to be a negative feedback loop (20). A dilemma in this model was that CK2 is not known to be regulated by these phospholipids directly or indirectly. The introduction of GSK3β solves this dilemma (Fig. 5c). This kinase is known to be inhibited by phosphorylation by Akt, suggesting that high levels of D3-phosphoinositides (e.g. in Jurkat T cells) may reduce the phosphorylation at Thr-366 (and perhaps Ser-380) of PTEN in cancer. Understanding how PTEN is regulated should provide new insight into cell signaling mechanisms and may suggest novel approaches to the treatment of PTEN-deficient tumors.

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