Acta Pharmaceutica Sinica B 2018;8(4):511–517

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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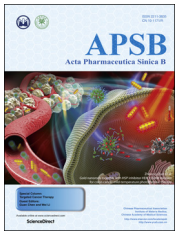

REVIEW

Targeting an oncogenic kinase/phosphatase signaling network for cancer therapy

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Received 28 February 2018; received in revised form 10 May 2018; accepted 10 May 2018

KEY WORDS

Kinase/phosphatase signaling network;
PDZ-coupled protein-protein complex;
Phosphorylation and dephosphorylation;
Cancer development and progression;
Targeted cancer therapy

Abstract Protein kinases and phosphatases signal by phosphorylation and dephosphorylation to precisely control the activities of their individual and common substrates for a coordinated cellular outcome. In many situations, a kinase/phosphatase complex signals dynamically in time and space through their reciprocal regulations and their cooperative actions on a substrate. This complex may be essential for malignant transformation and progression and can therefore be considered as a target for therapeutic intervention. p38γ is a unique MAPK family member that contains a PDZ motif at its C-terminus and interacts with a PDZ domain-containing protein tyrosine phosphatase PTPH1. This PDZ-coupled binding is required for both PTPH1 dephosphorylation and inactivation of p38γ and for p38γ phosphorylation and activation of PTPH1. Moreover, the p38γ/PTPH1 complex can further regulate their substrates phosphorylation and dephosphorylation, which impacts Ras transformation, malignant growth and progression, and therapeutic response. This review will use the p38γ/PTPH1 signaling network as an example to discuss the potential of targeting the kinase/phosphatase signaling complex for development of novel targeted cancer therapy.

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Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

https://doi.org/10.1016/j.apsb.2018.05.007

1. Introduction

Kinases play a critical role in transmission and amplification of oncogenic and extracellular signaling and are considered as an attractive target for therapeutic intervention[1]. Kinases are typically inactivated by dephosphorylation *via* a phosphatase, which is essential for termination of an upstream proliferative signal. A dysregulation of kinase/phosphatase signaling cross-talk contributes to malignant development and progression[2], and dissecting their signaling interaction events may reveal a novel strategy for cancer therapy by targeting cancer-specific pathways/networks[3].

The spatial and temporal organization of molecules within a cell is critical for the efficient coordination and integration of their activities into a specific response[3]. Scaffold proteins organize functional complexes, modulate enzyme activities, and fine-tune signaling output by locally concentrating relevant proteins and avoiding their non-specific interactions[4]. The kinase suppressor of Ras 1 (KSR1) scaffold, for example, assembles RAF, MEK1/2 (MAP2K1/MAPK2K2) and ERK1/2 (MAPK3/MAPK1) to increase signaling efficiency and to control the normal function of the ERK pathway[1,5]. Targeting scaffold proteins has been considered an efficient and novel approach for the development of cancer therapies[6].

PSD-95/Dlg/ZO-1 homology (PDZ) binding occurs between a PDZ-domain containing protein and a protein with a PDZ-binding motif[7] and is an important mechanism for scaffold protein formation[8]. p38γ (MAPK12) is a member of mitogen-activated protein kinases (MAPKs) with a unique C-terminal PDZ-binding motif (-ETXL)[9–11]. While early studies classified p38γ as a stress kinase[12,13], recent research has shown that p38γ plays an important role in transformation and cancer development and growth[9,14,15]. This review will present recent discoveries about p38γ signaling through PDZ-coupled interaction with its phosphatase protein tyrosine phosphatase H1 (PTPH1) and with their respective individual and common effectors with a focus on their signaling dynamics and integration. We hope that this knowledge may serve as a platform for developing novel cancer therapeutics by targeting an oncogenic kinase/phosphatase signaling network.

2. PDZ-coupled p38γ/PTPH1 interaction in Ras oncogenesis

p38 MAPK family proteins (α, β, γ, and δ) are encoded by four separate genes (*MAPK14*, *MAPK11*, *MAPK12*, and *MAPK13* respectively) and play overlapping, distinct, and even opposite roles in regulating cell growth, cell death, and differentiation [14,16,17]. Among 15 classical and nonclassical MAPKs, p38γ is the only MAPK with PDZ motif at C-terminus[18,19], structurally indicating its specific activities[20]. Early studies have shown that p38γ is involved in differentiation[18], stress response[11], and G2/M cell cycle transition[21]. Although p38γ depends on its C-terminal PDZ motif to interact with and phosphorylate several PDZ-domain proteins, including α1-syntrophin (SNTA1)[10], synapse-associated protein 90/postsynaptic density protein 95 (DLG4)[22], and SAP97 (DLG1)[23], the functional consequence of these complexes in cell growth or death remains mostly unknown.

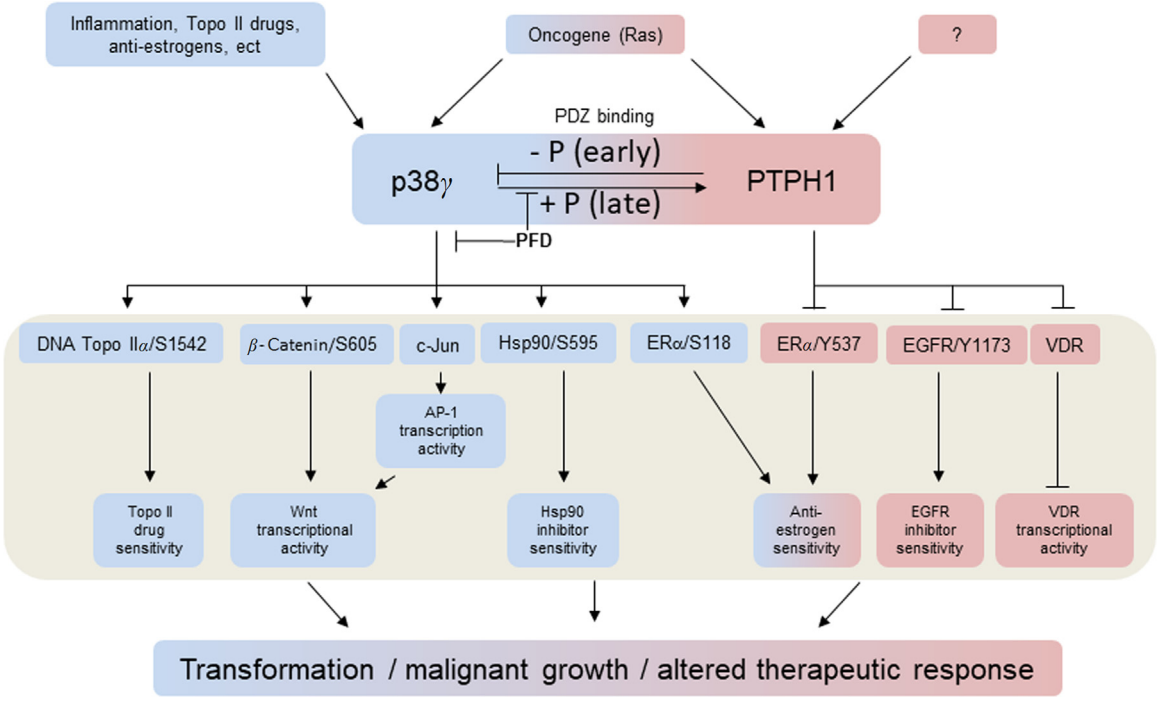

Figure 1 The p38γ MAPK/PTPH1 phosphatase signaling complex in regulation of transformation, malignant growth, and therapeutic response. p38γ and PTPH1 are activated in response to K-Ras oncogene and are both required for Ras transformation in which PTPH1 dephosphorylates p38γ (likely in early stage) and p38γ phosphorylates PTPH1 at S459 (likely in late stage). p38γ can be further activated by indicated extracellular stimuli, whereas activating signals for PTPH1 are unknown (?). Furthermore, p38γ can stimulate Topo IIα, β-catenin, Hsp90 and ER phosphorylation at indicated residues, whereas PTPH1 can catalyze tyrosine dephosphorylation of ER and EGFR, restore their natural cellular localization and increase VDR cytoplasmic accumulation. Through individual and common effectors, the p38γ/PTPH1 signaling complex regulates transformation, malignant growth and therapeutic responses, and may be targeted by the p38γ inhibitor PFD for therapeutic intervention.

p38γ RNA/protein expression is induced by the *K-Ras* (*KRAS*) oncogene in intestinal epithelial cells and the depletion of p38γ by siRNA blocks K-Ras transformation[24]. Of interest, transient co-expression analyses have shown that oncogenic K-Ras decreases p38γ phosphorylation but increases phosphorylation of its isoform p38α, indicating their coordinative and perhaps even opposite action in Ras transformation[24]. Because p38α is a tumor suppressor[25], these results indicate that upregulated p38γ may antagonize the p38α activity to promote K-Ras oncogenesis through a process involving p38γ dephosphorylation[24,26]. To search for a p38γ-specific phosphatase, wild-type and PDZ motif deleted p38γ were used for two-hybrid screening of human colon cDNAs. p38γ, but not its PDZ-deleted mutants, was found to interact with a PDZ-domain containing protein tyrosine phosphatase H1 (PTPH1) (gene name: *PTPN3*) by which p38γ is dephosphorylated *in vitro* and *in vivo*[27] (Fig. 1). PTPH1 is a nonmembrane tyrosine phosphatase containing a single PDZ domain[28]. Significantly, K-Ras transformation stimulates protein expression of both p38γ and PTPH1 and knockdown of either p38γ or PTPH1 or disruption of their interaction by a peptide or expressing a PDZ binding-deficient mutant inhibits the malignant transformation and/or growth in cell culture and/or in nude mice[27,29]. Furthermore, elevated p38γ in human colon cancer specimens is correlated with up-regulated PTPH1, highlighting the critical role of the p38γ/PTPH1 complex in K-Ras-dependent colon cancer development and growth[27].

To investigate if the PDZ-coupled complex reciprocally regulates the phosphatase activity, PTPH1 proteins were screened for potential phosphorylation by mass spectrometry after *in vitro* incubation with p38γ. PTPH1 was found to be phosphorylated at S459 by p38γ through PDZ binding[30]. Importantly, this phosphorylation is important for K-Ras transformation, for K-Ras dependent colon-cancer growth, and for stress-induced cell-death independent of other major MAPK pathways[30]. Since levels of phosphorylated forms of p38γ and PTPH1 proteins are both elevated in colon cancer cells containing mutated K-Ras as compared to those containing only wild-type K-Ras[30], these results indicate a critical role of p38γ phosphorylation of PTPH1, but not of p38γ dephosphorylation by PTPH1, in maintaining the transformed phenotype and malignant growth[15]. Of interest, PTPH1 dephosphorylates p38γ independent of phosphorylation at S459. This serine phosphorylation, however, is required for PTPH1 to catalyze Epidermal Growth Factor Receptor (EGFR) tyrosine dephosphorylation, thus propagating p38γ signaling by its stimulation of substrate-specific PTPH1 catalytic activity[30]. Reciprocal allosteric regulation of p38γ and PTPH1 *via* PDZ binding was recently further demonstrated by crystal-structure analysis[31]. Together, these results indicate a role of PTPH1 dephosphorylating p38γ in early stage of Ras transformation such as cell proliferation and morphological alterations[24,27] and a role of p38γ phosphorylating PTPH1 in late stage of Ras oncogenesis through maintaining the malignant phenotype and stimulating malignant invasion[30] (Fig. 1). Therefore, the PDZ-coupled p38γ/PTPH1 complex may promote K-Ras oncogenesis by a stage-specific mechanism. Because p38γ and PTPH1 can act on individual substrates and/or partners, below we will discuss their effector pathways to further understand their integrated biological activities.

3. p38γ phosphorylation of substrates and stimulation of key transcriptional programs

3.1. *c-Jun/AP-1 pathways*

c-Jun (JUN) and AP-1 (the transcription factor complex of which c-Jun is a part) play an important role in the regulation of gene expression by MAPKs[32,33]. p38γ is required for MAP3K8- and RHOA-induced activation of c-Jun promoter[34,35]. Moreover, p38γ expression alone is sufficient to stimulate c-Jun promoter activity through AP-1 and MEF2 binding sites[36,37]. The work by Loesch et al.[37] further showed that p38γ depends on both its C-terminal PDZ motif and phosphorylation to bind and to trans-activate c-Jun, which is essential for basal AP-1 transcription activity. Further studies revealed that p38γ increases cell invasion and stimulates matrix metalloproteinase 9 (*MMP9*) promoter activity *via* AP-1[37,38]. Recent analyses further showed that through interaction with c-Jun, p38γ is recruited to gene promoters of several oncogenic molecules at AP-1 sites, including *MMP9*[37], *cyclin D1* (*CCND1*)[39], *NANOG*[40] and *EGFR* [41]. These results together indicate that p38γ may further stimulate oncogenic processes through c-Jun/AP-1 dependent transcriptional activation, resulting in cancer-like stem cell (CSC) expansion, malignant invasion, and/or alterations in therapeutic response (Fig. 1).

3.2. *β-catenin/Wnt pathways*

β-Catenin (CTNNB1) is a central component of Wnt signaling and plays a critical role in colon cancer development and progression by stimulating Wnt transcription activity[42]. Conditional p38γ knockout (KO) from intestinal epithelial cells (IECs) decreases expression of pro-inflammatory cytokine, β-catenin, and Wnt target genes in colon tissues, and attenuates colon tumorigenesis in an azoxymethane(AOM)/dextran sodium sulfate (DSS) mouse model[29]. Studies with a whole body knockout of *p38γ*, *p38δ*, and both together also attenuate inflammation-induced colon and skin cancer[43,44]. Further analyses have shown that p38γ binds β-catenin and increases its protein stability by stimulating its S605 phosphorylation and thereby decreasing its proteasome-dependent degradation[29]. Moreover, inflammation stimulates p38γ and β-catenin phosphorylation and β-catenin/S605 is required for p38γ dependent stimulation of Wnt transcriptional activity and for colon cancer growth[29]. Because there is an active cross-talk between c-Jun/AP-1 and β-catenin/Wnt signaling[45] and p38γ binds both c-Jun and β-catenin proteins[29,37], p38γ MAPK may mediate the c-Jun/β-catenin signaling crosstalk through a complex formation to promote colon tumorigenesis (Fig. 1).

3.3. *Estrogen receptor α (ER) pathways*

Estrogen receptor α (ESR1) is a nuclear receptor of estrogens and an important target for antiestrogen therapy in breast cancer[46]. p38γ antagonizes ER activity downstream of Ras to stimulate breast cancer invasion[47]. Further studies have shown that p38γ binds and phosphorylates ER at S118 and forms a complex with ER and c-Jun on cyclin D1 promoter [39]. p38γ-induced ER/S118 phosphorylation is important for p38γ to inhibit the classical ER pathway and stimulate the non-classical ER (AP-1 dependent) pathway activities[39]. However, ER binds both c-Jun[48] and p38γ[39], the cellular outcome of p38γ/ER interaction may regulate breast cancer growth by a context and/or or

environment-specific mechanism. Indeed, treatment of breast cancer cells with the ER inhibitor tamoxifen (TAM) stimulates p38γ interaction with both ER and c-Jun, and p38γ overexpression increases while its depletion decreases breast cancer hormone sensitivity[39]. These results indicate a critical role of the p38γ/ER/c-Jun complex in determining sensitivity to antiestrogens. Moreover, the signaling interaction between p38γ and ER is reciprocally antagonistic, as they suppress each other's expression[39,47], and p38γ promotes invasion and metastasis in ER negative and triple-negative breast cancer (TNBC)[39,47,49,50]. However, whether there is a functional p38γ/ER/c-Jun complex in clinical breast cancer to regulate hormone sensitivity remains unknown. Of note, p38γ forced-expression alone induces TNBC transformation *via* stimulation of c-Jun/AP-1/Nanog-dependent cancer stem-like cell (CSC) expansion, whereas its silencing and pharmacological inhibition block TNBC growth and metastasis[40]. Thus, targeting p38γ may be a novel strategy for the treatment of TNBC, which warrants further investigations.

4. p38γ phosphorylating and activating other key signaling molecules in cancer

4.1. *Heat shock protein 90 (Hsp90)*

Hsp90 is an important chaperone to protect oncoproteins from proteasome-dependent degradation and its inhibitors are currently explored as novel agents in cancer therapy[51,52]. Proteomic analysis of p38γ precipitates identified a mutant K-Ras-dependent interaction of p38γ with Hsp90 in colon cancer cells[53]. Importantly, this complex contains mutated, but not wild-type, K-Ras protein, and p38γ protects the oncoprotein from degradation by phosphorylating Hsp90 at S595[53]. Further analysis showed that Hsp90/S595 is important for stabilizing mutated (but not wild-type) K-Ras protein against proteasome-dependent degradation[53]. Significantly, high levels of p38γ proteins in K-Ras mutant colon cancer cells are required to maintain endogenous mutant, but not wild-type, K-Ras protein expression, and targeting p38γ by shRNA or its specific pharmacological inhibitor pirfenidone (PFD) selectively inhibits K-Ras-dependent colon cancer growth *in vitro* and *in vivo*[53]. These results, together with the reported role of Hsp90 in stabilizing K-Ras oncoprotein[54,55], indicate that the Hsp90/S595 phosphorylation-dependent p38γ/Hsp90/K-Ras complex may functionally drive K-Ras-dependent malignant growth and thus may be a novel therapeutic target for K-Ras mutated cancer[53].

4.2. *DNA Topoisomerase IIα (Topo IIα)*

Topo IIα (TOP2A) is an important therapeutic target for cancer chemotherapy and inclusion of Topo II inhibitors (such as Adriamycin: ADR; etoposide: VP16) is a standard therapeutic regimen in clinic for many types of cancers[56]. However, critical determinants for therapeutic response to Topo II drugs are largely unknown[57]. Studies showed that treatment of breast cancer cells with Topo II inhibitors, but not with the anti-microtubule drug paclitaxel (taxol), increases p38γ, but not p38α, phosphorylation; p38γ expression increases (and its depletion decreases) breast cancer sensitivity to Topo II drugs[58]. Topo IIα is a nuclear DNA-associated protein [56]. In contrast to p38α, phosphorylated p38γ is mostly accumulated in the nucleus[26,59]. Further, the Ras oncogene stimulates both *p38γ*[24] and *Topo IIα* gene expression,[60] and Ras-transformed cells are more sensitive to Topo II inhibitors[61]. These findings suggest that p38γ activation may mediate a cellular positive feedback loop between Topo II and its inhibitors in which p38γ activation increases the growth inhibition by Topo II drugs by phosphorylating and activating their target enzyme Topo II. Indeed, p38γ binds, phosphorylates Topo IIα at S1542, and thereby increases its protein stability and catalytic activity[58]. In addition, elevated p38γ in breast cancer tissues is correlated with increased Topo IIα expression[58]. These results together indicate that increased p38γ expression in cancer cells may be a good marker for their sensitivity to Topo II inhibitors.

5. PTPH1 dephosphorylation of substrates and regulation of protein localization and/or activity

5.1. *PTPH1 dephosphorylates estrogen receptor α*

ER activity is regulated by phosphorylation at multiple residues[46]. Studies have shown that Y537 is important for ER dimerization[62] and nuclear export[63]. PTPH1 catalyzes ER/Y537 dephosphorylation *in vitro* and *in vivo* and thereby increases ER nuclear translocation and turnover[64]. Furthermore, PTPH1 increases breast cancer sensitivity to the antiestrogens tamoxifen and fulvestrant in cell culture and in breast cancer xenograft[64]. Because increased p-ER/Y537 is associated with a poor response to tamoxifen in clinic[65], these results suggest a novel therapeutic strategy to increase breast cancer hormone sensitivity by PTPH1-mediated ER/Y537 dephosphorylation.

5.2. *PTPH1 dephosphorylates EGFR*

Epidermal growth factor receptor (EGFR) belongs to the plasma membrane receptor tyrosine kinase family, plays an important role in cancer development and progression, and is a key molecule for targeted cancer therapy[66]. Previous studies showed that EGFR interacts with ER in breast cancer cells and thereby results in resistance to antiestrogens[67]. Ma et al.[68] showed that PTPH1 catalyzes EGFR/Y1173 dephosphorylation, disrupts the EGFR/ER interaction, and thereby increases breast cancer sensitivity to EGFR inhibitors (lapatinib and gefitinib). Of great interest, PTPH1 depends both on phosphatase activity and phosphorylation at S459 to dephosphorylate EGFR, to bind EGFR and ER, to increase ER nuclear and EGFR membrane localization, and thereby to increase breast cancer sensitivity to tamoxifen and/or lapatinib[68]. Together, these results suggest a strategy to sensitize breast cancer cells to EGFR/ER-targeted therapies through PTPH1-induced tyrosine dephosphorylation and disruption of their inhibitory complex leading to restoration of their natural localizations (nuclear ER and membrane EGFR)[68].

5.3. *PTPH1 regulates vitamin D receptor (VDR)*

VDR is a nuclear receptor of vitamin D3 which interacts with multiple proteins for its physiological and pathological activities[69]. VDR is typically expressed and functions in vitamin D3 target tissues and cells[70]. Studies have shown that VDR can be transactivated by p38 and JNK stress pathways[71] and in turn inhibits stress-induced cell death through interaction with c-Jun[72,73]. Thus, VDR may have broader biological effects. Nuclear localization is critical for classical VDR activity[74]. In contrast to stimulating ER nuclear translocation[64,68], PTPH1 binds VDR and increases cytoplasmic VDR accumulation in the presence and absence of vitamin D3, which is important for PTPH1-induced increases in breast cancer growth and for VDR transcriptional activity[75]. Mechanisms for PTPH1/VDR interaction, however, remain unclear. Together with the regulatory effects on ER and EGFR

as discussed above, these results indicate that an important property for oncogenic PTPH1 in cancer cells may involve regulation of cellular localization of key signaling proteins.

6. p38γ and PTPH1 cooperate to regulate ER and EGFR activity

ER/S118 phosphorylation is required for breast cancer sensitivity to antiestrogens [76] and stimulation of this phosphorylation by p38γ correspondingly confers the sensitivity to tamoxifen[39]. Increased ER/Y537 phosphorylation, on the other hand, is associated with clinical resistance to tamoxifen[65] and a decreased Y537 phosphorylation by PTPH1 increases breast cancer hormone sensitivity[64,68]. Thus, p38γ and PTPH1 may cooperate to increase breast cancer sensitivity to antiestrogens by respectively stimulating ER/S118 phosphorylation and ER/Y537 dephosphorylation (Fig. 1). Although p38γ clearly stimulates PTPH1/S459 phosphorylation in colon cancer cells and in intestinal tissues[29,30,41,53], it remains to be determined if p38γ actively and positively regulates p-PTPH1/S459 expression in breast cancer cells and if it thereby stimulates ER/Y537 dephosphorylation.

EGFR is dephosphorylated by PTPH1, but trans-activated by p38γ, resulting in increased levels of non-phosphorylated EGFR protein in K-Ras mutant colon cancer cells[41]. Moreover, p38γ and PTPH1 are both responsible for EGFR inactivation and for intrinsic resistance to lapatinib in K-Ras mutant colon cancer cells by stimulating c-Jun-dependent *EGFR* transcription and PTPH1-dependent EGFR dephosphorylation[41]. Because p38γ depends on its phosphorylation and C-terminal PDZ motif to bind c-Jun [37], PTPH1[27] and EGFR[41], these results indicate a role of the p38γ-driven and PDZ-coupled p38γ/PTPH1/c-Jun/EGFR complex to coordinatively stimulate *EGFR* transcriptional activation and posttranslational dephosphorylation. Thus, the p38γ/PHPH1 complex may confer the insensitivities of K-Ras mutant cancer cells to tyrosine kinase inhibitors through a scaffold with the membrane receptor EGFR and the nuclear transcription factor c-Jun[41] (Fig. 1).

7. Targeting the p38γ/PTPH1 signaling complex for cancer therapy

p38γ and *PTPH1* increase the malignant growth in cell culture and xenografts as demonstrated by knockdown and overexpression experiments with colon and breast cancer cells[27,29,30,40,41,64,68,75]. Because p38γ depends on its phosphorylation to bind and activate its proliferative effectors, including PTPH1, c-Jun, Hsp90, ER, Topo II and β-catenin (Fig. 1), inhibition of its activity by pirfenidone (PFD), a pharmacological inhibitor, has been tested *in vitro* and *in vivo* for cancer prevention and treatment. PFD is a selective inhibitor of p38γ with minimal effects on p38α or p38β or other MAPKs[77]. Because of its strong anti-fibrotic and anti-inflammatory activities, PFD is FDA-approved for the treatment of idiopathic pulmonary fibrosis (IPF)[78–82]. Therefore, investigation of regulatory effects of PFD in cancer development and growth depending on p38γ will have a great translational potential.

In colon cancer cells, treatment with PFD inhibits growth in cell culture[30] and in xenografts[53]. Because phosphorylated p38γ is up-regulated in K-Ras mutant cells over those without K-Ras mutation[30], the growth-inhibitory activity of PFD appears to be more evident in colon cancer expressing the mutated oncoprotein[53]. However, in human breast cancer cells, PFD suppresses TNBC growth[40] but attenuates ER positive breast cancer sensitivity to tamoxifen[39]. Therefore, PFD may only have therapeutic potentials in breast cancer without ER expression. Recent studies showed that chronic application of PFD attenuates inflammation-induced colon cancer in mice but not in those with conditionally p38γ knockout from intestinal epithelial cells[29]. Moreover, PFD increases the sensitivity of K-Ras mutant colon cancer xenograft to lapatinib in mice[41]. Because PFD is relatively nontoxic, its cancer therapeutic potentials warrant further investigation.

Disruption of the PDZ-coupled p38γ/PTPH1 complex by a peptide (targeting the p38γ C-terminus) was previously shown to inhibit K-Ras mutated colon cancer growth *in vitro*[27]. Because K-Ras mutant colon cancer cells contain higher levels of total and phosphorylated p38γ proteins with an increased PTPH/p38γ complex-formation[30], a specific disruption of the p38γ/PTPH1 complex by a peptide may be a novel therapy for the treatment of K-Ras mutant colon cancer. This potential is further suggested by a growth inhibitory activity of a PDZ peptide targeting the PDZ domain of PTPN4 in tumor cells[83] and by the unique reciprocal regulatory effect of the PDZ-coupled p38γ/PTPH1 complex[31].

8. Perspectives of targeting the p38γ/PTPH1 signaling complex

Proteins signal in time and space, which may be typically displayed by dissecting the dynamic, antagonistic and cooperative relationship between a kinase and a phosphatase[3]. PTPH1 is the only known p38 MAPK isoform-specific phosphatase which interacts through PDZ binding[27]. In Ras oncogenesis, this kinase/phosphatase nodule is one-way regulated by a stage-specific mechanism, as PTPH1 dephosphorylates and inactivates p38γ as an early event, whereas p38γ phosphorylates and activates PTPH1 and other substrates (but not itself) later in the process (Fig. 1)[27,30]. Although p38γ phosphorylating PTPH1 has been demonstrated in different systems, PTPH1-induced p38γ dephosphorylation is only observed in limited situations[24,27]. Moreover, p38γ and PTPH1, and their effectors may be further activated and inactivated by yet unknown partners. It is therefore critical to further define when this PDZ-coupled complex promotes cancer growth through p38γ-induced PTPH1 phosphorylation and when through PTPH1-induced p38γ dephosphorylation in a more clinical relevant model to further demonstrate its therapeutic target activity (Fig. 1).

Acknowledgements

This was supported by grants (R01 NIH CA91576, Departments of Veterans Affairs (VA) Merit Review Grant 1I01BX002883, Department of Defense Grant BC141898, and Cancer Center of Medical College of Wisconsin). We sincerely thank the former Chen lab members for their great contributions.

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