Targeting protein phosphatase methylesterase 1 to induce apoptosis in NSCLC cells through its ubiquitylation and proteasomal degradation

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Abstract:

Protein phosphatase methylesterase 1 (PPME1) negatively regulates protein phosphatase 2A (PP2A) by demethylating its catalytic subunit. In our previously study, we found afatinib has antitumor activity in certain NSCLC cell lines by regulating Elk-1/CIP2A/PP2A/Akt pathway. Here, we found nintedanib, an inhibitor of angiokinase, can enhance the antitumor activity of afatinib by suppressing PPME1 expression. The detail mechanism of the synergistic effects is unclear. Therefore, we aim to characterize the mechanism for the antitumor effects related to PPME-1 inhibition. Four EGFR wild-type NSCLC cell lines were used in vitro to test the cytotoxicity of the combination treatment. The PPME1 expressions in NSCLC cells were significantly down-regulated by combined treatment. Flow cytometry, western blot, and caspase activity ELISA assay were used to detect the anti-tumor effects. PP2A activity assay, siRNA strategy, QPCR, and Co-IP assays were performed to determine the mechanism of combination efficacy. Furthermore, in vivo efficacy of combination treatment against xenografts tumors in nude mice were performed. The regulation of PPME1 was studied to analysis the levels of PPME1, demethylated PP2A, and ERK phosphorylation in IHC samples from NSCLC patients. Higher levels of PPME1 and demethylated PP2A were associated with poor survival in patients with NSCLC. Combination treatment increased the anti-tumor efficacy through PPME1 inhibition in a dose- and time-dependent manner. Combination treatment showed synergistic apoptotic effect by enhancing PP2A activity and reducing phospho-ERK. Attenuated PPME1 accelerates antitumor activity that involved the ubiquitin-proteasome degradation process. Our results provide in vitro and in vivo evidences for the therapeutic potential of afatinib and nintidanib combination in EGFR wild type NSCLC treatment. Our findings disclose the therapeutic mechanism and suggest developing PPME1 inhibitor as a novel anti-cancer strategy.

Introduction:

Phosphatases and kinases control the reversible phosphorylation of proteins, a central mechanism in the regulation of cellular signal transduction. The four classes of phosphatases are 1) protein serine/threonine phosphatases, 2) protein tyrosine phosphatases (PTPs), 3) dual specificity phosphatases, and 4) histidine phosphatases. The class of the phosphoprotein phosphatase (PPP) family is the largest of the serine/threonine family and includes PP1, PP2A, PP2B, PP4, PP5 and

PP6 (Shi, 2009). The combined activity of the PPP family makes up the majority of the serine/threonine phosphatase activity within a cell.

Since protein phosphorylation has profound effects on every aspect of cell biology, precise balance between the activities of kinases and phosphatases is required for the proper regulation of cell function. Strikingly, such balance is not seen in scientific literature, where published articles with “kinase” outnumber those with “phosphatase” in their titles by a factor of almost ten to one. There are several possible explanations for such bias. By definition phosphorylation is an energy-dependent process requiring the consumption of ATP. For reasons of energy economy, evolution may have favored use of protein phosphorylation as a means of response to perturbation, and dephosphorylation as a means of restoring or maintaining equilibrium. This generalization seems broadly true, although many readers will readily think of exceptions. In this perspective, phosphatase activity may be seen as rather non-specific, unregulated, and consequently not very interesting as a subject of study. Recent advances suggest that these are all misconceptions. Inhibition of kinases to prevent harmful cell activation is more conceptually and practically straightforward than stimulation of phosphatases to promote restoration of homeostasis. Much like the scientific literature, patent literature demonstrates strong bias towards targeting of kinases rather than phosphatases. In this review we argue that improved understanding of cell signalling may open up new opportunities to exert therapeutic effects via activation of the phosphatase(s) PPME1.

Methods:

Protein phosphatase methylesterase 1 (PPME1) negatively regulates protein phosphatase 2A (PP2A) by demethylating its catalytic subunit. In our previously study, we found afatinib has antitumor activity in certain NSCLC cell lines by regulating Elk-1/CIP2A/PP2A/Akt pathway. Here, we found nintedanib, an inhibitor of angiokinase, can enhance the antitumor activity of afatinib by suppressing PPME1 expression. The detail mechanism of the synergistic effects is unclear. Therefore, we aim to characterize the mechanism for the antitumor effects related to PPME-1 inhibition.

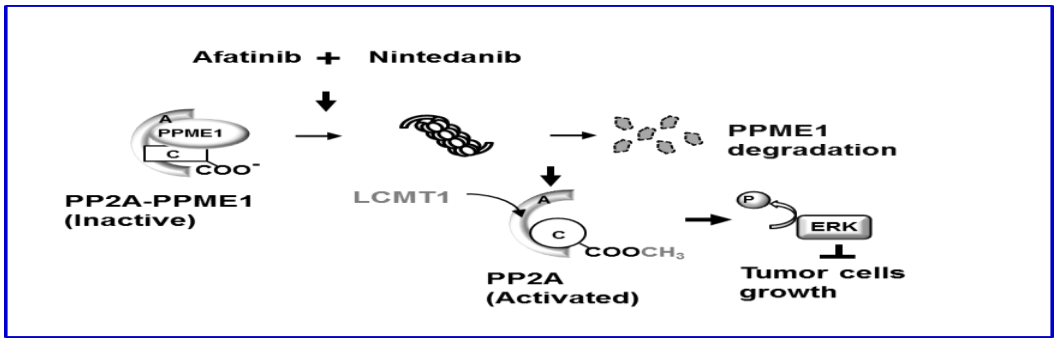
Four EGFR wild-type NSCLC cell lines were used in vitro to test the cytotoxicity of the combination treatment. The PPME1 expressions in NSCLC cells were significantly down-regulated by combined treatment. Flow cytometry, western blot, and caspase activity ELISA assay were used to detect the anti-tumor effects. PP2A activity assay, siRNA strategy, QPCR, and Co-IP assays were performed to determine the mechanism of combination efficacy. Furthermore, in vivo efficacy of combination treatment against xenografts tumors in nude mice were performed. The regulation of PPME1 was studied to analysis the levels of PPME1, demethylated PP2A, and ERK phosphorylation in IHC samples from NSCLC patients.

PPME1 and cancer:

As reviewed in depth elsewhere, impairment of PPME1 function is a very common feature of many cancers, therefore PPME1 may be considered as a tumor suppressor (Carratu, Signorile, De Rasmo, Reale, & Vacca, 2016; Grech et al., 2016; Haesen et al., 2014; Janssens & Rebollo, 2012; O'Connor, Perl, Leonard, Sangodkar, & Narla, 2017; Ruvolo, 2016; Westermarck & Hahn, 2008). Dysregulated phosphorylation and function of PPME1 substrates contributes to several hallmarks of cancer (Hanahan & Weinberg, 2011), including inflammation as discussed in detail below. In cancer, many different routes to functional impairment of PPME1 have been described, reflecting the magnitude of the advantages that can be gained in terms of growth, survival and metastasis. (1) Okadaic acid, calyculin A and microcystin-LR are toxins derived from aquatic microorganisms, which exert tumor-promoting effects by directly inhibiting PPME1 (Fujiki & Suganuma, 2009). These and related compounds can be useful for investigating functions of PPME1, although their effects must be interpreted with caution because of their imperfect specificity for PPME1 (Swingle, Ni, & Honkanen, 2007). (2) Small and middle T antigens of DNA tumor viruses also directly inhibit PPME1 function, promoting proliferation of infected cells and conferring a replicative advantage to the virus (Chen et al., 2007; Cho et al., 2007; Guergnon et al., 2011; Sablina & Hahn, 2008). (3) Oncogenically dysregulated tyrosine kinases impair PPME1 function (Chen et al., 1994; Zonta et al., 2015), although the precise mechanism remains uncertain as noted above. (4) Expression of specific B subunits is decreased through chromosomal deletion, epigenetic or micro-RNA-mediated silencing (Ruvolo, 2015).

Results:

Higher levels of PPME1 and demethylated PP2A were associated with poor survival in patients with NSCLC. Combination treatment increased the anti-tumor efficacy through PPME1 inhibition in a dose- and time-dependent manner. Combination treatment showed synergistic apoptotic effect by enhancing PP2A activity and reducing phospho-ERK. Attenuated PPME1 accelerates antitumor activity that involved the ubiquitin-proteasome degradation process.



Conclusion:

Our results provide in vitro and in vivo evidences for the therapeutic potential of afatinib and nintidanib combination in EGFR wild type NSCLC treatment. Our findings disclose the therapeutic mechanism and suggest developing PPME1 inhibitor as a novel anti-cancer strategy.