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All roads lead to PP2A: Exploiting the therapeutic potential of this phosphatase

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Abstract

Protein phosphatase 2A is a serine/threonine phosphatase involved in the regulation of many cellular processes. A confirmed tumor suppressor protein, PP2A is genetically altered or functionally inactivated in many cancers highlighting a need for its therapeutic reactivation. In this review we will discuss recent literature on PP2A: the elucidation of its structure and the functions of its subunits, and the identification of molecular lesions and post-translational modifications leading to its dysregulation in cancer. A final section will discuss the proteins and small molecules that modulate PP2A and how these might be used to target dysregulated forms of PP2A to treat cancers and other diseases.

Introduction

Complex processes in cell signaling require a set of molecular tools to modulate the activity and localization of specific proteins. Many of these responsibilities are regulated by kinases and phosphatases, whose opposite actions reversibly phosphorylate proteins. Due to their involvement in the progression of many cancers, the study of kinases has become an important field and one that has drawn considerable attention from the pharmaceutical industry over the past decade and a half. Despite their abundance and variety of substrates, the 518 human kinases display a high degree of similarity, and most features of their structure are conserved. In contrast, phosphatases exhibit considerably more structural variety with only a few enzymes performing the majority of the work. In the past, phosphatases were considered to play a more passive, housekeeping role and received less

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attention. Recent studies solidify their importance but reveal the inherent difficulties in designing tools to improve their function.

There are multiple families of phosphatases with diverse active sites and mechanisms. The major classes of phosphatases are protein tyrosine phosphatases (PTPs) and protein serine/threonine phosphatases (PSPs). The protein serine/threonine phosphatases consist of three families: phospho-protein phosphatases (PPPs), metal-dependent protein phosphatases (PPM), and DxDxT phosphatases [1, 2]. PPP, the phosphoprotein phosphatase family, is the largest containing several members including PP1, PP2A, PP2B, and PP4.

Structure of the PP2A holoenzyme

PP2A is a heterotrimeric complex. It consists of a scaffolding subunit (A), a regulatory subunit (B), and a catalytic subunit (C) (Fig. 1). The A and C subunits each exist with two possible variants α and β, with Aα and Cα accounting for the majority of each subunit in most cells [3–5]. The four classes of the B subunit are: B (B55/PR55), B′ (B56/PR61), B″ (PR48/PR72/PR130), and B‴(PR93/PR110)/Striatin. Each class contains 2–5 isoforms and additional splice variants. This predicts over 80 distinct combinations of the PP2A holoenzyme. This multitude of forms regulates PP2A's activity and cellular localization and imparts specificity towards different substrates.

Structural contributions to PP2A activity

The PP2A A subunit is composed of 15 tandem HEAT repeats (Fig. 1). HEAT repeats are named for the set of four cytoplasmic proteins first recognized to contain them (Huntingtin, EF3, PP2A A subunit, and TOR1). Repeats contain approximately 40 amino acid residues organized into two anti-parallel α-helices. The helices are hydrophobic in nature enforcing their mutual attraction. In PP2A, the combination of these repeats forms a characteristic crescent structure [6]. The C subunit binds to HEAT repeats 11–15 of the A subunit [7]. The C subunit embodies a globular structure with an α/β fold. This structure is homologous to other PPP catalytic subunits, however, the subunits are not interchangeable among the different enzyme types [8]. The molecular basis for these interactions was described when the AC dimer, also known as the "Core Enzyme" crystal structure was solved [9]. The C subunit active site contains 2 manganese atoms that bind to phosphate and facilitate the hydrolysis of serine/threonine phosphate esters. The active site is positioned away from the ridge of the A subunit HEAT repeats and proximal to the site where the B subunits bind.

The core enzyme interacts differently with each class of B subunit (Fig. 2A). Crystal structures have been solved for the B, B′, B″ family of subunits while less is known about the B‴ family. The B/PR55 family contacts the scaffolding subunit via two extended interfaces (Fig. 2B). The first is a seven bladed propeller, each composed of a WD40 repeat. The second is a β-hairpin handle with additional secondary structures. The bottom face of the propeller binds to the HEAT Domains 3–7 of the A subunit, and the β-hairpin handle interacts with HEAT repeats 1 and 2. The top face of the propeller is the proposed substrate binding site. While proximal to it, the B/PR55 subunit makes very few contacts with the C subunit [10–12]. The structure of the B′/PR61 family of subunits is strikingly similar to the A subunit, containing 8 HEAT-like repeats (Fig. 2C). These interact with the A subunit at

HEAT repeats 2–8 and also interact with the C subunit. The B/PR55 and B′/PR61 subunits bind similarly to the core enzyme such that the substrate binding site lies on the top face of the B subunit proximal to C subunit active site. The proximity of these B subunits to the active site explains their role in conferring specificity for substrate proteins [9, 13].

The structures of the holoenzyme with B″ subunits (PR70/PR72) were solved recently (Fig. 2D). The B″ family consists of linear arrangements of different functional motifs with a substrate binding region near the C-terminus. This arrangement differs from the B and B′ whose substrate binding sites are found on their top surfaces and involve their structural repeats. The B″ family also includes an N-terminal hydrophobic motif with two EF hand calcium-binding motifs that bind to the A subunit at HEAT repeats 1–7. The subunit also contacts the C subunit near the active site via a helix (439–446) positioning the substrate binding site next to the active site [14].

The A subunit orchestrates formation of the active holoenzyme through its conformational flexibility. Binding with the C subunit shifts HEAT repeats 13–15 by 20–30 Å, while binding with the B′ subunit forces an N-terminal repeat to twist up to 50–60 Å and rearranges the hydrophobic core within A [9, 13]. For the B″ family, the A subunit adopts a compact conformation relative to other holoenzymes, reducing its width and increasing its height. An additional helix domain of PR70 extends beyond the A subunit making it wider than the other holoenzymes [14].

Subunit stoichiometry and activity

PP2A constitutes approximately 1% of total cellular protein. The nature of its production, assembly, and subunit stoichiometry is still unclear. To address these questions, studies were conducted in yeast, which contain a smaller repertoire of subunit variants. The ratio of subunits (A:B:C) in yeast was originally determined to be 1:4:8, suggesting that production of A is a limiting factor [15, 16]. However, another study looking at global protein expression suggested the ratio was quite different; the ratio of subunits A:B:C instead being 17:9:10 [16, 17]. In mammalian cells, the A subunit is expressed in excess of the other subunits [18]. There is consensus that the monomeric C subunit is unstable and requires binding to the A subunit or other non-canonical B subunits to preserve its activity [19–21]. Moreover, binding to the A subunit may enhance the stability of the B subunits and fulfill other housekeeping roles [21–23].

Dysregulation of PP2A in diseases

PP2A related pathways are perturbed in many diseases. In both cancer and neurodegeneration, the common pathological mechanism involves activated kinase signaling pathways combined with loss of PP2A activity. In the case of neurodegeneration, PP2A dysfunction leads to increases in hyperphosphorylated tau. Tau protein normally stabilizes microtubules. Hyperphosphorylated tau is thought to play an important role in the etiology of Alzheimer's disease by forming neurofibrillary tangles. The PP2A Bα isoform is the primary tau binding phosphatase. PP2A mediates ~71% of total tau phosphatase activity in the human brain [24] and dephosphorylates abnormally phosphorylated tau at Ser46, Ser199, Ser202, Ser396, and Ser404 [25]. Alterations in PP2A regulating proteins, its catalytic

activity, subunit expression, methylation and phosphorylation patterns have been reported in Alzheimer's disease-affected brain regions. For example, reduced PP2A mRNA [26], protein levels [27], and phosphatase activity [25, 28] were observed in the brains of Alzheimer's patients. Increased phosphorylation of PP2A at Tyr307 has been found in phospho-tau-rich, tangle-bearing neurons [29]. Attempts to reduce this phosphorylation through kinase inhibition generated limited success clinically. Consequently, there is growing interest in developing PP2A-targeted therapies for Alzheimer's disease. These include disruption of inhibitory protein-protein interactions, modulation of disease relevant post-translational modifications, and allosteric activation [30, 31].

In contrast, PP2A performs the opposite role in diabetes. Where these pathways are functioning normally, glucose homeostasis requires the insulin-mediated activation of PI3K-AKT and downregulation of PP2A [32]. This leads to stimulation of the GLUT4 transporter resulting in uptake of glucose into skeletal muscle tissues. This decreased PP2A activity is absent in skeletal muscle tissues of individuals with type II diabetes and leads to impaired insulin sensitivity [33]. A plant derived natural product, carnosic acid, also discussed below, stimulates proper glucose metabolism by activation of AKT via C subunit demethylation [34]. Therefore, PP2A deactivation might be a useful target for ameliorating dysregulated glucose metabolism that is characteristic of type II diabetes.

There are additional reports that implicate a positive role for PP2A in inflammatory lung diseases like asthma and COPD [35] and in heart function [36]. These effects are attributed to PP2A's inhibitory effects on the mediators of inflammation, and on a variety of substrates involved in cardiac muscle contraction, respectively. Given that cancer, Alzheimer's disease, diabetes, asthma, and COPD claim millions of lives each year and cost billions to treat in the United States alone, developing drugs that modulate relevant targets such as PP2A in these indications is an attractive option.

Mechanisms of PP2A inactivation in cancer

PP2A's essential role in homeostasis can be inferred from the increasing number of disease states in which it is functionally inactivated. Numerous studies highlight the role of PP2A as a tumor suppressor and suggest that disruption of the PP2A holoenzyme may contribute to the development of cancer. In cancer, PP2A is inactivated through several mechanisms including: somatic mutation, phosphorylation and/or methylation of the C terminal tail of the catalytic subunit (Fig. 3) and through increased expression of endogenous PP2A inhibitors (Fig. 6) [37]. Several of the genetic alterations prevent the A subunit from binding to the B and/or C subunits, resulting in disruption of the core enzyme and complex [5, 38–40]. Understanding these defects will enhance the future development of PP2A targeted therapeutics by facilitating selection of the correct patient cohorts and suggesting effective combination therapies.

PP2A subunits and cancer: Mutation, deletion, inactivation, and aberrant expression

Scaffolding subunit: PP2A Aα and Aβ

Mutations have been detected in all subunits of PP2A in cancer, but the gene encoding the Aα subunit, PPP2R1A, has the highest mutation rate. PP2A is an essential enzyme, therefore cancer-associated PP2A Aα mutations in clinical specimens typically involve only a single allele [23, 41]. These mutations create a state of haploinsufficiency. Point mutations most commonly occur in the Aα subunit and ~30% of these mutations occur at a mutational hotspot within HEAT repeat 5. While the functional relevance of some of the identified Aα mutations have been studied, the significance of the mutations in this hot spot region has yet to be elucidated [39, 42]. Most reported PP2A Aα mutants are unable to bind the regulatory subunits, and, in particular, to members of the B′ family (B56) [38]. For example, when R418W and Δ171–589, two point mutations in Aα that were first detected in melanoma and breast carcinomas respectively, were studied, these mutations led to reduced binding to the C subunit and to all of the B subunits tested. Two additional mutants, E64D and E64G, specifically lost efficient binding to the B56 family subunits [5, 39] and resulted in a state of happloinsufficiency in transgenic mice [23]. In addition, mice containing the E64D mutation showed an increased incidence of lung cancer when exposed to benzopyrene and decreased survival when crossed with $KRAS^{G12D}$ mice [39]. To date, mutations in PPP2R1A have been identified in breast, lung, melanoma, ovarian, endometrial, uterine, and colon cancers [41, 43–47] and decreased expression of Aα was detected in human gliomas [48]. (Table 1)

Akin to PP2A Aα mutations, cancer-associated Aβ mutations also induce haploinsufficiency and impaired binding to the B and C subunits [38–40]. The gene that encodes the β isoform of the A subunit, PPP2R1B, is located on a chromosomal band frequently deleted in cancer cells, 11q23 [72, 73]. According to a seminal paper by Wang *et al.,* which was the first to demonstrate PPP2R1B to be mutated in human cancers, the 11q23 gene locus displayed loss of heterozygosity in 30–50% of breast, lung, ovary, cervical carcinomas, melanomas, and in 15% of non-Hodgkin's lymphomas (NHL) and chronic lymphocytic leukemias (CLL) [49]. Many of the mutations in PPP2R1B are missense mutations including G8R, P65S, G90D, L101P, K343E, D504G, V545A, V448A. One contains the double mutant L101P/V448A and one contains an in frame deletion ΔE344–E388 [40, 74]. The ΔE344–E388 mutant was found to be incapable of binding to any of the B subunits tested [40, 74]. One mechanism of inactivation unique to the β isoform of the scaffolding subunit is abnormal RNA splicing leading to aberrant transcripts of PPP2R1B. Alternative splice variants of PP2A Aβ were observed in B-cell chronic lymphocytic leukemia (B-CLL). These aberrant transcripts were incapable of binding B and C subunits, which subsequently led to a loss of PP2A activity [54]. In addition, 29% of hepatocellular carcinoma (HCC) tumors and 3% of corresponding non-tumor tissues tested showed co-expression of wild-type and aberrant mRNA of PPP2R1B, suggesting that alternative splicing may facilitate the development of HCC [55].

Analogous to AKT, the GTPase RalA participates in transcription, migration, transport, apoptosis, and cell proliferation. PP2A Aβ binds and regulates the activity of RalA by dephosphorylating RalA at Ser183 and Ser194. This dephosphorylation leads to inactivation

of RalA, again highlighting that PP2A Aβ functions as a tumor suppressor. Mutations in Aβ disrupt this interaction leading to a constitutive activation of RalA that results in transformation [74].

In summary, extensive sequencing of human samples and cancer cell lines has revealed PPP2R1B to be mutated in many solid cancers including breast, lung, colon, melanoma, ovarian, cervical, HCC, NHL, CLL, and B-CLL. Although it is 40 times less abundant than Aα [75], PP2A Aβ clearly has a role in the tumor suppressor capabilities of PP2A as mutations and loss of expression of the PPP2R1B gene inhibit this activity. There has been some speculation that PP2A Aα can compensate for Aβ mutation and or loss of expression in cancer. However, several reports suggest that this is unlikely. While Aα and Aβ are 85% identical, they have vastly different B and C subunit affinities and have distinct biochemical properties [13, 40]. For instance, Aβ mutations show decreased binding to the B and some of the B′ regulatory family members but predominantly affect processes involving the catalytic subunit and regulatory PR72-containing holoenzyme. In contrast, Aα subunit mutations mostly affect pathways where B′-containing holoenzymes are instrumental [5, 38, 40]. In addition to this, complexes involving Aβ and regulatory subunits regulate the phosphorylation of specific substrates involved in cellular transformation that are distinct from pathways regulated by the Aα-regulatory subunit complexes [38]. Lastly, in experiments in transgenic mice, overexpression of Aα could not revert tumorigenesis that was induced by suppression of Aβ [5]. Together, this evidence strongly suggests that PP2A Aα and Aβ are functionally different and cannot compensate for each other.

Regulatory subunits: PP2A B, B′, B″, and B‴

Mutations in the regulatory subunits of PP2A occur at much lower frequencies than those in the A subunit and most commonly result in decreased expression of the B subunits. Other methods of inactivation include deletion, DNA hypermethylation, and one point mutation found in lung cancer. The F395C mutation detected in lung cancer occurs in a region necessary for PP2A-B56γ-p53 interaction. In cell culture based studies, the B56γ mutant protein was unable to interact with p53, thereby inhibiting its p53 dependent tumor suppressive functions [67]. A study of 141 prostate cancer samples using an Affymetrix SNP array found that PPP2R2A, which encodes the regulatory subunit, B55α, was deleted in 67.1% of the tumor samples tested. Moreover, homozygous deletions occurred in three of the prostate cancer samples [57]. Deletions in the PPP2R2A gene have also been reported in breast cancer and myeloma [56, 58].

In addition to mutations, loss of B subunit protein expression has been linked to cancer progression. Decreased expression of both PPP2R5A and PPP2R5C, which encode B56α and B56γ, respectively, were reported in melanoma, with the lowest levels of expression in metastatic tissues [65, 66, 76]. In addition, immunohistochemical analysis of PPP2R2C protein levels in primary prostate tumors determined that loss of PPP2R2C, which encodes B55γ, was highly correlated with metastasis and prostate cancer specific mortality (PCSM) [62]. Furthermore, in a cohort of 231 patients with acute AML, B55α expression was inhibited and was associated with increased AKT phosphorylation at threonine 308 and loss of complete hematological remission. B55α dephosphorylates AKT at T308 and when

suppressed, it leads to constitutive activation of AKT and enhanced proliferation. In a study by Ruvolo *et al.,* remission duration was evaluated in 231 newly diagnosed AML patients evaluated at the MD Anderson Cancer Center. Patients were divided into two groups based on their B55α protein expression level: B55α-high and B55α-low. Kaplan–Meier survival curves illustrated the effect of B55α expression level on remission duration. Patients in the B55α-low group experienced significantly shorter complete remission duration than those in the B55α-high group [59]. The identification of B55α as a specific regulator of AKT phosphorylation at Thr308 as well as B55α expression's correlation with remission duration highlight its potential to serve as a biomarker in AML.

Lastly, epigenetic alterations are a method of PP2A inactivation that is unique to the regulatory subunits. PPP2R2B, which encodes B55β, may be inactivated through epigenetic silencing according to a study by Muggerud *et al.,* which detected an increase in DNA methylation of the PPP2R2B gene in ductal carcinoma in situ and locally advanced breast tumors [60]. Furthermore, B55β is epigenetically inactivated by DNA hypermethylation in colorectal cancer (CRC). This inactivation was shown to effect MYC signaling. A study by Tan *et al.* demonstrated that epigenetic inactivation of PPP2R2B occurred in >90% of patient derived CRC tumor samples tested. They found that loss of PPP2R2B expression led to the induction of PDK-1 dependent MYC phosphorylation at serine 62 by the mTOR inhibitor, rapamycin, which subsequently led to resistance. Restoration of PPP2R2B expression abrogated MYC phosphorylation, resensitizing CRC cells to rapamycin. As clinical responses to rapamycin are quite variable, better biomarkers are needed to predict which patients are most likely to respond to treatment. Tan's study highlighted the potential of PPP2R2B to act as such a biomarker for selecting patients who may respond best to rapamycin treatment [61]. In summary, given the critical role the regulatory subunits play in determining the substrate specificity of the PP2A heterotrimeric complex, identifying mutations in these subunits and understanding their functional implications remains an active area of research.

Catalytic subunit: PP2A Cα and Cβ

To date, the only reports of mutations in the C subunit have been in prostate cancer and AML. A genome wide expression study identified significant downregulation of PPP2CA, which encodes the α isoform of the catalytic subunit, in androgen insensitive prostate cancer cell lines compared with androgen sensitive lines [71]. This finding was subsequently confirmed at the protein level and in human clinical samples [70]. In addition, PPP2CA was found to be downregulated in a cohort of patients with P53 mutant AML[53, 69]. There have been no reports to date of mutations in PPP2CB, the gene that encodes the β isoform of the catalytic subunit.

Post-translational modifications critical to PP2A activity

Methylation at L309 on the C subunit C-terminal tail

The C subunit C-terminus undergoes methylation at L309 (Fig. 4A and 4B). This modification is regulated by two enzymes: LCMT-1, an S-adenosylmethionine (SAM)-dependent methyltransferase that is expressed in the cytoplasm and PME-1 (Fig. 5A), a

lipase-like methylesterase, that is expressed in the nucleus. While LCMT-1 mediated activation of PP2A is reversible (Fig. 5B), PME-1 mediated demethylation is not truly reversible because it denatures the active site [77–79]. The role of this methyl modification is complicated, and both enzymes are essential [80, 81], and likely exhibit control on cell cycle and development [82]. Decreased methylation typically corresponds with increases in cancer progression because methylation enhances holoenzyme assembly, specifically of the preformed AC core enzyme with B subunits. L309 methylation is not essential for every variant (PR61/B′, PR72/B″), but is essential for some (PR55/B) [2, 10, 83–88].

The role of L309 methylation gained in popularity in part due to its putative role in neurodegeneration. PP2A/PR55α is a predominant brain expressed phosphatase that requires L309 methylation for holoenzyme formation. Folate is a key nutrient in the production of the LCMT-1 cofactor, S-adenosylmethionine (SAM), which supplies the enzyme's methyl donor. In folate deprived neuroblastoma cells, both PR55α and LCMT-1 expression levels are diminished, and PP2A exists in demethylated forms. This state leads to tau hyperphosphorylation and cell death, a signature of neurodegenerative diseases [89]. In mouse models of Alzheimer's, levels of LCMT-1, methylated C subunit, and PR55α are decreased. Restoration of methylation by overexpression of LCMT-1, and induced expression of PR55α restores neurite outgrowth, a signature of disease remission [90]. Methylated PP2A is typically found associated with unphosphorylated tau, localized to the plasma membrane. In contrast, demethylated PP2A is improperly localized and not associated with tau [91]. An herb-derived compound, cornel iridoid glycoside, reverses tau phosphorylation by inhibiting C subunit demethylation [92]. The development of natural products and small molecules to modulate tau hyperphosphorylation would provide much needed tools and therapeutics for Alzheimer's disease.

There is also evidence that PME-1 inhibits PP2A independently of its role on C subunit demethylation. Association with PME-1 possibly stabilizes PP2A in an inactive conformation and creates a cellular pool of enzyme that can be activated when required. This association is regulated by the interplay of PP2A with another protein, Phosphatase Activator (PTPA) which activates the PME-1 bound form of PP2A (Fig 5C) [93]. PTPA is a protein with an elusive functional role; however, it is commonly associated with PP2A and possesses a chaperone-like function for the correctly folded C subunit [94]. It also possesses a peptidyl-prolyl cis / trans isomerase activity that acts on Pro190 of the C subunit, inducing a conformational change that may contribute to the reactivation of PME-1 bound PP2A [95, 96]. Additionally, like PME-1 and PTPA, several other proteins associate with the PP2A core or trimer that are not classifed as B subunits. The α4 protein is a PP2A binding protein that stabilizes PP2A and other PPP enzymes by binding to the C subunit and preventing its degradation [97, 98]. The α4 protein is essential and plays a role in cellular adaptation to stress by preserving stocks of PP2A. This PP2A store ultimately dephosphorylates the accumulated products of stress responses [17].

Phosphorylation of the C subunit C-terminal tail

The phosphorylation of multiple residues on the C subunit C-terminus is also critical for modulating PP2A activity via B subunit interactions (Fig. 4B). Y307 phosphorylation

inhibits methylation of L309 by LCMT-1 and limits specific B subunit binding to the core enzyme. Phosphorylation of Y307 itself increases T304 phosphorylation. T304 phosphorylation does not appear to influence methylation, but, like Y307, it may selectively inhibit B subunit binding and dissociate B subunits from fully formed trimers [10]. Y307 and T304 phosphorylation selectively inhibit the binding of all PR55 subunits. Phosphorylation of Y307 prevents assembly of the holoenzyme with PR61/B′αβγε subunits, but not with the B′δ, PR72/B″, or PR70/B″ subunits. As a note, these latter experiments were performed with uncharged (Y307F) or phosphomimetic (Y307D) point mutants, which could not undergo L309 methylation [10, 85, 99].

Exogenous inhibitors of PP2A

There are a number of microbial, marine, and insect derived natural products that bind and inhibit PP2A and other PPP members. These include okadaic acid, fostriecin, microcystins, calyculins, cantharidin, and dragmacidins. Many were isolated from screens of natural product extracts for cytotoxins. Others were discovered in pulldown studies of biologically active extracts, as PP2A binding small molecules [100]. While it seems counterintuitive to inhibit a tumor suppressor like PP2A, at the time, the potency of these compounds in cytotoxicity assays generated much interest in their potential clinical uses for cancer. Their toxicity underscores PP2A's essential role in regulation, and several of these compounds provided extremely useful tools for exploring PP2A's functions. The natural product toxins bind into or adjacent to and obstruct the C subunit active site. [9, 101] The same could be said for the Simian Virus 40 (SV40) small t antigens (ST). SV40 ST consists of an N-terminal J domain and a C-terminal unique domain that contains two separate zinc-binding motifs. SV40 ST interacts with the core enzyme by binding to the B56 subunit binding site on PP2A Aα (HEAT repeats 3–7), causing displacement of the B subunits [102]. This displacement perturbs the function of PP2A and its activity towards multiple substrates [103, 104]. While not directly tumorigenic in humans, these viruses transform cells and can promote tumor growth.

Endogenous inhibitors of PP2A

PP2A is commonly inactivated in cancer by the overexpression of its endogenous inhibitors. The most prominent deactivation mechanism, this occurs in up to 90% of cases in lung and breast cancers and is often associated with poor response to current therapies. There are several endogenous inhibitory proteins that inactivate PP2A (Fig. 6). Inhibitor 1 of PP2A (I1PP2A), also known as ANP32A, inhibits PP2A activity in human umbilical vein endothelial cells [105, 106]. I1PP2A also binds to sphingosine, and this interaction abrogates its binding to PP2A resulting in PP2A activation [107]. Additionally, the greatwall kinase (Gwl) might function as an inhibitor of PP2A. Gwl activates ENSA and Arpp19, which are phosphorylation-dependent inhibitors of PP2A [108, 109]. GWL mediated inhibition of PP2A-B55 leads to phosphorylation of Cdk1 substrates and mitotic entry. Furthermore studies have shown that HOX11, a homeobox gene rearranged in T-cell leukemia by chromosomal translocation, inhibits PP2A [110]. However, the two endogenous inhibitors of PP2A most overexpressed in human cancers and best characterized are SET and CIP2A [106, 111]. SET, also known as inhibitor-2 of PP2A (I2PP2A), binds to the C subunit of PP2A. It was discovered as a chimeric protein in a patient with acute undifferentiated

leukemia. In this case, SET was a translocated gene fused with nucleoporin (CAN gene) [112–114]. SET displays increased expression or increased activity in several cancers such as CML, AML, and B-cell CLL, colorectal cancer, breast cancer, and lung cancer [115–121]. In addition to its overexpression, altered phosphorylation of SET also inactivates PP2A [122, 123]. Studies in Alzheimer's disease have elucidated that Val92 at the amino-terminal fragment and the amino acids 176–277 on the C-terminal region of SET are important for PP2A binding. Furthermore, accumulation of SET in the cytoplasm is regulated by phosphorylation of Ser9 in the nuclear localization signal [124–126].

The association of SET with cancer inspired several attempts to target this inhibitor for PP2A activation. One strategy to inhibit SET involves ApoE (apolipoprotein E), a multifunctional holoprotein with a role in cholesterol transport [127–129] and immunoregulatory functions [130–132]. ApoE and apoE-mimetic peptides, COG112 and COG449 (OP449), bind to SET resulting in activation of PP2A [133–136]. SET antagonism with OP449 results in cytotoxic activity with demonstrable efficacy in the treatment of CML and AML [133].

FTY720 (Fingolimod/Gilenya®), originally approved for use in multiple sclerosis by Novartis, activates PP2A via inhibition of SET. FTY720 was derived from a fungal metabolite [137, 138] and acts as an immunosuppressant by modulating the sphingosine-1-phosphate (SIP) receptor [139–141]. FTY720 exerts anti-tumor activity in breast, HCC, glioma, and multiple myeloma models. Specifically in CML, activation of PP2A [117, 142–144] by FTY720 induces apoptosis through the inactivation of BCR-ABL1 and negative regulation of several survival factors including ERK. Finally, ceramide is a sphingolipid that activates PP2A in several cancers and induces apoptosis [145–149]. Some reports implicate a direct interaction of ceramide with PP2A. Others suggest that ceramide activates PP2A by inhibiting the interaction between PP2A and SET [150]. Ceramide induces apoptosis in prostate cancer cells through PP2A mediated induction of p27 [147].

CIP2A (Cancerous Inhibitor of PP2A) is a PP2A interacting protein. CIP2A is most strongly associated with inhibiting the activity of PP2A on c-MYC resulting in c-MYC stabilization and consequential proliferation. Inhibition of PP2A by CIP2A is also associated with the stabilization of other pro-survival and pro-growth proteins including E2F1, mTOR, and DAPk, resulting in the inhibition of senescence, autophagy and apoptotic pathways respectively [151–153]. Conversely, the depletion of CIP2A results in a decrease in cancer cell viability. It is not understood how CIP2A inhibits PP2A but some reports suggest that it interacts with the A subunit and perhaps the C subunit, preventing the interaction of the active site with target proteins [154]. Encoded by the KIAA1524 gene, CIP2A is overexpressed and may be prognostic in lung cancer, breast cancer, pancreatic cancer, bladder cancer, osteosarcoma, esophageal cancer, gastric cancer, ovarian cancer, cervical cancer, prostate cancer, hepatocellular carcinoma, and colorectal cancer [155–172]. This abundant clinical relevance makes CIP2A an important therapeutic target.

Several natural products possess activities that are relevant to PP2A activation via CIP2A inhibition. Celastrol (tripterine) caused a proteasome-mediated degradation of CIP2A resulting in inhibition of proliferation and induction of apoptosis in lung cancer [173, 174].

Celastrol induced rapid degradation of CIP2A through the interaction of the E3 ligase, CHIP. In vivo studies showed that celastrol potentiated the effects of cisplatin suggesting that celastrol could have therapeutic implications in lung cancer. Ethoxysanguinarine (ESG), a benzophenanthridine alkaloid, downregulates CIP2A resulting in an increase in PP2A activity and a consequential downregulation of c-MYC and AKT in lung cancer. The downregulation of CIP2A subsequently results in inhibition of proliferation and induction of apoptosis in lung cancer [175, 176]. Combined treatment with ESG enhanced the effects of cisplatin in lung cancer.

The anti-cancer drug bortezomib might provide another strategy for activating PP2A via CIP2A inhibition. Bortezomib, a dipeptidyl boronic acid, is a proteasome inhibitor first approved for treatment of multiple myeloma. It blocks degradation of IκB, an inhibitor of NF-κB [177, 178]. In subsequent studies, bortezomib negatively regulated transcription of CIP2A resulting in decreased AKT phosphorylation and induction of apoptosis in breast cancer [179]. It enhances PP2A activity in HCC [180]. Finally, derivatives of erlotinib that were devoid of anti-EGFR activity, inhibited production of CIP2A causing subsequent decreases in AKT phosphorylation and cell growth inhibition [181].

Promethylating agents

One strategy to activate PP2A could be the induction of methylation. As mentioned previously, PP2A methylation at residue Leu309 enhances the affinity of the PP2A core enzyme for the regulatory subunit [79, 182–186]. Some reports suggest that PP2A methylation is linked to PP2A activity and that PP2A methylation induced by an agonist, such as xylulose-5-phosphate, would cause an increase in PP2A activity [185, 187, 188]. This increase in PP2A activity results in a decrease in AKT and c-MYC expression and a decrease in proliferation [189]. Furthermore, DNA damaging agents such as chloroethylnitrosourea (CENU), induce PP2A methylation, increasing PP2A activity resulting in inhibition of AKT and c-MYC [189, 190].

PME-1 inhibitors

PME-1 garnered increasing attention due to its role in cancer and its association with PP2A [191]. To develop probes for annotating enzymatic function, the Cravatt laboratory used its activity based protein profiling technique to screen for inhibitors of serine hydrolases. Their methods generated two independent small molecule inhibitors of PME-1. The first, an azalactam (ABL127, IC_{50} = 10 nM) [192], and the second, a sulfonyl acrylonitrile covalent inhibitor derived from a complementary screening library [193]. These will hopefully provide useful leads to validate PME-1 as a drug target.

Agents with undefined mechanism of action

Several compounds activate PP2A by unknown mechanisms. Forskolin, derived from the root of Coleus forskohlii, is a diterpenoid natural product used for many conditions including cancer. By activating adenylate cyclase, forskolin increases intracellular concentration of cyclic adenosine monophosphate (cAMP) [194]. Forskolin treatment reduces phosphorylation at Y307 on the C-terminal tail of PP2A C thereby activating PP2A [195–197]. In AML, forskolin increased PP2A activity resulting in decreases in

proliferation, induction of apoptosis, and changes in the phosphorylation of AKT and ERK [195]. Perphenazine and other phenothiazine containing tricyclic neuroleptics, activate PP2A in T-ALL cells through direct binding of the PP2A Aα subunit [198]. Carnosic acid is a polyphenolic diterpene that activates PP2A in prostate cancer. This activation results in negative regulation of the AKT/IKK/NF-κB pathway [199]. Vitamin E analogs, such as α-tocopheryl succinate, inhibit proliferation in several cancers [200–210]. α-Tocopheryl succinate promoted activation of PP2A inactivates JNK signaling [211, 212].

Conclusion

PP2A is one of the most abundant cellular proteins, a prominent phosphatase tumor suppressor that regulates the activity of numerous kinases. With a high degree of sequence conservation among yeast, drosophila, and mammals, PP2A controls many cellular functions ranging from metabolism, cell cycle, DNA replication, growth, and apoptosis. It is commonly dysregulated and deactivated in a variety of cancers and other diseases. The biochemical and clinical studies presented here demonstrate that each of its three subunits can be genetically altered and functionally inactivated. Where achievable, restoration of PP2A function inhibits cancer progression, and notably, by a mediator that is downstream of the oncogenic kinases that initiate and drive cancer progression. Several strategies in development accomplish this goal by affecting PP2A's posttranslational modifications and its endogenous inhibitory proteins. There are likely small molecules capable of direct activation of the protein. These will provide additional tools for determining PP2A targets and as potential therapeutics. Further development and combinations of these with other targeted therapies will enable the translation of these findings to patients.

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Abbreviations

PP2A	protein phosphatase 2A
PTP	protein tyrosine phosphatase
PSP	protein serine/threonine phosphatase
PPP	phospho-protein phosphatase
PPM	metal-dependent protein phosphatase
HCC	hepatocellular carcinoma
NHL	non-Hodgkin's lymphoma
CLL	chronic lymphocytic leukemia
B-CLL	B-cell chronic lymphocytic leukemia

CRC	colorectal cancer
LCMT-1	leucine carboxyl methyltransferase-1
PME-1	Protein phosphatase methylesterase-1
SAM	S-adenosyl methionine
PTPA	PP2A phosphatase activator
I1PP2A/ANP32A	Inhibitor 1 of PP2A
ANP32A	acidic (leucine-rich) nuclear phosphoprotein 32 family member A
Gwl kinase	greatwall kinase
ENSA	endosulfine alpha
Arpp19	cAMP-regulated phosphoprotein, 19kDa
CIP2A	cancerous inhibitor of PP2A
SET/I2PP2A	inhibitor-2 of PP2A
ApoE	apolipoprotein E
ERK	extracellular-signal-regulated kinase
mTOR	mammalian target of rapamycin
ESG	Ethoxysanguinarine
EGFR	epidermal growth factor receptor
CENU	chloroethylnitrosourea
cAMP	cyclic adenosine monophosphate

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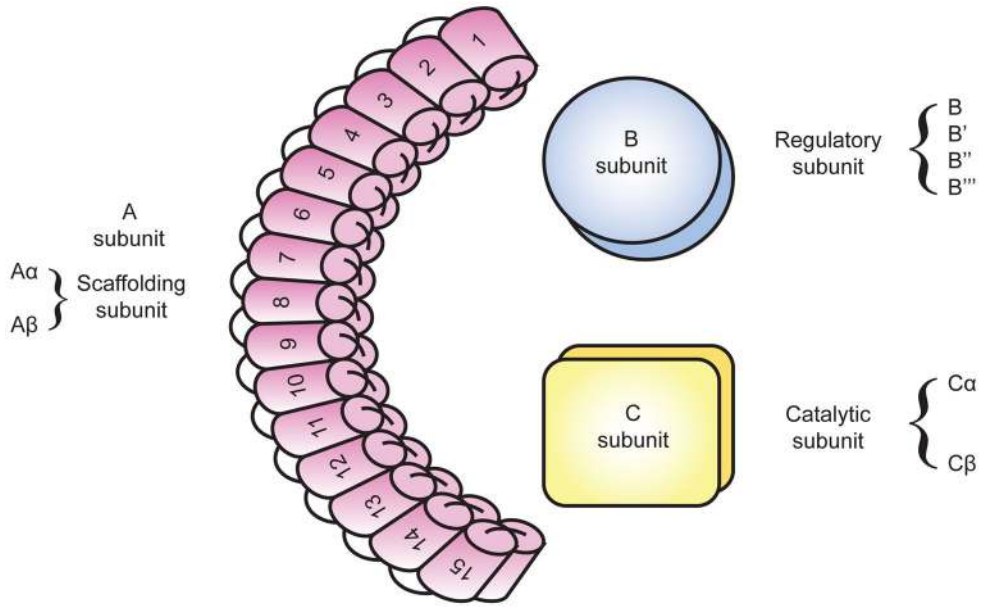

Figure 1.

PP2A is a heterotrimeric complex consisting of a scaffolding subunit (A), a regulatory subunit (B), and a catalytic subunit (C). PP2A A subunit is composed of 15 tandem HEAT repeats in two isoforms α and β. PP2A C subunit also exists in two possible isoforms α and β. PP2A B subunit consists of four classes: B (B55/PR55), B′ (B56/PR61), B″(PR48/PR72/PR130) and B‴(PR93/PR110).

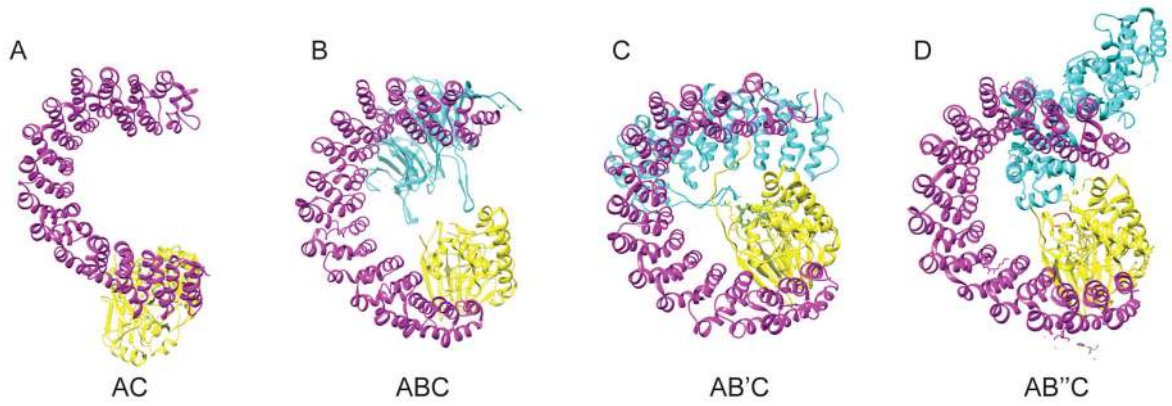

Figure 2.

Structures of PP2A core enzyme and holoenzyme. (**A)** (PDB code: 2IE3) Core Enzyme consisting of Aα (in magenta) subunit and Cα (in yellow) subunit. The C subunit binds A at Heat Repeats 11–15. The active site of the C subunit consists of 2 manganese atoms and is positioned away from the ridge of the A subunit HEAT Repeats. Binding with the catalytic subunit shifts HEAT repeats 13–15 by 20–30 Å (**B)** (PDB code: 3DW8) Core Enzyme binding to B family subunit (in cyan), Bα/PR55α. Members of this subunit family bind the A subunit at two interfaces. The first is via a seven bladed propeller, composed of WD40 repeats. The bottom face of the propeller binds to A subunit HEAT domains 3–7. The second is through a β-hairpin handle that interacts with A subunit HEAT repeats 1 and 2. Upon binding to the holoenzyme, the B subunit substrate binding site lies on the top face proximal to the active site of the catalytic subunit. **(C)** (PDB code: 2IAE) Core enzyme binding to B′ family subunit (in cyan), Bγ1/PR61γ1. The B′ structures are similar to the A subunit, composed of 8 HEAT-like repeats. These interact with HEAT repeats 2–8 of the A subunit, and with the C subunit. Much like binding to B family subunits, the substrate binding site of B′ is proximal to the active site of the catalytic subunit upon holoenzyme formation. Binding to the B′ subunits forces the N-terminal repeat of the A subunit to twist 50–60 Å, rearranging the hydrophobic core of the scaffolding subunit. **(D)** (PDB code: 4I5L) Core Enzyme binding to B″ family subunit PR72 (in cyan). B″ subunits consist of a linear arrangement of different functional motifs that include an N-terminal hydrophobic motif and 2 EF hand calcium binding motifs. The N-terminal hydrophobic motif and one EF hand bind to the A subunit at HEAT repeats 1–7 and binds to the catalytic subunit via a helix on the subunit at residues 439–446 near the active site, positioning the substrate binding site near the active site. The resulting conformation of this holoenzyme is wider and taller than that which forms with B or B′ subunits.

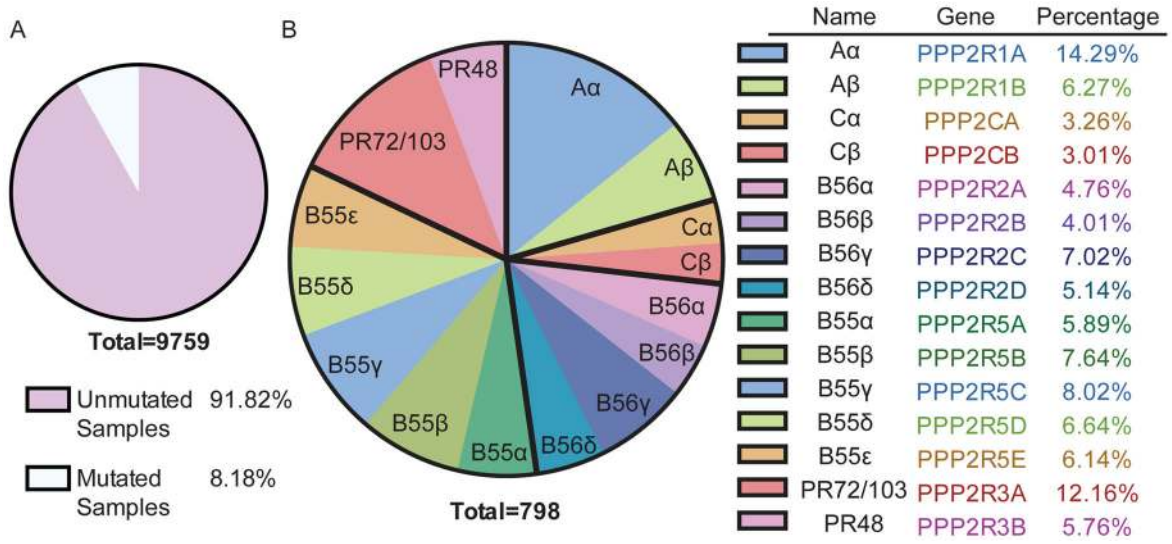

Figure 3.

(A) Pie chart of the frequency of PP2A mutations across 9,759 tumor samples. Mutational information was analyzed from Cbioportal.org, which includes 85 different sequencing studies, including TCGA data. Studies with targeted sequencing or expression only data were excluded from the total number. **(B)** Pie chart of the frequency of PP2A mutations divided by PP2A subunit families: A, B, B′, B″, and C. Bold black lines divide each subunit. Mutational information was analyzed from Cbioportal.org, which includes 85 different sequencing studies, including TCGA data. Studies with targeted sequencing or expression only data were excluded from the total number. The results shown here are in part based upon data generated by the TCGA Research Network: http://cancergenome.nih.gov/.

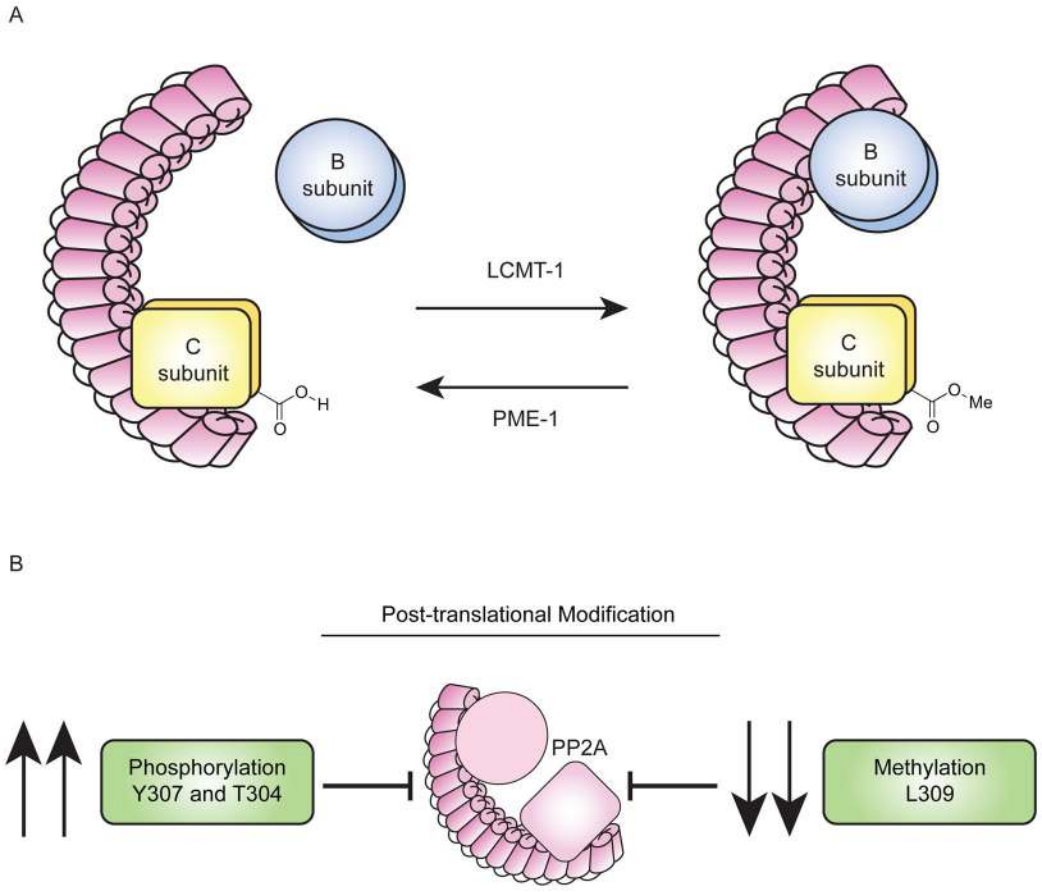

Figure 4.

(A) The C-terminus of the catalytic C subunit undergoes methylation at L309 via LCMT, a SAM-dependent methyltransferase. PME-1 mediates demethylation. (B) Decreased methylation at L309 and increased phosphorylation of Y307 and T304 of the catalytic C subunit are posttranslational modifications that inhibit PP2A.

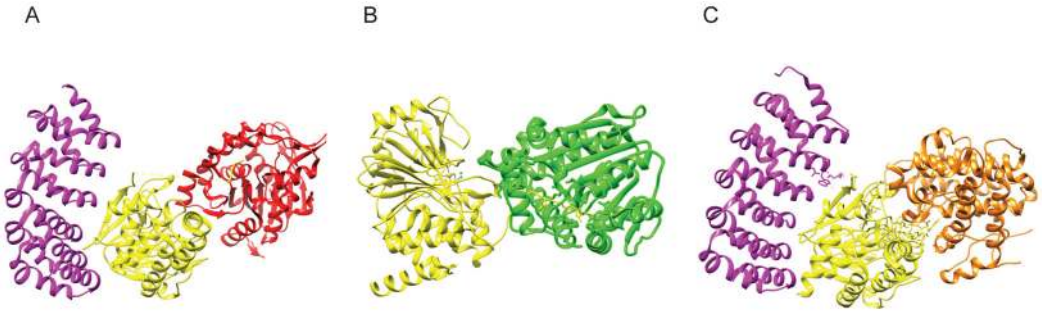

Figure 5.

(A) (PDB code: 3C5W) Structure of PP2A and PME-1 complex with scaffold subunit in magenta, catalytic subunit in yellow, and PME-1 in red. (B) (PDB code: 3P71) Structure of PP2A and LCMT complex with catalytic subunit in yellow and LCMT in green (C) (PDB code: 4LAC) Structure of PP2A and PTPA complex with scaffold subunit in magenta, catalytic subunit in yellow, and PTPA in orange.

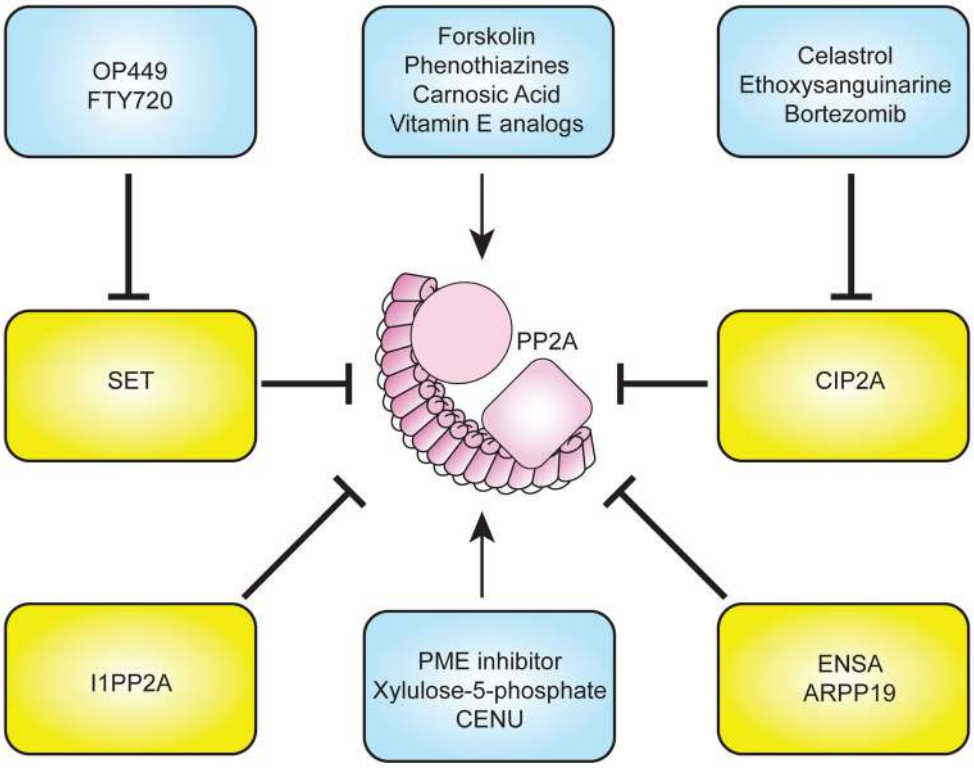

Figure 6.

Activators of PP2A. Several strategies to activate PP2A include: decreasing PP2A Y307 phosphorylation, inhibiting endogenous inhibitors (SET and CIP2A), inhibiting PME-1, and using promethylating agents.

Table 1

PP2A subunit alterations in cancer

Subunit	Gene	Isoform	Alteration	Disease	Reference
A	PPP2R1A	Aα	Point mutation	breast, lung, melanoma, ovarian, endometrial, uterine, colon	[41], [43], [44], [45], [46], [47]
			Deletion	Breast	[41]
			Decreased expression	Glioma	[48]
			Missense mutation	breast, colon, lung	[41], [49], [50], [51], [40]
			In-frame deletion	breast	[40]
A	PPP2R1B	Aβ	LOH	breast, lung, ovarian, cervical, melanoma, NHL, CLL	[49], [52]
			Decreased expression	AML	[53]
			Abberant transcription	HCC, B-CLL	[54],[55]
B	PPP2R2A	B55α	Deletion	breast, prostate, myeloma	[56], [57], [58]
			Decreased expression	AML	[59]
B	PPP2R2B	B55β	DNA hypermethylation	breast, colon	[60], [61]
B	PPP2R2C	B55γ	Decreased expression	breast, prostate	[62], [63], [64]
B′	PPP2R5A	B56α	Decreased expression	melanoma	[65]
B′	PPP2R5C	B56γ	Decreased expression	melanoma	[66]
			Point mutation	lung	[67]
B′	PPP2R5E	B55ε	SNP	soft tissue sarcoma	[68]
C	PPP2CA	Cα	Decreased expression	AML, prostate	[53], [69], [70], [71]