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PTEN regulation of ERK1/2 signaling in cancer

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Abstract

Since its discovery, the tumor suppressor phosphatase and tensin homolog (PTEN) has become a molecule with a wide spectrum of functions, which is typically mediated through its lipid phosphatase activity; however, PTEN also functions in a phosphatase-independent manner. It is well established that PTEN regulates several signaling pathways, such as phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT), janus kinase (JAK)/signal transducers and activators of transcription (STAT), focal adhesion kinase (FAK), and more recent, extracellular signal-regulated kinase (ERK)1/2, where activation of these pathways typically leads to cancer development and progression. In regard to most of these pathways, the underlying molecular mechanism of PTEN-mediated regulation is well established, but not so much for the ERK1/2 pathway. Indeed, accumulating evidence has shown an inverse correlation between PTEN expression and ERK1/2 in several malignancies. However, the detailed mechanism by which PTEN regulates ERK1/2 is poorly understood. In this review, we discuss the role of PTEN in regulating ERK1/2 by directly targeting shc/Raf/MEK and PI3K/AKT cascades, and a putative cross-talk between the two.

Keywords

ERK1/2; PTEN; PI3K/AKT; Raf; Ras; cancer

Introduction

Tumorigenesis is the result of genetic alterations and/or epigenetic modifications to oncogenes and tumor suppressors. Aberrations in oncogenes results in an increase in mitogenic functions, such as cell proliferation and survival signaling; alterations in tumor suppressor genes are associated with unrestrained cell proliferation. Thus, tumor suppressor proteins generally inhibit cancer development and progression by regulating a spectrum of cellular survival functions, including cell cycle, apoptosis, DNA repair, signal transduction, and cell adhesion. One frequently inactivated tumor suppressor, found in a variety of human cancers, is the phosphatase and tensin homolog deleted on chromosome 10q23 (*PTEN*) gene. In fact, phosphatase and tensin homolog (PTEN) is the second most mutated tumor suppressor, only to p53, in several malignancies such as prostate, breast, lung, and brain (1–4).

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Declaration of Interest

None.

Loss of PTEN in cancer cells is typically associated with increased activation of the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) pathway, thereby facilitating cancer malignant transformation and progression. PTEN negatively regulates AKT through its lipid phosphatase activity; PTEN dephosphorylates phosphatidylinositol 3,4,5-triphosphate (PIP3), a secondary messenger for AKT, to PIP2, thereby preventing the activation of AKT and subsequent downstream survival pathways (5). Other pathways that are under regulation by PTEN are (1): signal transducers and activators of transcription (STAT); (2) Jun N-terminal kinase (JNK); and focal adhesion kinase (FAK) (6–8). Emerging studies have suggested that PTEN may also negatively regulate the extracellular signal-regulated kinase (ERK)1/2 pathway. To date, several studies have established an inverse correlation between PTEN expression and ERK1/2 inhibition, but have not fully investigated the underlining mechanism(s) by which PTEN inhibits ERK1/2 activity. In fact, we have demonstrated that PTEN reconstitution in PC3 prostate cancer cells inhibited ERK1/2 phosphorylation (2). Herein, we will briefly focus on the tumor-suppressive role of PTEN, as well as an emerging target of PTEN regulation, ERK1/2 kinase.

Regulation of PTEN

In 1997, Li *et al.* (9) and Steck *et al.* (10) first identified a high frequency of PTEN mutations and deletions in cancers of the brain, bladder, breast, and prostate, implicating PTEN as a novel tumor suppressor. Likewise, Yu *et al.* demonstrated that inactivation of PTEN was the result of point mutations, epigenetic silencing, and deletions. Apart from its tumor-suppressive role, PTEN aberrations also associate with an array of diverse diseases, such as Cowden disease, autism, Lhermitte Duclos disease, and Bannayan–Zonana syndrome (11).

PTEN is a member of type-I protein tyrosine phosphatase (PTP) family and consists of five domains: (1) N-terminus phosphoinositol 4, 5 bisphosphate (PIP2) binding domain; (2) the phosphatase domain; (3) lipid binding C2 domain; (4) PDZ domain (post synaptic density protein [PSD95], Drosophila disc large tumor suppressor [Dlg A], and zonula occludens-1 protein [zo-1] motif; and (5) a C-terminal tail containing a PEST motif (12). The N-terminus phosphatase domain has dual-specificity activity, which dephosphorylates proteins and phosphoinositides substrates (13). The peptide phosphatase activity is targeted against tyrosine, serine, and threonine residues on proteins, while the lipid phosphatase activity targets PIP3. This dual phosphatase function indicates that PTEN targets a wide range of molecules, and indirectly, molecules that are downstream of these targets, thereby regulating tumorigenic functions, such as apoptosis, cell cycle, cell adhesion, and cell migration (14,15).

PTEN is a potent tumor suppressor; therefore, it is expected that PTEN expression and function would be well regulated to maintain cellular homeostasis. For instance, PTEN expression and enzymatic activity are regulated through transcriptional regulation, microRNA (miRNA) targeting, and post-translational regulation. Transcriptionally, PTEN expression is mediated by growth regulated transcription factor 1 (EGR1), peroxisome proliferator activated receptor γ (PPAR γ), and p53, through direct binding to the PTEN promoter region, leading to its gene transcription (16–18). Conversely, PTEN transcriptional silencing is enhanced by NF- κ B and Jun in several cancer models; while promoter hypermethylation, another form of repressing gene expression, was identified in lung, thyroid, breast, and ovarian cancers (19–22). Moreover, miRNA targeting, specifically miRNA21 (miR-21), miR-22 and miR-25a, reduced PTEN expression (23,24). As with most proteins, PTEN is also regulated through post-translational modifications, commonly phosphorylation, acetylation, ubiquitylation, and active site oxidation. For instance, Torres *et al.* (25) demonstrated that phosphorylation of the C-terminus end of PTEN by casein

kinase 2 (CK2) rendered PTEN resistant to proteasomal degradation, ultimately, enhancing stability. PTEN degradation is enhanced by the E3 ligase NEDD4-1 through ubiquitin-mediated proteasomal degradation (26). Finally, PTEN activity was regulated by reactive oxygen species (ROS), where accumulation of ROS has been shown to oxidize PTEN within the catalytic domain by forming disulfide bridges, rendering PTEN inactive (27). An interruption in PTEN expression and activity will increase, and enhance expression and catalytic activity of growth-promoting kinases, such as AKT, and consequently, encourage phenotypic behaviors that allow tumor cells to survive and become mobile (28).

Tumor-suppressive functions of PTEN

PTEN is classically associated with inhibiting the PI3K/AKT pathway and ultimately cell survival, proliferation, and migration (5). Accumulating studies have indicated that PTEN exerts its tumor-suppressive functions through its phosphatase activity as well as protein interactions. For instance, PTEN promoted cell cycle G1 phase arrest by downregulating cyclin D1 through its protein phosphatase activity, while upregulating p27 through its lipid phosphatase activity in breast cancer cells. This study suggested that the tumor-suppressive function of PTEN does not depend on the lipid phosphatase function or the protein phosphatase function independently, but coordinating activities of both phosphatases (29). Despite this conclusion, current evidence supports the notion that both phosphatase activities can suppress tumor development independent of each other. Through the peptide phosphatase activity, PTEN targeted the tyrosine residue on FAK, which disrupted cell adhesion and migration (30). As an example of protein phosphatase function, Freeman *et al.* (31) demonstrated that PTEN regulated p53 stabilization and transcriptional activity by competing with MDM2 for direct binding with p53.

The continued investigation of PTEN has revealed its multi-faceted role in regulating cell proliferation, gene expression, metabolism, migration, and survival, by acting on targets involved in these processes, explaining its potent tumor-suppressive role (32,33). PTEN utilizes its phosphatase domain, as well as its protein-protein domain, to regulate cell signaling and the function of cognate molecules. The ERK1/2 pathway has become a novel target of PTEN regulation (2). Weng *et al.* (34) reported one of the initial studies to support a cross-talk between PTEN and ERK1/2, where reconstitution of PTEN in breast cancer cells inhibited ERK1/2-mediated phosphorylation of ETS-2, thereby preventing cancer progression. Since then, more studies have provided evidence that ERK1/2 is a target of PTEN, but not enough to fully elucidate the molecular mechanism.

ERK1/2 signaling pathway

The ERK1/2 signaling pathway plays a critical role in regulating cell proliferation, differentiation, survival, and apoptosis of various cancer types such as bone, prostate, breast, and lung. In fact, it has been estimated that ERK1/2 targets more than 180 different molecules that are responsible for cell growth, survival, and differentiation, thus aberrant regulation greatly affects cell growth (35). ERK1/2 kinase couples the signals from cell surface receptors to molecules that transmit cell proliferative signals. Following stimulation of appropriate receptors, the Src homology 2 domain-containing protein (shc), becomes associated with the activated receptor (36). The coupling of shc and a stimulated receptor recruits the growth factor receptor-bound protein 2 (Grb2), and son of sevenless (sos) to form a complex. This complex then exchanges GDP with GTP onto membrane-bound Ras, thereby activating Ras function. Subsequently, Ras-GTP recruits Raf serine/threonine-specific kinase to the membrane, which then becomes activated. Raf then mediates activation of ERK kinase 1 (MEK1) through phosphorylation on two of its serine residues within the activating loop (36). Finally, MEK1 phosphorylates ERK1/2, rendering it active,

which then targets a variety of substrates through its kinase activity. ERK1/2 kinase activity then leads to the upregulation of various transcription factors such as Ets-1, c-Jun, c-Myc, and HIF1 α (37,38). Each of these transcription factors regulate genes that promote cancer cell survival in some way—from metabolism, to proliferation, to metastasis, and distal colonization (39). Independent of direct activation by ERK1/2 kinase, constitutive activation of molecular events upstream of ERK1/2 can also involve the kinase in cancer progression. For instance, the continuous activation of epidermal growth factor receptor (EGFR) resulted in constitutive activation of downstream target ERK1/2. Likewise, a mutation in the proto-oncogene B-Raf rendered it constitutively active, and as a result, downstream targets like the ERK1/2 pathway also became constitutively active and tumorigenic (40).

PTEN regulation of the ERK1/2 signaling pathway

In cancer cells, an assortment of signaling pathways leads to neoplasia, and eventually, tumor development and progression of the tumor to a malignant disease. Tumor development primarily depends on the homeostatic imbalance of activated oncogenes, and repressed tumor suppressors. It is well established that the tumor suppressor PTEN negatively regulates the oncogenic signaling pathway PI3K/AKT; however, literature suggests that PTEN may also regulate the ERK1/2 pathway to combat neoplastic development. Bouali *et al.* (41) and Chetram *et al.* (2) independently demonstrated that reconstitution of PTEN in null prostate cancer cells reduced the phosphorylation of both AKT and ERK1/2, resulting in reduced kinase activity. Inversely, ROS-mediated catalytic inactivation of PTEN resulted in increased phosphorylation of both AKT and ERK1/2 (28), rendering them active. A putative mechanism by which PTEN regulates ERK1/2 may be due to an interaction between PI3K and ERK1/2. An early link between the two kinases was observed in *Xenopus laevis* oocytes, which demonstrated that an increase in the catalytic subunit (p110 α) of PI3K stimulated ERK1/2 activity (42). Likewise, a subsequent investigation in *Simia aethiops* kidney cells (COS-7) revealed that overexpression of the p110 γ subunit of PI3K also facilitated the activation of ERK1/2 (43). In support of these studies, Liu *et al.* (44) demonstrated that pharmacological inhibition of PI3K suppressed ERK1/2 activity in hepatocellular carcinoma, breast, and prostate cancer cells. It remains unclear, however, whether stimulation of ERK1/2 activity depends on direct interaction with PI3K, or indirectly through a molecule that interacts with both the kinases.

A putative indirect mechanism by which PI3K regulates ERK1/2 is through protein kinase C (PKC) (44). Studies have shown that PKC isoforms, such as PKC ϵ , PKC δ and PKC η , were directly activated by PIP3 or through AKT activation (45,46), which in turn led to the phosphorylation of Raf, and ultimately, lead to ERK1/2 activation (47,48). These observations further suggested that the conversion of PIP3 to PIP2 by PTEN would negatively regulate ERK1/2 by preventing the activation of the AKT/PKC/Raf signaling axis (Figure 1), which indirectly connected PTEN and ERK1/2.

Lee *et al.* (49) demonstrated that AKT kinase directly associated with Raf and caused its inactivation by phosphorylating serine-259 in prostate cancer cells. A similar observation was made in breast cancer cells, where a high dose of insulin-like growth factor I (IGF-I) stimulated AKT and inhibited Raf through serine-259 phosphorylation, while a low dose of IGF-I abrogated these effects (50). In the same study, phorbol 12-myristate 13-acetate (PMA), a differentiation-inducing agent, activated the Raf/ERK pathway, but mildly activated the PI3K/AKT pathway, which did not trigger any cross-talk with Raf (50). It is possible that the differential regulatory role of AKT on the Raf/ERK1/2 pathway may be ligand and/or concentration-dependent.

Another mechanism by which PTEN may regulate ERK1/2 is through the Ras superfamily of small GTPases, which activates Raf. The coupling and phosphorylation of shc adaptor protein to a ligand-stimulated receptor recruits the Grb-SOS complex to the plasma membrane, which activates Ras, then Raf, and ultimately, ERK1/2 (51). Gu *et al.* (52) demonstrated that reconstitution of PTEN in glioblastoma cells dephosphorylated shc adaptor protein, which inhibited activation of ERK1/2. Complementary studies in glioblastoma models confirmed these observations and even reported a correlation between PTEN-mediated shc dephosphorylation and cell migration (53). Further, a study by Nogueira *et al.* (54) suggested that loss of PTEN is correlated with Ras activation and consequently resulted in melanoma development. The latter studies did not further define the involvement of ERK1/2 in cell migration and melanoma development; however, it is plausible to suggest that increased Ras activation lead to downstream ERK1/2 activity. Therefore, these results support that PTEN regulates ERK1/2 signaling, although indirectly by targeting upstream kinases.

Conclusion

Current knowledge of PTEN-mediated regulation favors its effects on the PI3K/AKT pathway. Emerging studies support a new paradigm that PTEN may also target the ERK1/2 signaling pathway; however, very little information definitely describes how this mechanism may occur. The diverse regulatory molecules of the ERK1/2 signaling pathway suggests its importance in cellular survival that can encourage tumor development; and for this reason, the involvement of PTEN would be integral in regulating these functions. PTEN-mediated regulation appears to be indirect through several molecules, such as PI3K/AKT, shc, PKC, Ras, and Raf, all of which cross-talk and dictate the activation status of the ERK1/2 (Figure 1). For instance, PI3K/AKT may activate ERK1/2 through PKC, while the phosphorylation of Raf by AKT may inhibit ERK1/2 function. The activity status of signaling molecules connected to ERK1/2 may differ among tissue types; hence, the role of PTEN in regulating ERK1/2 pathway may differ among tumor types. Considering that PTEN may modulate both AKT and ERK pathways, it is crucial to evaluate both the pathways in future research. Therefore, assessing the status of PTEN expression in tumors may improve treatment options; current treatments for PTEN-null tumors focus on antagonizing the PI3K/AKT pathway, but should now consider antagonizing ERK1/2 in a combinatory manner. To substantiate our observations, however, further studies are warranted to understand the detailed mechanisms by which PTEN regulates the ERK1/2 pathway.

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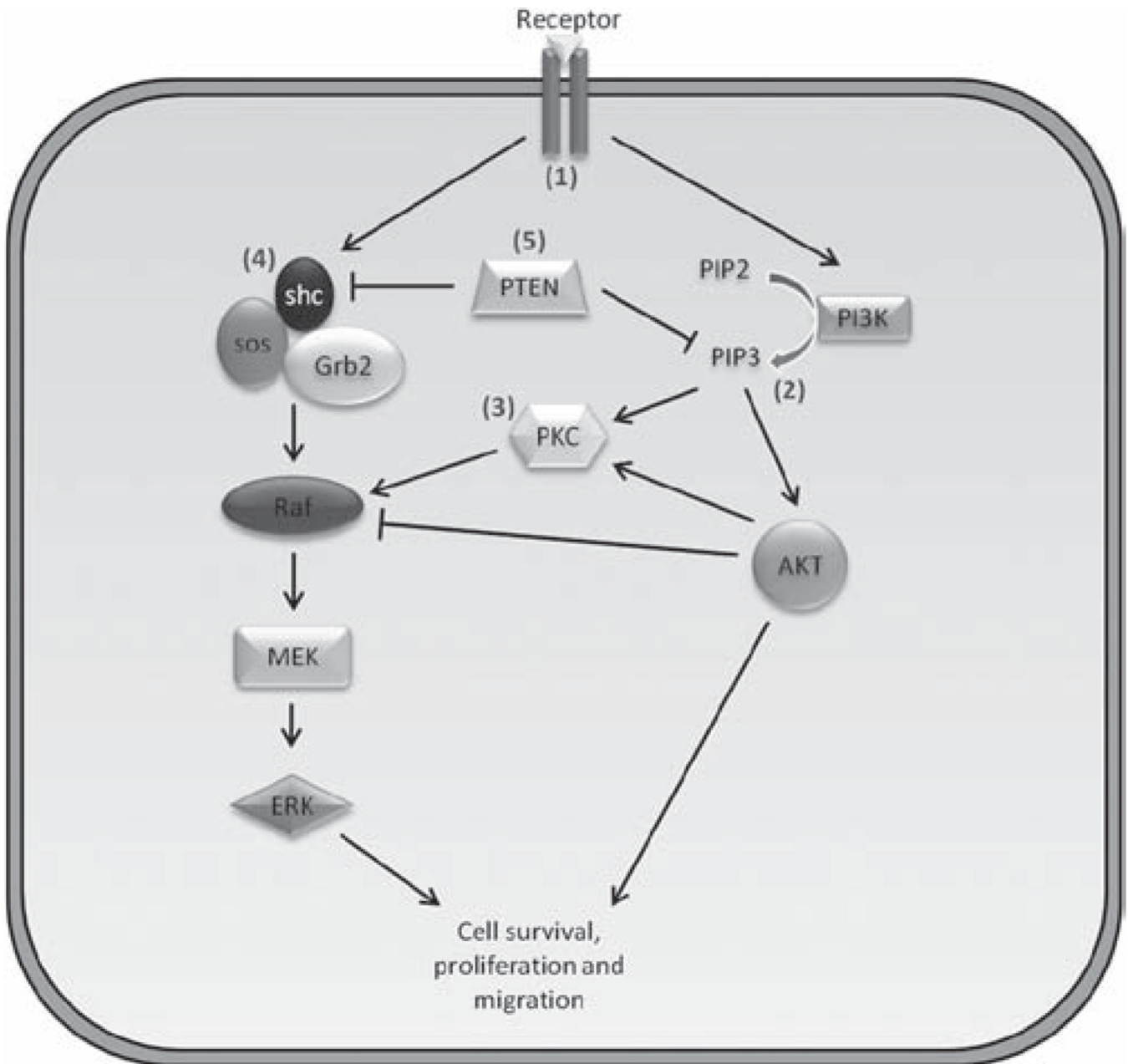


Figure 1.

A schematic of PTEN-mediated regulation of ERK1/2 in cancer cells. (1) The stimulation of a cell surface receptor results in the activation of PI3K (44) and shc complex (51). (2) An increase in PI3K function leads the accumulation of PIP3, which subsequently activates PKC directly (45) or indirectly through AKT (46) activation. (3) Increased PKC activity results in ERK1/2 activation and subsequent downstream functions (47). (4) Furthermore, the stimulation of shc results in the activation of Ras, Raf and ultimately, ERK1/2 (51). (5) PTEN regulates ERK1/2 by dephosphorylating shc and by reducing PIP3-mediated activation of PKC and AKT. PI3K, phosphoinositide 3-kinase; PIP3, phosphatidylinositol 3,4,5-triphosphate; AKT, protein kinase B; PKC, protein kinase C, shc, Src homology 2 domain-containing protein.