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REVIEW

To be or not to be: PP2A as a dual player in CNS functions, its role in neurodegeneration, and its interaction with brain insulin signaling

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Abstract

Accumulating evidence has reached the consensus that the balance of phosphorylation state of signaling molecules is a pivotal point in the regulation of cell signaling. Therefore, characterizing elements (kinases–phosphatases) in the phosphorylation balance are at great importance. However, the role of phosphatase enzymes is less investigated than kinase enzymes. PP2A is a member of serine/threonine protein phosphatase that its imbalance has been reported in neurodegenerative diseases. Therefore, we reviewed the superfamily of phosphatases and more specifically PP2A, its regulation, and physiological functions participate in CNS. Thereafter, we discussed the latest findings about PP2A dysregulation in Alzheimer and Parkinson diseases and possible interplay between this phosphatase and insulin signaling pathways. Finally, activating/inhibitory modulators for PP2A activity as well as experimental methods for PP2A study have been reviewed.

Keywords Protein phosphatase 2A · Alzheimer disease · Parkinson disease · Insulin · Insulin resistance · Brain

Introduction

Phosphorylation/dephosphorylation of cellular proteins such as signaling molecules, enzymes, and receptors is one of the most crucial mechanisms, which regulates a broad spectrum of critical signaling pathways and physiological mechanisms [1]. Two classes of enzymes, protein kinases and protein phosphatases, govern this shuttle like process in the cell. Protein kinases add a phosphate group (PO_4^{3-}) to specified sites and protein phosphatases remove these groups and reverse the functions of protein kinases [2]. Therefore, maintaining the precise balance between the opposing activities of kinases and phosphatases is of great importance in the determination of phosphorylation state of proteins. This balance in the phosphorylation/dephosphorylation state of target proteins is mostly inclined to a set point nearer to dephosphorylation state, and therefore, it is believed that phosphatases are more prominent and even dominant in maintaining the balance of phosphorylation state of target proteins in physiological conditions (reviewed in [3, 4]). In addition, overwhelming evidence has confirmed that dysregulation in the activity of these specific enzymes could participate in different pathological states including diseases of the central nervous system (CNS) [5]. On the other hand, as an important hormone in the control of metabolism, insulin also plays very important roles in the CNS and its disruption is known to be an underlying mechanism in the pathogenesis of neurological disorders [6]. Moreover, insulin signaling transmission is greatly dependent on the phosphorylation/dephosphorylation [7]. Herein, we reviewed the latest knowledge about protein phosphatases and more specifically on *PP2A* as the most important member of the phosphatase family. In addition, we also summarized its interplay with insulin signaling pathway in physiological and pathological conditions with an emphasis on the CNS and neurodegenerative disorders.

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Protein phosphatases

In general, protein phosphatases classification is based either on their substrate specificity or on their structural properties. As depicted in Fig. 1, based on the type of substrates, protein phosphatases are categorized into three broad groups; serine/threonine (Ser/Thr) specific, tyrosine (Tyr) specific, and dual-specific phosphatases (DSP) [4].

1. The first subgroup of protein phosphatases in the substrate categorization is Ser/Thr phosphatases. This subgroup is responsible for a large percentage of dephosphorylation reactions in the eukaryotic cells on Ser/Thr residues [8]. Ser/Thr phosphatases themselves are structurally subdivided into three main subtypes:

   (a) Phosphoprotein phosphatases (PPPs), which are the largest group and encompass some of the most important protein phosphatases such as PP1, *PP2A*, PP2B (also known as calcineurin), PP4, PP5, PP6, and PP7. PP1 is a major member of this family which regulates a wide range of cellular activities including cellular division, metabolism, and protein production machinery as well as the membrane activity (reviewed in [8]). The next members of this family are PP2A and PP2B in which PP2A will be discussed in more details in succeeding parts. PP2B (also named calcineurin) is mostly known for its involvement in cellular processes that carried out in a calcium-dependent manner. Neural development, learning and memory, immune response, and signaling process are among the most important calcium-dependent processes that are regulated by PP2B [9]. The activity of this enzyme is triggered by association with calcium–calmodulin complex [8]. Another member of PPP family is PP5. This member is just encoded by a single gene (in contrast to others) and is highly expressed in the brain. The catalytic and regulatory subunits of PP5 are resided within a same polypeptide sequence. It has been reported that PP5 is a key regulator for cellular fate as well as cellular differentiation and migration [10]. Furthermore, PP5 also has a close interaction with glucocorticoid receptors and participates in the cellular response to stress [11, 12]. PP4 and PP6 are two other members of PPP family which have a catalytic subunit with high homology to PP2A catalytic subunit and contribute in the recruitment of a series pivotal cellular responses [8]. Finally, PP7 is exclusively expressed in plants, which has some significant structural differences with other members. For instance, PP7 has three insertions in their catalytic domains which the longest one induces auto-inhibitory function on its activity. In fact, when the longest insertion is cleaved, PP7 is activated. Interestingly, PP7 such as PP2B has

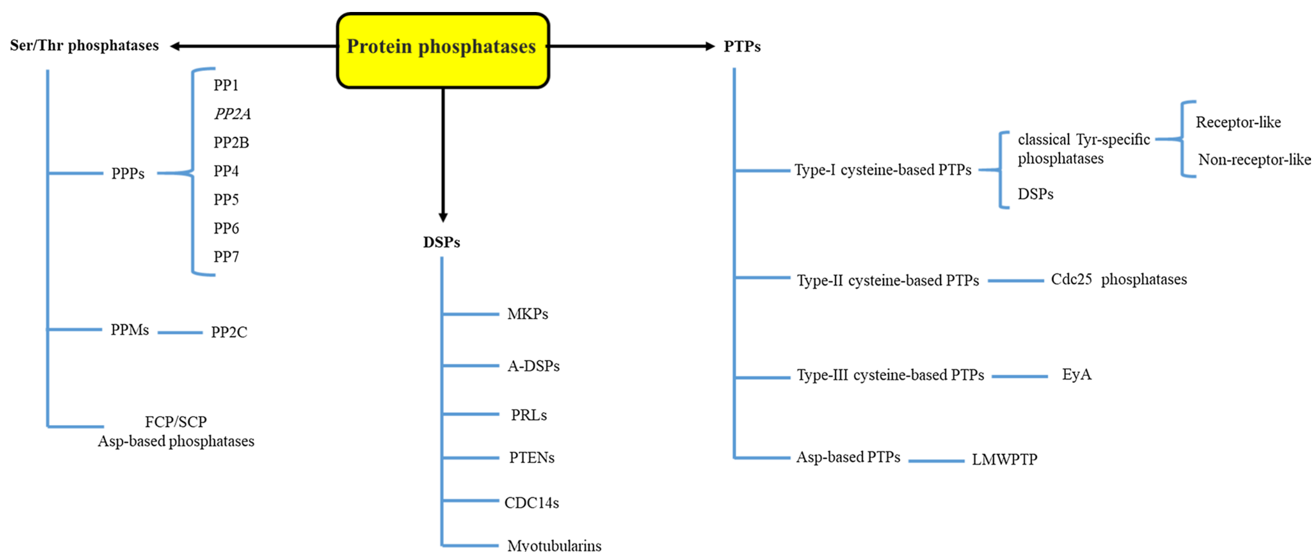

Fig. 1 Schematic diagram showing protein phosphatases categorization. *Ser/Thr phosphatases* serine/threonine phosphatases, *PTPs* protein tyrosine phosphatases, *DSPs* dual-specific protein phosphatase, *PPPs* phosphoprotein phosphatases, *PPMs metal* metal-dependent protein phosphatases, *Asp-based* aspartate-based, *Cdc* cell division cycle, *LMWPTP* low-molecular-mass protein tyrosine phosphatase, *EYA* eyes absent, *A-DSP* atypical DSP, *MKP* mitogen-activated protein kinases (MAPK) phosphatase, *PRLs* phosphatases of regenerating liver

interaction with calcium–calmodulin complex; however, this interaction is inhibitory [13].

(b) Metal-dependent protein phosphatases (PPMs) are the second subtype of Ser/Thr phosphatases, which are characterized by their dependence on manganese/magnesium (Mn^{2+}/Mg^{2+}). PP2C and pyruvate dehydrogenase are two members of this group from which PP2C itself has at least 22 different isoforms in the human cells. These isoforms participate in different cellular functions such as regulation of cell differentiation, growth, and apoptosis. It seems that despite distinct functions of PP2C in cells, all have conserved active sites and identical catalytic mechanisms [8, 14].

(c) FCP/SCP aspartate-based (Asp-based) phosphatases are the third member of Ser/Thr phosphatases family that mainly dephosphorylate C-terminal domain (CTD) of RNA polymerase II such as Fcp1 and Scp1 (reviewed in [8]).

2. Another subgroup in the substrate categorization of protein phosphatases is Tyr phosphatases which mostly dephosphorylate tyrosine residues and contribute in multiple signaling pathways that govern several essential functions like cell movement, apoptosis, and cell growth. Based on their protein sequences and functions, this subfamily is also divided into four groups: (A) Type-I cysteine-based protein tyrosine phosphatases (PTPs) which encompass classical Tyr-specific phosphatases such as receptor-like (also called transmembrane) and non-receptor-like PTPs. Interestingly, because of structural similarities, DSPs are also categorized as a member of this group. (B) Type-II cysteine-based PTPs, which have both Tyr and Thr-specific phosphatase activity and include cell cycle regulators called cell division cycle (Cdc25) phosphatases. Three members of this small group are Cdc25A, Cdc25B, and Cdc25C. (C) Type-III cysteine-based PTPs are broadly expressed in most bacteria and nearly all eukaryotic cells. Low-molecular-mass PTP (LMWPTP) is an example of this group which is expressed in human genomes. (D) As a member of haloacid dehalogenase (HAD) family, the last group of PTPs are aspartate-based group which represented by a Tyr-specific phosphatase named as eyes absent (EyA) (reviewed extensively in [3, 4]).
3. Finally, as their names imply, DSPs are a group of phosphatases that dephosphorylate both Tyr and Ser/Thr residues. These enzymes have pivotal roles in immune system, brain functions, organogenesis, and development. Moreover, some of them contribute in the cancer development and some others are identified as tumor suppressors. Up to our knowledge, based on sequence identity, they are categorized into six subgroups: One of the most well-known members of this group is mitogen-activated protein kinases (MAPK) phosphatase or MKP, which terminates the diverse effects of MAPK signaling pathway. The second member of this family is atypical DSP (A-DSP). This member has some similarities with MKPs and even can dephosphorylate MAPKs. Other subgroups are phosphatases of regenerating liver (PRLs), phosphatase, and tensin homologues deleted on chromosome 10 (PTENs), cell division cycle 14 proteins (CDC14 s), and myotubularins (reviewed in [3, 15, 16]).

As it was evident, in contrast to older categorization based on substrate specificity, the newer division of protein phosphatases based on their structural properties does not fully coincide with substrate-based categorization; however, some overlaps exist between them. For instance, some Thr-specific phosphatases are categorized in type-II tyrosine phosphatases or DSPs are grouped with classical tyrosine phosphatases.

As mentioned before, more than %98 of key regulatory proteins are phosphorylated in Ser/Thr residues. This implies an important role of PPP in the regulation of cellular functions. Among the PPP family members, PP2A is an omnipresent Ser/Thr phosphatase in mammalian cells, which catalyzed the majority of Ser/Thr phosphatase reactions and constitutes about 0.3–1% of the total cellular [17, 18]. In succeeding sections, we reviewed the latest evidence about this phosphatase and the role that PP2A plays in the pathophysiology of the CNS and its interaction with insulin signaling in the brain.

Protein phosphatases 2A

PP2A contributes to numerous physiological and biological functions. For instance, processes such as metabolism, neural development, transcription, cell cycle, proliferation, and apoptosis are regulated by PP2A activity [18].

Two distinct forms of PP2A exist in the cells, namely, a core dimeric form ($PP2A_D$) which is composed of scaffolding (A) and catalytic (C) subunits and a heterotrimeric holoenzyme form ($PP2A_T$) that is composed of scaffolding (A), catalytic (C), and regulatory (B) subunits.

The A subunit has a flexible horse-shoe like structure that contains 15 Huntingtin-Elongation-A subunit-TOR (HEAT) repeats. Each HEAT repeat is a sequence of 39 amino acids and comprises two α-helices that connected to each other by inter-helical loops. 11–15 HEAT repeats are recognized by catalytic subunit, whereas 1–10 HEAT repeats interact with regulatory subunit [19], so that A subunit prepares appropriate interaction between regulatory and catalytic subunit to catalyze the distinct cellular process. PP2AC subunit binds

to A and B subunit with a specific conserved sequence that mostly has been replaced in other PPPs [8].

Furthermore, PP2AC subunit has specific sequences to binds substrate, post-translational modifications, and tumor suppressing toxins like okadaic acid and calyculin A [20].

Each A and C subunits could be found in two α and β isoforms (Aα, Aβ or Cα, Cβ), α isoforms (Aα and Cα) being the predominant subunit in most cells (reviewed in [18, 21, 22]). The variety in regulatory B subunit is more than that of two mentioned subunits. As its name implies, the regulatory B subunit is the main modulator for $PP2A_T$ holoenzyme and brings about temporal and spatial specificity (reviewed in [22]). B subunit is generally categorized into four subfamilies (B/B55/PR55, B′/B56//PR61, B″/PR48/PR130/PR72, B‴/PR93/PR110) (reviewed extensively in [22]). In fact, what generate various forms of PP2A in diverse tissues, and determine tissues and substrate specificity as well as subcellular distribution is the various post-translational modification and alternative combinations of these isoforms. By this way, the enormous number of phosphoproteins and signaling pathways can be affected by PP2A [23].

Regulation of protein phosphatases 2A

Different ways and regulators could modulate the activity of PP2A. These include molecular mechanisms that control PP2A stability, alternative holoenzyme assembly, and/or its enzymatic activity.

PP2AC subunit which contains 309 amino acids is an important target protein in the regulation of PP2A activity. From a regulatory viewpoint, an important portion of this subunit is its c-terminal tail (Thr304-Pro-Asp-Tyr-Phe-Leu309) that is highly conserved in eukaryotes. Modifications like methylation and phosphorylation are taken place in this portion play an important role in the regulation of PP2A activity. These modifications mainly adjust interaction with B subunit and, therefore, affect holoenzyme assembly. Accordingly, studies have reported that phosphorylation of PP2AC at Tyr_{307} and Thr_{304} sites, which is catalyzed by a cyclin-dependent kinase (CDK) and a Src-like or receptor tyrosine kinase (RTK), plays an inhibitory role in the regulation of PP2A activity (reviewed in [24–26]).

On the other hand, reversible carboxyl methylation on leu_{309} is another regulatory mechanism, which affects holoenzyme assembly, a process that eventually controls the catalytic activity of PP2A. In this process, leucine carboxyl methyltransferase (LCMT) and protein phosphatase methylesterase (PME-1) act as two opposing enzymes that catalyze methylation and demethylation reactions, respectively [25–27]. Gene deletion studies have revealed that the absence of either of these two enzymes results in the activation of pro-apoptotic pathway that even causes perinatal lethality [28, 29]. Phosphatase 2A phosphatase activator (PTPA) is another important regulator for PP2A activity within the cells. This protein was first cloned as an ATP/Mg^{2+}-dependent enzyme that activates both phosphotyrosyl phosphatase and Ser/Thr phosphatase activity of PP2A [30, 31]. Normally, a fraction of PP2AC subunit is synthesized in the cell in the inactive conformation, a form that is stabilized by binding to PME-1. PTPA is able to reactivate this native inactive complex and, therefore, plays role as a stimulatory modulator of PP2A activity [30, 31]. Moreover, PTPA also participates in the phosphorylation-mediated regulation of PP2A activity. In accordance, it has been documented that PTPA restores PP2A activity by reducing the inhibitory phospho-PP2AC subunits level. It is demonstrated that this dephosphorylation of PP2AC is achieved via upregulating protein tyrosine phosphatase B1 (PTPB1) [32]. PTPA also increase LCMT-mediated methylation of PP2AC [33]. Therefore, PTPA is considered as an essential regulator component for PP2A. Two isoforms of PP2A C subunits (α and β) are expressed in a fine-tuned manner, and once expressed, they have a very short half-life in their monomeric forms [34]. It has been demonstrated that a protein named as immunoglobulin-α-binding protein 1 (IGBP1) or simply α-4 has the ability to bind to PP2AC subunit and protect PP2AC from proteasomal degradation [35]. Despite the certainty about the effect of α-4 on PP2A activity, the net effects remain ambiguous and are believed to be substrate dependent [26, 36]. Another cellular regulator, which interacts with and takes part in PP2A biogenesis and activity, is the target of rapamycin signaling pathway regulator-like (TIPRL-1). In accordance, it has been reported that TIPRL-1 (or TIP in yeast) binds to PP2AC subunit and inhibits PP2A activity [37].

Two heat-stable proteins named as Inhibitor-1 and -2 of PP2A (I1 and I2) which were first extracted from bovine kidney are also involved in the negative regulation of PP2A [38]. However, with unequal preferences, both I1 and I2 (SET) bind to PP2A regulatory subunits and serve as endogenous inhibitors for PP2A phosphatase activity [39].

In addition to mentioned mechanisms and molecules involved in the regulation of PP2A, several other modulators are also introduced which could modulate PP2A activity in discrete cellular conditions. For instance, Ensa, Arpp-19, and Bod1 are mitosis-related proteins, which inhibit PP2A activity during normal mitosis process [40–42]. This long-list of mechanisms and molecules that not closed and is being continuously updated are been schematically summarized in Fig. 2.

Dysregulation of PP2A in neurodegenerative diseases

PP2A and Alzheimer's disease

Alzheimer's disease (AD) is one of the most prevalent neurodegenerative diseases which are associated with cognitive

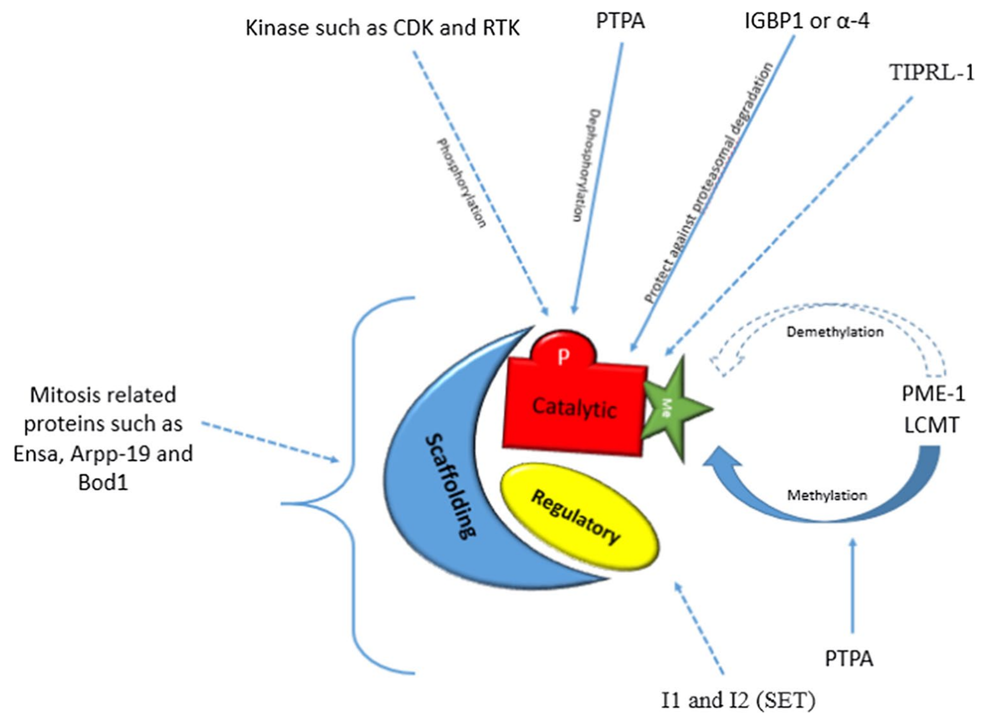

Fig. 2 Schematic diagram showing different PP2A regulatory molecules. *P* phosphate group, *Me* methyl group, *PME-1* protein phosphatase methylesterase-1, *LCMT* leucine Carboxyl Methyltransferase 1, *PTPA* protein phosphatase 2 phosphatase activator/phosphotyrosyl phosphatase activator, *TIPRL-1* target of rapamycin signaling pathway regulator-like, *IGBP1 or α-4* immunoglobulin-α-binding protein 1, *CDK* cyclin-dependent kinase, *RTK* src-like or receptor tyrosine kinase, *I1 and I2 (SET)* inhibitor-1 and -2 of PP2A. Inhibitory pathways are represented by dash lines and activatory pathways are shown by solid lines

and memory dysfunctions at the phenotype level. From the neuropathological point of view, this disease is characterized by the accumulation of extracellular senile plaques and intracellular neurofibrillary tangles (NFT) along with oxidative stress, local neuronal dysfunction, and degeneration of dendritic processes [43].

Beside mentioned characteristics, studies in recent decades have revealed that disturbance of the insulin signaling is also an inseparable part of the AD pathology. Consistently, epidemiological evidence has shown a higher risk for AD development in diabetic patients [44, 45]. Moreover, several lines of evidence have shown that insulin expression both in mRNA and protein level has decreased in CSF. In addition, overwhelming proofs also have reported that tyrosine kinase activity of insulin receptors and its sensitivity are significantly decreased in comparison to aged-match groups. Furthermore, the expression and activity of downstream signaling elements have disturbed in favor of insulin resistance. For instance, decreased activity of insulin receptor substrate (IRS), phosphatidylinositide 3-kinase (PI3K/Akt), or increased glycogen synthase kinase 3-beta (GSK-3β) activity has reported in numerous studies [6, 46].

As it is predictable, disturbance in the phosphorylation levels of the proteins is an important underlying mechanism in the development of the pathological hallmark of AD [47]. Therefore, it is conceivable to assume that dysregulation of PP2A, as the most important Ser/Thr phosphatase in the brain, may contribute to the development of AD. In accordance, it has been demonstrated that PP2A activity is decreased in different areas of AD brains [48, 49]. Consistent with this decrease in activity, decrements in various PP2A subunits (such as PP2AC) are also reported in gene and protein expression levels (reviewed in [47]). In addition, changes in PP2A regulatory mechanisms like methylation, phosphorylation, and endogenous regulators are also evident in AD [47, 50]. Multiple lines of studies have been done on PP2A in AD patients and animal models that suggest a causal relation between dysregulation of PP2A and incidence of different hallmarks of AD. In subsequent sections, we reviewed the latest evidence linking PP2A dysfunction with each main characteristics of AD (summarized in Fig. 3).

PP2A and tau hyperphosphorylation

Tau is a cytoskeletal protein that normally promotes microtubule assembly and maintains its stability. Neuronal tau can be phosphorylated at more than 40 serine and threonine residues and hyperphosphorylation of these residues negatively impress its interaction and regulatory roles on the microtubules assembly. In fact, followed by abnormal tau phosphorylation, its sequestration, microtubules binding, and dissociation all would be negatively affected and tau aggregation in the shape of intraneuronal neurofibrillary tangles (NFTs) develops. These processes eventually interrupt microtubule network [51]. Thereafter, disruption in neural plasticity, cytoskeleton integration, and neural transport are inevitable consequents of tau hyperphosphorylation. As mentioned in the preceding part, Tau hyperphosphorylation is one of the main hallmarks of AD and it has been shown

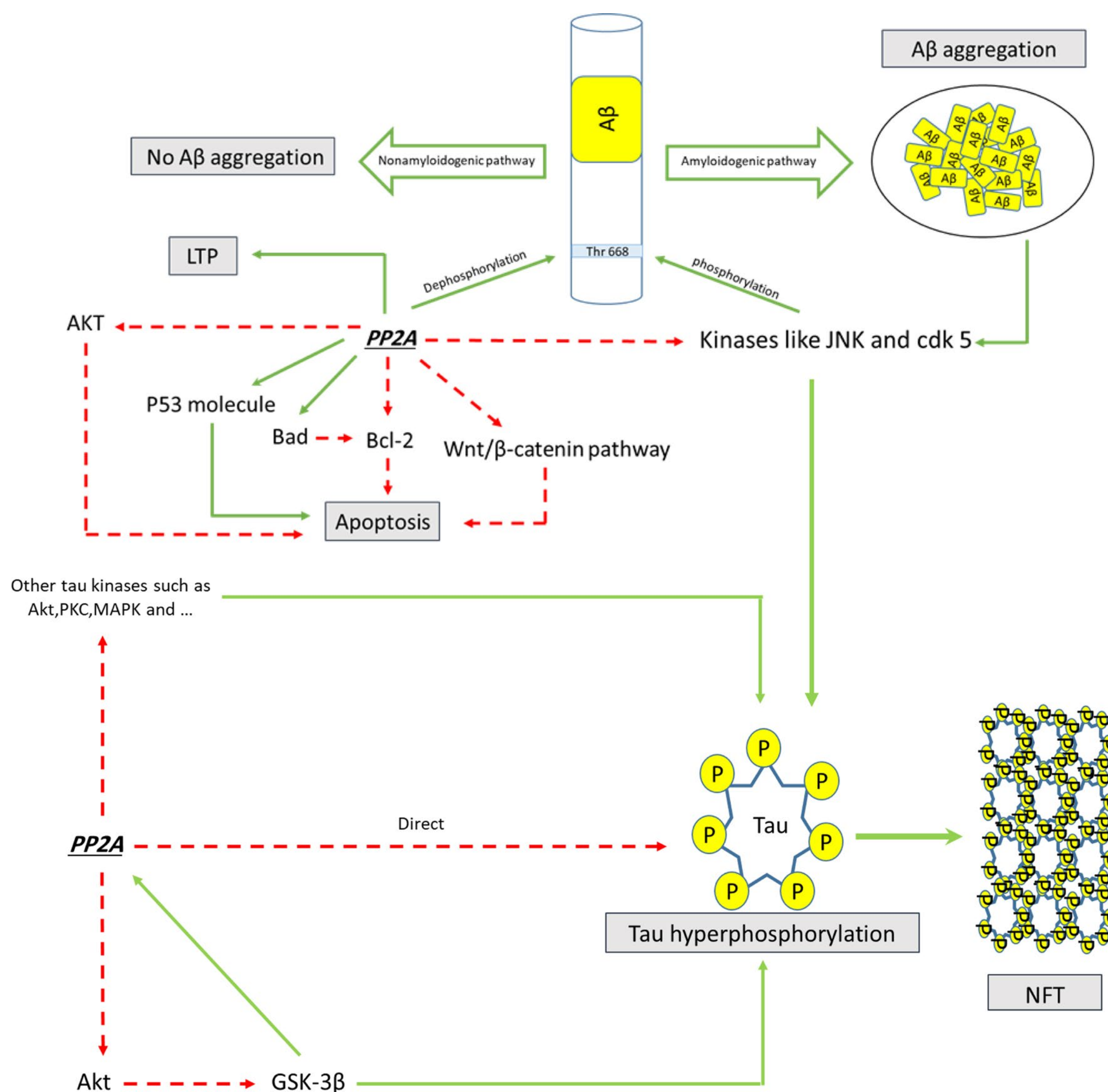

Fig. 3 Schematic diagram showing the interplay among PP2A, kinases and status of AD-related proteins. *Aβ* amyloid beta, *JNK* c-Jun N-terminal kinases, *cdk5* Cyclin-dependent kinase 5, *Bcl-2* B-cell lymphoma 2, *LTP* long-term potential, PKC protein kinase C, *MAPK* mitogen-activated protein kinase, *PP2A* protein phosphatase 2A, *GSK-3β* Glycogen synthase kinase 3β, *NFT* neurofibrillary tangle. Inhibitory pathways are represented by dash lines and activatory pathways are shown by solid lines

that tau phosphorylation is three-to-fourfold higher in AD brain than normal subjects [52]. As phosphorylation level of proteins depends on the activity of kinase/phosphatase enzymes, then it seems possible that an imbalance in the activity of tau phosphatase and kinase might play a determinant role in tauopathies like AD. Major tau kinases are categorized in three main groups; proline-directed protein kinase (PDPK), and non-PDPK and tyrosine protein kinases (TPKs). Each mentioned class includes several protein kinases; however, GSK-3β from PDPK class is considered as the most important tau kinase. In the opposing site, the major tau phosphatase is PP2A that it is responsible for more than 70% of tau Ser/Thr dephosphorylation [53–55]. In this regards, molecular and mass spectrometric studies have recognized about 18 PP2A-regulated and more than 80 tau kinase-regulated phosphorylation sites in the tau protein [56, 57]. Accordingly, several lines of evidence have shown that compromised PP2A activity is involved in the tau hyperphosphorylation and therapeutic strategies that revert PP2A activity are able to dephosphorylate tau [58, 59]. In addition to the direct effect of PP2A on tau, there are mutual regulatory communications between PP2A and tau kinases and these interplays make the conclusion somehow complex. For instance, a mutual regulatory relation between PP2A and GSK3-β has been reported previously. GSK3-β activity is controlled by phosphorylation at Ser_9 and Tyr_{216},

in which phosphorylation at Ser_9 is inhibitory [60, 61]. As mentioned, GSK3-β is a main kinase for tau hyperphosphorylation; therefore, it seems that PP2A and GSK3-β are two opposing enzymes which their balance would determine tau phosphorylation state; however, the interplay between these molecules is not such simple. On one hand, PP2A directly dephosphorylates tau and opposes GSK3-β effect on tau. On the other hand, PP2A dephosphorylates and inactivates the upstream inhibitor of GSK3-β (PKB/Akt) and also it has been shown that PP2A directly remove the inhibitory phosphate group on GSK3-β [53]. Both these effects favor GSK3-β activation and, as a result, increase tau phosphorylation. Importantly, this is not a one-way street. In fact, GSK3-β also affects PP2A activity by methylation of PP2AC via LCMT and suppressing PME-1 expression [62]. This intricate interplay between PP2A and GSK3-β on tau phosphorylation seems bewildering that if PP2A inhibition is more beneficial or its activation in preventing tau hyperphosphorylation? The answer to this question seems to reside in the sites of tau phosphorylation. In this regard, Qian et al. have shown that, however, with some overlaps, PP2A activity in tau dephosphorylation and GSK3-β activity on tau phosphorylation do not match exactly. They showed that PP2A mostly dephosphorylate $Thr_{205/212}$ and $Ser_{214/262}$, while GSK3-β phosphorylates tau mostly on $Ser_{199/396/205}$. Therefore, they concluded that overall changes in tau phosphorylation state that happen after PP2A disruption is a mixed effect, directly through PP2A inhibition and indirectly through GSK3-β [53]. Furthermore, the activity of GSK3-β is not controlled solely by PP2A activity and other factors also affect its activity. Therefore, the net effect of PP2A on GSK3-β and eventually tau phosphorylation also depends on the activity of other GSK3-β modulators [53]. The effects of PP2A on tau kinases are not limited just to GSK3-β and it has been depicted that PP2A also have a negative regulatory role on the activity of other tau kinases. The activation/inactivation of many protein kinases is modulated by their phosphorylation state. Phosphatase function of PP2A acts as an off switch for the activity of several protein kinases including Protein kinase C (PKC), Akt (PKB), P70S6 kinase, MAPK, and extracellular signal-regulated kinase (ERK) [63]. In this regard, Kins et al. demonstrated that chronic inhibition of PP2A leads to activation of ERK and c-Jun N-terminal kinase (JNK) signaling pathways that may produce tau hyperphosphorylation [64]. Moreover, it has been reported that the major cause of frontal and temporal lobe (AD-affected brain regions) vulnerability to tau hyperphosphorylation in AD is probably high expression level of tau kinases, whereas the PP2A level is similar to other regions [55]. These data imply that, through affecting tau kinase activity or dephosphorylating tau, dysfunction of PP2A could directly or indirectly participate in tau hyperphosphorylation seen in AD.

PP2A, Aβ neurotoxicity and apoptosis

Formation of extracellular senile plaques from β-amyloid protein (Aβ) is another histopathological change in AD brains. Accumulation of aggregated Aβ polypeptide initiates a wide range of neurotoxic cascade in the brains of AD subjects followed by impairment in learning and memory. The amyloid precursor protein (APP) is a transmembrane protein that is cleaved either in amyloidogenic or non-amyloidogenic pathways [65]. In the amyloidogenic pathway, continuous processing of APP by β-secretase (BACE-1) and γ-secretase leads to Aβ generation. It is documented that APP metabolism in both amyloidogenic and non-amyloidogenic pathways is regulated by phosphorylation states of APP protein [66]. For instance, Lee et al. have demonstrated that APP has several phosphorylating sites; among them, phosphorylation on Thr_{668} residue is an effective factor in APP processing, so that phospho-$APP_{(Thr668)}$ promotes colocalization of APP and BACE-1 that finally leads to Aβ generation [67]. In this regard, PP2A and JNK are considered as two fundamental players, so that JNK phosphorylates APP on this specific residue and treatment with JNK inhibitor significantly shifts APP processing toward non-amyloidogenic pathway [68]. In the opposite way, PP2A decreases the phosphorylation level of APP. Consistently, it has been indicated that the activity of β-secretase and γ-secretase is increased in cells treated with PP2A inhibitor (okadaic acid) [69]. Therefore, it seems that decreased activity of PP2A in AD could be a critical factor in inclining APP processing toward amyloidogenic pathway. In addition, in accordance to studies proposing a direct inhibitory role of PP2A on JNK activity [63], it can be presumed that decreased expression and activity of PP2A in AD could lead to activation of JNK, which would result in Aβ overproduction [68, 69]. In addition to Aβ overproduction, in vitro molecular studies have demonstrated that JNK activation leads to apoptosis without any effect on γ-secretase and BACE-1 [70]. These results collectively imply that reduction in PP2A expression and activity might contribute to Aβ-induced neurotoxicity either directly or indirectly. PP2A is widely known as tumor suppressor molecule that in different levels can activate apoptotic pathways. One of these pathways is the suppression of AKT-induced pro-survival pathways by dephosphorylating of AKT on Thr_{308} [71, 72]. Moreover, PP2A can stabilize P53 molecule and triggers P53-dependent apoptotic pathways [73]. Moreover, Deng et al. reported that activation of PP2A by either ceramide or expression of PP2AC subunit results in Bcl2 dephosphorylation and enhanced p53/Bcl2 interaction which in turn leads to the loss of Bcl2 anti-apoptotic activity [74]. Furthermore, PP2A dephosphorylates Bad (pro-apoptotic member of Bcl-2 family) and causes its mitochondrial

retention; consequently, Bad inhibits Bcl-2. All of these processes result in the cytochrome *c* release which is necessary for the apoptotic procedure [71, 72, 75].

Meanwhile, another signaling pathway which contributes to cell growth and development is Wnt/β-catenin pathway. Despite some controversy, it has been demonstrated that PP2A affects Wnt/β-catenin pathway. Some of these studies believed that PP2A activity stabilizes β-catenin and prevents its degradation and some others reported that PP2A has an inhibitory effect on this pathway, so that the activity of PP2A leads to β-catenin degradation [72, 76]. It is believed that this PP2A mediated β-catenin degradation is carried out both directly through dephosphorylating it or indirectly through activation of GSK-3β (as a negative modulator for β-catenin) (see the interaction between GSK-3β and PP2A) [72]. Given that Wnt/β-catenin signaling pathway is actively involved in preventing apoptosis and even progression of oncogenes [77], and considering that PP2A contributes to the regulation of this pathway either directly or indirectly, therefore, it is conceivable to conclude that Wnt/β-catenin pathway is another way that may contribute in the mysterious effects of PP2A on apoptosis.

Furthermore, there is some evidence, showing that PP2A participates in acute Aβ-induced LTP impairment and its subsequent cognitive deficits in AD [78].

Possible mechanisms responsible for PP2A disruption in AD

The underlying mechanisms that lead to the decrease in PP2A activity and expression in AD have not been clarified yet. Theendakara et al. have shown that attachment of ApoE4 to the promoter region of PPP2R5E (B56ε), a kind of PP2A regulatory B′ subunit, suppresses its expression. In addition, this transcription factor causes demethylation of PP2AC subunit which leads to the decrease in PP2A activity, so that ApoE4 can be considered as a causative factor [79].

Moreover, emerging evidence has reported that the mRNA expression level and activity of two endogenous PP2A inhibitor, I1 and I2 (SET), are up-regulated in AD-affected brain regions [80]. In normal conditions, I1 localizes in neural cytoplasm and nucleus; however, I2 is mostly confined to the nucleus. There is convincing evidence showing that cytosolic PP2A and its two inhibitors, I1 and I2, are abnormally co-localized with hyperphosphorylated tau proteins in the cytoplasmic compartment [80]. Thereafter, the question that raised was why and how I2 translocates to the cytoplasm and how it inhibits the PP2A activity? In response to this question, Islas et al. suggested that cellular acidosis might be the determinant factor. In accordance, factors which damage lysosomal membranes and increase their permeability lead to the release of active endopeptidases, Asparaginyl Endopeptidase (APE). APE in turn, cleaves I2 to the N-terminal motif (NTF) and C-terminal motif (CTF) that both of them bind to PP2A and inhibit its activity [81]. In line with this study, another experiment has revealed that a mutated nuclear localization signal (NLS) in CTF of I2 is sufficient for its translocation [51]. In addition, Chasseigneaux et al. proposed that full-length cytoplasmic I2 without any cleavage can also impair PP2A methylation [82].

Furthermore, an increase in activity-dependent synaptic zinc in the amygdala, hippocampus, and cortical area of AD is correlated with amyloid deposits [83]. Interestingly, studies reported that, through activation of Src kinase, zinc phosphorylates PP2A at Tyr_{307} and inhibits its activity [84].

Therefore, it seems that cellular damages which bring about lysosomal instability and its subsequent protein degradation and re-localization of PP2A inhibitors and also the accumulation of zinc might be responsible mechanisms for PP2A disturbance in AD.

PP2A and Parkinson's disease

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease, which is characterized by progressive and substantial loss of dopaminergic (DA) neurons within the substantia nigra. At the molecular level, this debilitating disease is associated with accumulation of alpha-synuclein (α-Synuclein) protein and at the phenotypic level; PD is characterized with symptoms like bradykinesia (slowness and minimal movement), rigidity, resting tremor, and postural instability [85]. Alpha-synuclein is a chaperon-like pre-synaptic protein in the brain that normally contributes to neuroplasticity. However, in the disordered neurons, accumulation of intracellular α-synuclein plays a pivotal role in the pathology of a group of neurodegenerative disorders, collectively named as α-synucleinopathies. PD, Parkinson's disease dementia (PDD), dementia with Lewy bodies (DLB), and the multiple.

Molecular studies in the synucleinopathy brains revealed that, in one hand, α-synuclein phosphorylation at Ser_{129} residue stimulates the febrile formation and causes α-synuclein aggregation [86], and on the other hand, aggregated forms of α-synucleins are much more susceptible to phosphorylation [87]. Taken together, there is a positive association between α-synuclein phosphorylation, aggregation, and disease progression [88]. Interestingly, in PD and DLB, there is also a trace of disturbance in PP2A activity. In normal situations, PP2A with B55α subunit is the major phosphatase of α-synuclein at Ser_{129}, which dephosphorylates α-synuclein and prevents its accumulation [89, 90]. Accordingly, studies have demonstrated that treatment of cells with PP2A inhibitor, okadaic acid, aggregates intracellular α-synuclein [88]. PP2A and α-synuclein are both co-localized in mitochondria and there is a mutual relation between them. Accordingly, there is emerging evidence that soluble α-synuclein binds to PP2AC and activates PP2A holoenzyme. It seems that

structural change in aggregated α-synuclein reduces the tendency of binding to PP2A, so that aggregated α-synuclein has 50% less stimulatory effect on PP2A function [91]. Furthermore, as we discussed in the previous sections, the phosphatase activity of PP2A increases with its carboxyl methylation. Lee et al. reported that α-synuclein transgenic mice that received PME-1 inhibitor, the level of phospho-α-synuclein S_{129}, has significantly decreased and hence, these animals have the lower aggregation of α-synuclein [89]. On the other side of this mutual relation, other studies have investigated the expression of enzymes involved in methylation/demethylation of PP2A in synucleinopathies particularly PD. For example, Park et al. have indicated that, while the expression level of LCMT is reduced in frontal cortex and substantia nigra, the expression of PME is increased. Therefore, the ratio of methylated/demethylated PP2A is reduced causing lower activity of PP2A in PD and DLB [90]. Consistently, in a recent study, Tian et al. have investigated the effect of α-synuclein on the PP2A activity and reported that increased expression of α-synuclein not only inhibits LCMT-1 expression, but also upregulates PME-1. A process which inclines the balance between the activity of LCMT-1/PME-1 toward reduction in PP2A methylation and phosphatase activity. They further demonstrated that the level of PPP2R2 regulatory subunit is also decreased in the brain of animals with α-synuclein overexpression. This α-synuclein-mediated inhibition of PP2A activity brings about α-synuclein hyperphosphorylation at Ser_{129} and its aggregation [92]. In addition to PP2A methylation, it has been demonstrated that PP2AC inhibitory phosphorylation at Tyr_{307} is also increased in neurodegenerative diseases such as PD. Accordingly, Arief et al. have reported that β-*N*-methylamino-L-alanine (BMAA), an environmental factor involved in PD etiology, is able to increase PP2A phosphorylation at Tyr_{307} residue in a metabotropic glutamate receptor 5 (mGLUR5)-dependent manner. They concluded that this mGLUR5 mediated inhibition of PP2A may contribute to Parkinsonism dementia of Guam, a neurodegenerative disease which has representative signs of idiopathic Parkinsonism with early emergence of dementia [93]. Collectively, these studies imply that unbalanced phosphorylated/dephosphorylated state of proteins also participates in PD pathology, and PP2A, as a main cellular phosphatase, is a central factor in maintaining the physiologic balance of phosphorylation/dephosphorylation state.

PP2A and insulin signaling

Over 4 decades, since the discovery of insulin discovery in the brain, compiling evidence has shown that insulin signaling disruption plays role as a central underlying mechanism in the development and progression of neurodegenerative disorders particularly AD [6]. On the other hand, insulin signaling inside the target cells is greatly dependent on phosphorylation/dephosphorylation cycles of signaling molecules [7]. Therefore, kinase enzymes that participate in either activatory or inhibitory phosphorylation of insulin signaling elements (such as IRS, PI3K/Akt…) and phosphatase enzymes, which reverse these phosphorylation reactions, are greatly involved in the net response of insulin signaling.

In this regards, several lines of evidence have proven that PP2A is one of the phosphatase enzymes participating in the insulin response regulation (summarized in Fig. 4). For instance, it has been shown that PP2A is one of the enzymes, which dephosphorylates and inactivates Akt as a central element in the insulin signaling [94]. Therefore, it is conceivable to assume that PP2A dysregulation may be involved in the disruption of insulin signaling, known as insulin resistance. Accordingly, controversial documents have been published about the interplay of PP2A and insulin signaling pathway mainly in peripheral tissues and less in the CNS. Beg and colleagues have shown that increased PP2A activity is involved in the hyperinsulinemia-induced insulin resistance in the adipocytes cells. Concurrently, they also reported that the expression of PP2A regulatory subunit is increased in the insulin-resistant adipocytes [95]. In support to these results, some other studies have proven that elevation of PP2A activity participates in the induction of insulin resistance either by high-fat diet in the visceral adipose tissue or palmitate in the skeletal muscle [96, 97].

On the other hand, several lines of evidence have shown that insulin also affects PP2A activity. For instance, insulin exposure is shown to rapidly inactivate PP2A by promoting the Tyr phosphorylation of its catalytic subunit in the skeletal cell culture [98, 99]. In agreement, Hojlund et al. have demonstrated that insulin treatment in healthy humans down-regulated PP2AC alpha expression in skeletal muscle; however, in diabetic patients, this effects was not detectable [100]. Collectively, these records imply that a physiological balance exists between insulin signaling and PP2A activity, but upon disturbance, a vicious cycle may form between them. In this paradigm, PP2A over-activity by any cause promotes insulin resistance and lack of insulin-mediated inhibition of PP2A in insulin-resistant cells may intensify PP2A activity, a process that exacerbates insulin resistance.

In contrast to the results discussed above, some studies are also available, showing that activation of PP2A improves insulin signaling and opposes developing insulin resistance. For instance, in a study conducted on human adipocytes, it was reported that PP2A dephosphorylates and inactivates JNK, a member of MAPK family involved in developing insulin resistance, and nullifies its negative effects on insulin signaling [101].

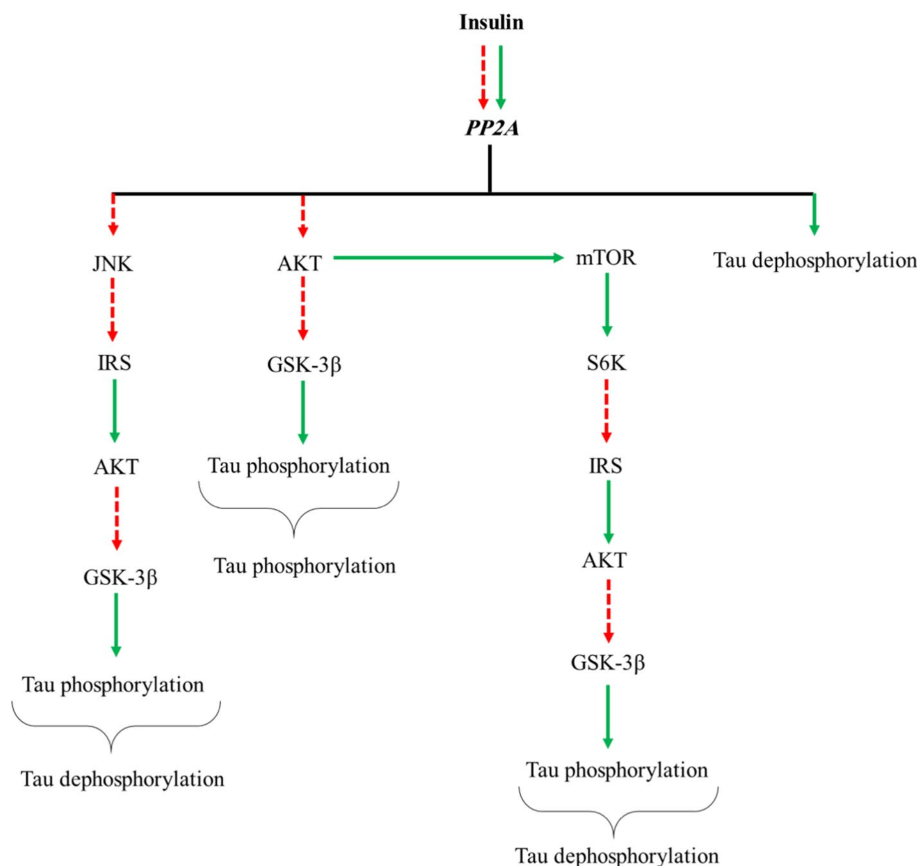

Fig. 4 Schematic diagram showing PP2A and insulin signaling pathway interaction. This figure shows that PP2A activity could either promote tau phosphorylation or its dephosphorylation. *PP2A* protein phosphatase 2A, *JNK* c-Jun N-terminal kinase, *mTOR* mammalian target of rapamycin, *IRS* insulin receptor substrate, *GSK-3β* glycogen synthase kinase 3β. Inhibitory pathways are represented by dash lines and activatory pathways are shown by solid lines

In a more complicated way, in another study, Carlson et al. have demonstrated that inhibition of PP2A could increase mammalian target of rapamycin (mTOR) mediated IRS-1 inhibition, indicating that PP2A serve as a negative regulator for mTOR activity in phosphorylating IRS-1 on Ser_{307}. The authors concluded that PP2A-induced inhibition of Akt, as an upstream activator of mTOR, may be responsible for the negating effect of PP2A against mTOR stimulated IRS-1 inhibition [102]. In contrast to this view, Hartley and Cooper reported that inhibition of PP2A is directly associated with mTOR and increased phosphorylation and degradation of IRS-1. They also showed that inhibition of mTOR by rapamycin significantly increases PP2A activity. Therefore, they suggested that mTOR-mediated regulation of IRS-1 results from its inhibitory effects on PP2A activity [103]. In support of later view, it has been suggested that, normally, PP2A binds to and from a complex with IRS-1 that protect it against excessive phosphorylation and degradation [104]. In addition, Galbo et al. proposed that inhibition of PP2A, as the main Akt phosphatase, may positively help insulin transmission, but they encountered a paradoxical situation in which PP2A inhibition improved insulin sensitivity and increased Akt activation in the white adipose tissue, but this accompanied by whole-body and hepatic insulin resistance. Authors concluded that, apart from Akt, other PP2A target proteins, which are involved in the normal insulin transmission, may be affected by PP2A inhibition and bring about such situations [105]. As mentioned, and up to our knowledge, there are a very limited number of studies investigating the interaction of insulin and PP2A in the brain. In one of these studies, Gratuze et al. have reported that STZ-induced insulin deficiency as an animal model of diabetes inhibits PP2A in the brain and results in tau hyperphosphorylation; insulin pretreatment was able to reverse these effects [106]. On the other side of the coin, it has been suggested that tau pathology could disrupt insulin signaling activity and bring about neuronal insulin resistance (reviewed in [45]). These data imply that PP2A may also participate in insulin resistance development in the CNS and vice versa. However, some very serious questions in this regard remain to be addressed. For instance, if PP2A reduction is associated with the development of AD pathology, then is this reduction related to insulin resistance seen in AD? How it can be described that reduction in PP2A activity plays role in insulin resistance? What are the differences between peripheral and central nervous system?

Targeting PP2A expression and activity

Considering the point that PP2A dysregulation has been reported in several lines of evidence, determining new features of PP2A and identifying its targeting molecules and/or cellular functions seem to be of great importance. In this regard, natural and/or new synthetic compounds with the ability to control the PP2A activity are widely used in the experimental studies.

Based on the modulatory approach of these compounds, they can be generally classified in three distinct categories: a group, which displaces or changes the expression of PP2A-associated negative regulatory elements [such as I2 (SET) and cancerous inhibitor of PP2A (CIP2A)]. Another group that changes the activity and/or expression of enzymes involved in post-translation modification of PP2A such as PME-1 and LCMT and, finally, a group of pharmacological allosteric agonists/antagonists.

In this section, we briefly introduced some of these modulators, which are being used in the experimental studies to modulate PP2A activity either negatively or positively (summarized in Fig. 5).

PP2A activators

Sphingolipid analogue FTY720

One compound which has activatory effects on PP2A is FTY720 (Fingolimod, Myriocin analogue). As a FDA-approved sphingosine analogue, FTY720 is generally prescribed as an immune suppressor for treatment of multiple sclerosis. From mechanistic point of view, FTY720 targets both SET and CIP2A. Moreover, FTY720 decreases the inhibitory phosphorylation level of PP2A at Tyr_{307}. These all make FTY720 as an activator for PP2A [107–109]. In this regard, MP07-66 and OSU-2S are two functional derivatives of FTY720 that activate PP2A and show anti-cancer effect without suppression on immune system [110, 111].

ApoE mimetics

ApoE mimetics are another group of PP2A activators. The human APO apolipoprotein E (ApoE) is a 299 amino acid holoprotein. It is expressed approximately in almost all cells, but its higher expression level can be detected in the liver and brain, respectively. The popular and well-known function of APOE is in cholesterol transport (reviewed in [112]). In addition, other effects are also attributed to ApoE

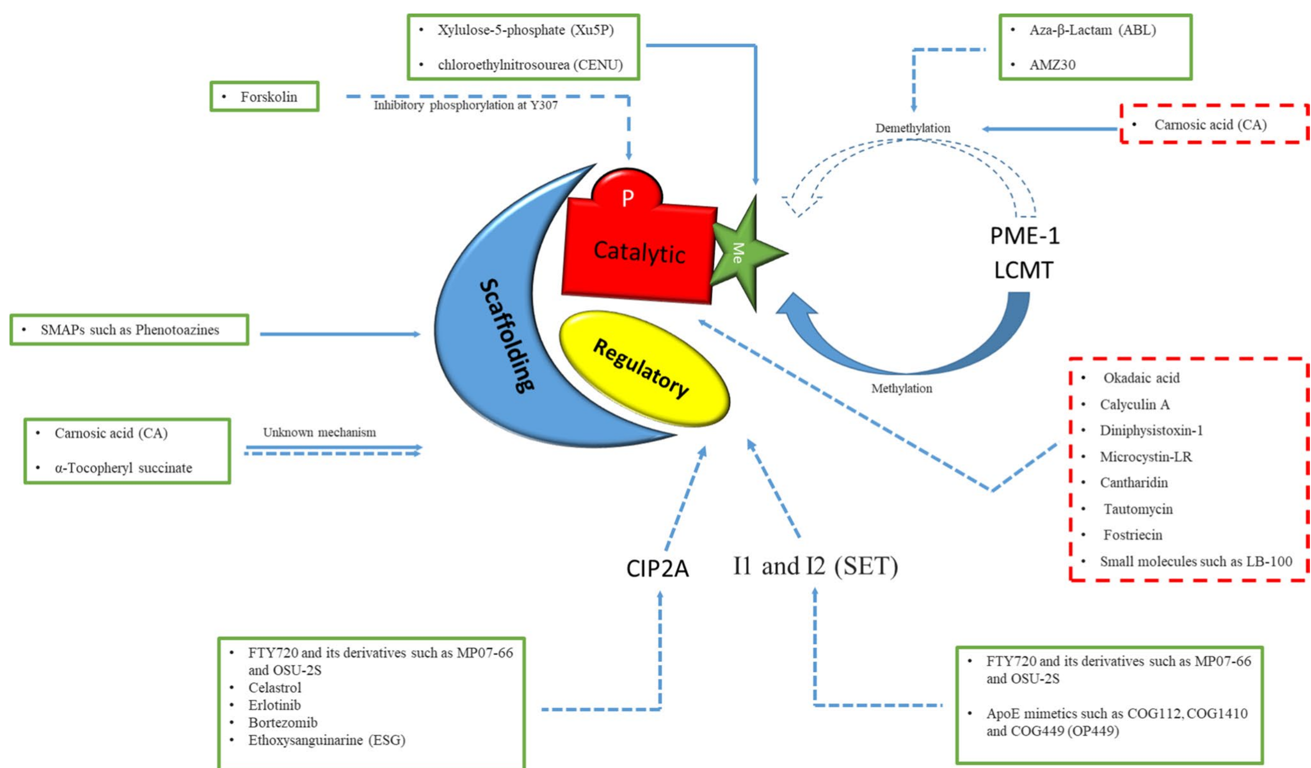

Fig. 5 Schematic diagram showing PP2A targeting compounds. *P* phosphate group, *Me* methyl group, *CIP2A* cancerous inhibitor of PP2A, *SMAPs* small molecule activators of PP2A. Inhibitory compounds are presented in dash line squares and activatory compounds are presented in solid squares

isoforms, for instance; in the CNS, it has been shown that carriers of ApoE4 are more susceptible to AD pathology, while other isoforms (ApoE2 and ApoE3) are protective. Accordingly, several peptides (named as ApoE mimetics) are designed to imitate these protective effects [112]. Some of ApoE mimetics used in studies are COG112, COG1410, and COG449 (OP449) [113]. Interestingly, it has been found that modulation of PP2A is one mechanism, which participates in the protective effects of ApoE mimetics [114, 115]. These compounds bind to C-terminal end of SET, dissociate it from PP2A, and disinhibit PP2A [116, 117].

Celastrol

Celastrol (tripterine) is a bioactive compound that is extracted from a Chinese herb called *Tripterygium wilfordii* [118]. This compound promotes interaction of CHIP, the E3 ligase, with CIP2A and, by this way, induces fast proteasomal degradation of CIP2A and subsequently increased PP2A activity [119]. In addition to the promotion of CIP2A degradation, there are some proofs showing that celastrol is also able to negatively modulate CIP2A expression [119, 120].

Erlotinib

Erlotinib (Tarceva) is originally an inhibitor of epidermal growth factor receptor (EGFR) Kinase; however, the recent studies described a new EGFR independent function for this FDA-approved drug [121]. In this regard, treatment with erlotinib is shown to activate PP2A via inhibition of the CIP2A expression. In a mechanistic point of view, it has been shown that erlotinib downregulates a transcriptional factor called Elk-1, which binds to CIP2A promoter and induces CIP2A transcription [122, 123].

Bortezomib

Bortezomib (Velcade) is another compound with CIP2A inhibitory properties. Bortezomib is a dipeptidyl boronic acid that is principally an FDA-approved proteasome inhibitor. However, it has been revealed that it can induce a negative regulatory function on CIP2A expression. Therefore, by downregulation of CIP2A, this compound also is able to activate PP2A [124, 125].

Ethoxysanguinarine (ESG)

Another natural compound which has antiviral and antibacterial properties [126, 127] and also modifies PP2A activity is a benzophenanthridine alkaloid known as ethoxysanguinarine (ESG) [128]. Accordingly, Liu et al. have demonstrated that ESG downregulates CIP2A both on mRNA and protein level in lung cancerous cells, a process which ultimately activates PP2A [128].

Compounds affecting PP2AC methylation

As mentioned in the earlier part, the balance between LCMT and PME-1 is a determinant factor in the activatory methylation of PP2AC carboxyl terminal, PP2A holoenzyme assembly and activity [26]. Aza-β-Lactam (ABL) and an individual group of sulfonyl acrylonitrile inhibitors, AMZ30, are two structurally distinct compounds which selectively inhibit PME-1 and disinhibit PP2A [129, 130]. Besides mentioned compounds which mainly inhibit PME-1-mediated demethylated of PP2A, a number of compounds are shown to elevate PP2A methylation in PME-1 independent manner [131]. One of these compounds is xylulose-5-phosphate (Xu5P), an endogenous metabolite produced in hexose monophosphate pathway, which increases the transcription of several lipogenic genes via a transcription factor named carbohydrate-responsive element-binding protein (ChREBP). Mechanistically, Xu5P activates PP2A that, in turn, dephosphorylates ChREBP and facilitates the transcription of target genes [132]. Another such compound is chloroethylnitrosourea (CENU) which belongs to a group of alkylating anti-cancer drugs with DNA damaging effects [133]. Similar to Xu5P, CENU also shows PP2A activatory properties through induction of PP2AC methylation. In this regards, Guénin et al. have demonstrated that treatment of B16 melanoma cells with CENU and Xu5P induces cell death in a PP2A-dependent way [131].

Forskolin

Forskolin is an Indian natural product extracted from the root of *Coleus forskohlii*. It seems that Forskolin induces cell death by both PKA/adenylate cyclase-dependent and -independent pathways [134]. Furthermore, forskolin treatment in cancer models brings about a reduction in PP2AC C-terminal phosphorylation at Tyr_{307} and, consequently, increases in PP2A activity and results in the alteration in PP2A phosphorylation level of substrates such as AKT and ERK [22, 135, 136].

Alpha-tocopheryl succinate

Alpha-tocopheryl succinate (α-TOS), an analogue of Vitamin E, has also the ability to activate PP2A. From a mechanistic perspective, this compound increases the activity of PP2A and produces pro-apoptotic effects in cancer models [137, 138].

Small molecule activators of PP2A

Recent studies are focused on developing small molecules for direct stimulation of PP2A activity. So far, several small molecules have been introduced. Phenothiazines are an example of this group. This FDA-approved drug is an antagonist of dopaminergic receptors and has been used in antipsychotic interventions, while a series of studies using chromatography and mass spectrometry described anti-cancer effects for this compound and these effects are related to PP2A reactivation [139]. This group of small molecule activators of PP2A (SMAPs) compounds interact with scaffolding Aα subunit of PP2A and change its conformation in a way that leads to activation of PP2A [139].

PP2A inhibitors

Okadaic acid and related compounds

Okadaic acid (OA) group is a class of PP2A inhibitors that interact directly with the catalytic subunit of PP2A.

This group includes OA (a polyester containing 38 fatty acids isolated from the black sponge *Halichondria okadai*), Calyculin A (extracted from a marine sponge *Discodermia calyx*, Dinophysistoxin-1 (also named 35-methylokadaic acid extracted from the mussel *Mytilus edulis*), Nodularin, and Microcystin-LR (both extracted from toxic blue–green algae *Cyanobacteria*) [140].

Similar to mentioned compounds, Cantharidin, Tautomycin, and Fostriecin are three other inhibitors which bind to PP2AC and inhibit PP2A activity [141]. A limiting characteristic of these inhibitors is their cross-reactivity with other protein phosphatases such as PP1, PP4, PP5, and PP6 activity [141–143]. However, this unspecific inhibition of different protein phosphatases is concentration dependent, so that almost all of them inhibit PP2A, PP4, and PP6 (phosphatase with highly similar amino acid sequences) activity in low concentration, while higher concentrations are needed to inhibit PP5 and PP1 [141, 143]. Among these inhibitors, fostriecin binds more selectively to PP2A versus PP1 and seems that their binding site in PP2AC subunit is different from other inhibitors [144]. Recent Structural and biochemical findings demonstrated that minor alteration in the structure of mentioned inhibitors could produce new compounds with desired sensitivity and potency (for more details, see [145]).

LB-100

LB-100 is a kind of water-soluble small molecule inhibitors of PP2A. This recently developed molecule can selectively bind to PP2AC subunit and potently inhibit its activity. LB-100 development and studying in the human clinical trial started when PP2A oncogenic role was discovered. In fact, it was revealed that downregulation of PP2A could be a target to overcome tumor resistance (extensively reviewed in [146]).

Bifunctional modulators

Carnosic acid (CA)

Another modulator for PP2A activity is carnosic acid (CA). CA is a polyphenolic diterpene which is extracted from the plant rosemary. This compound has beneficial properties such as anti-oxidative function, and improves insulin resistance and hyperglycemia in obesity [147, 148]. The important point about CA is its bidirectional modulatory effects on PP2A. On one hand, CA treatment in skeletal muscle cells leads to a decline in PP2A activity through an increment in PME-1 induced PP2A demethylation [148]. On the other hand, it has been shown that CA inhibits PP2A activity and shows anti-proliferative effect on androgen-independent human prostate cancer PC-3 cells [149].

Measuring PP2A activity in researches

Considering the importance of PP2A in regulating various cellular functions and the undeniable role that PP2A disruption plays in the pathological conditions, measurement of this phosphatase will aid investigators to get more clear insights about the role of PP2A in the physiology and pathology of CNS. To investigate changes in PP2A activity in different conditions, several practical methods have been introduced; each of them has some pros and cons. In continue, we summarized and discussed some of the custom methods used in measuring PP2A activity.

Western blotting

As we mentioned before, methylation and phosphorylation are two critical post-translational modifications that determine PP2A activity (reviewed in [24, 25]). Furthermore, expression of each subunit is an important factor in determining PP2A activity. Accordingly, over the last decades, popular companies have designed various antibodies, which aid measuring PP2A expression and/or modifications at the protein level. Accordingly, various experimental studies have used these antibodies (listed in Table 1) as an indicator of PP2A activity. However, availability of PP2A subunits is an essential step in the assembly of the holoenzyme, but post-translational modifications of subunits (particularly C subunit) finally determine the activity level of the enzyme. Therefore, evaluating the expression of subunits is necessary but it is not sufficient to judge the activity of the enzyme. On the other hand, quantifying either the level of methylation

Table 1 A summary of antibodies used in western blotting to investigate PP2A activity and/or expression

Antibodies	Sources	Cat number	References
Anti-PP2A antibody (C subunit)	Millipore	05-421, 06-222 05-545, 07-324	[82, 90, 150, 151]
	Cell Signaling	2038, 2259	[152, 153]
	Santa Cruz	sc-6110	[91]
Anti-PP2A antibody (A subunit)	Cell signaling	2041, 2260, 2039	[152, 154]
	Millipore	05-657, 07-250	[155, 156]
Anti-PP2A antibody (B subunit)	Millipore	05-592	[50]
	Cell Signaling	2290,4953	[153, 157]
Anti-methyl-PP2A (C subunit)	Millipore	04-1479	[82]
Anti-demethyl-PP2A (C subunit)	Millipore	05-577	[83]
Anti-PP2A methylesterase (PME-1) antibody	Millipore	07-095	[158]
Anti-PTPA antibody	Millipore	05-941	[158]
p-PP2ACα/β antibody (Tyr 307)	Santa Cruz	sc-12615	[159]

PP2A protein phosphatase 2A, *PTPA* PP2A phosphatase activator

or phosphorylation in different residues alone could not determine overall PP2A activity; instead, each modification just facilitates or inhibits PP2A activity and the overall balance of these modifications determines the enzyme activity. Therefore, besides the measurement of expression and post-translational modification, some complementary methods could be employed to reach a more precise judgment about PP2A activity. One of the most prevalent methods is using PP2A assay kits which are discussed in the next sections.

Colorimetric phosphatase assay

One common way to measure enzyme activity in biological systems is via enzyme assay kits. Accordingly, serine/threonine phosphatase assay kits are developed to study PP2A activity. In this method, first, the free endogenous phosphate should be removed from cell/tissue extract, and then, a synthetic phosphopeptide substrate (as a substrate for PP2A enzyme) is added to the samples. After stopping the reaction, the amount of free phosphate generated in this reaction is revealed by the measuring the absorbance of a molybdate malachite green phosphate complex. At the final step, PP2A activity is calculated based on the released phosphate amount per µg of protein per minute. One supplementary method to reach a more precise result in phosphatase assays method is to separate protein phosphatase by Immunoprecipitation and then perform protein phosphatase assay. In this method, the PP2A is isolated from the lysate by specific PP2A antibodies which bind to a site that does not interfere with its activity (For instance anti-PP2AC subunit), and thereafter, the purified PP2A activity is measured.

The important point regarding this kit is that the Ser/Thr phosphopeptide substrate, [RRA(pT)VA] can be dephosphorylated by PP2A, PP2B, and PP2C. Accordingly, a question raised that how this kit can detect PP2A activity in a specific manner. The response to this question is the difference in co-enzyme requirements for PP2 isoforms, while PP2B and PP2C are cation-dependent, but PP2A is not. Therefore, by eliminating cations from buffer, PP2B and PP2C activity will be inhibited. Table 2 represents two PP2A assay kits used in studies.

Another method that use para-nitrophenyl phosphate (pNPP) as substrate and could be employed to assess the activity of PP2A is pNPP method. pNPP is a non-proteinaceous chromogenic substrate which could be used to assess phosphatase activities in ELISA and conventional spectrophotometric assays. However, this method is less specific for PP2A and activity of other phosphatases could affect the results, but it is easier and cheaper than other methods [160]. One way to overcome the problem of unspecific results in phosphatase assays such as pNPP is to include a

Table 2 Samples of PP2A assay kits

Source	Promega	MERCK
Cat. number	V2460	17-313
Components	Molybdate dye solution Molybdate dye Additive Phosphate Standard, 1 mM Ser/Thr Phosphopeptide RRA(pT)VA 96-Well Plate (½-area, flat-bottom) Spin columns, Reservoirs and adaptors Sephadex® G-25 Phosphate-Free Water	Malachite green solution A Malachite Green Additive Phosphate standard Threonine phosphopeptide (K-R-pT-I-R+R) Anti-PP2A,C subunit, clone 1D6 pNPP Ser/Thr Assay Buffer Protein A Agarose 96-Well Microtiter Plate
Refs.	[83]	[161, 162]

control group with specific inhibition of PP2A (for instance by okadaic acid) and subtraction of the phosphatase activity from the whole results (in the absence of inhibitor) could be helpful .

Fluorescence-based PP2A activity assay

Another way to determine PP2A activity is fluorescent-based methods, which are much more sensitive than colorimetric and let researchers to monitor enzyme activity continuously. One substrate for phosphatase enzymes including PP2A that has the property to emit fluorescence and is used in fluorescent-based methods is 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP). In this method, phosphatase enzymes including PP2A convert the phosphorylated form, DiFMUP, which has low fluorescence to a high fluorescence product (DiFMU) [162]. DiFMU exhibits fluorescence at 358/452 nM, and therefore, the continuous production of this fluorescent product (as an indicator for phosphatase activity) could be calculated either by fluorescence reading or by measuring absolute DiFMU in solution [161, 162]. As DiFMU is a substrate for a wide variety of phosphatases; therefore, the results could not be solely attributed to PP2A activity. As mentioned, one way to extract the PP2A activity from other phosphatases is to perform a control experiment with a specific pharmacological inhibitor of PP2A (e.g., okadaic acid). In this way, subtracting fluorescence result in samples treated with okadaic acid from the whole sample could show the exact effect of PP2A in observed DiFMUP dephosphorylation. In another fluorescent method, a phosphorylated substrate (Rhodamine 110 peptide) which is nonfluorescent is added to Ser/Thr phosphatase-containing sample.

Next, the solution is left for 10 min at 22–25 °C to allow phosphatase reaction to go forward, and then, a protease solution is added to R 110 solution + sample to stop phosphatase reaction. As phosphorylated substrates (R110) are resistant to protease reaction, protease enzyme just digests dephosphorylated substrates and produces a highly fluorescent product. Therefore, the amount of fluorescence produced at the end of this reaction correlated with the phosphatase activity of our sample. As this method is not specific to PP2A and other relative proteases such as PP1, PP2B, and PP2C are also involved in the dephosphorylation reaction, therefore, the inhibitors of PP2A (okadaic acid) are used to extract the exact amount of PP2A activity.

Concluding remarks and perspectives

Although, in recent 2 decades, PP2A as a main phosphatase enzyme has gain attention of scientists and several lines of evidence have been published about its functions, but its exact role in CNS physiology remains elusive. Furthermore, it has been shown that disruption of PP2A contributes in the pathology of neurodegenerative diseases such as AD and PD. However, there is controversy and needs more investigation. As insulin signaling disruption is a key element in the pathology of above-mentioned disorders and phosphorylation/dephosphorylation cycle (balance) is a pivotal event in the insulin signaling pathway. Then, the possible involvement of PP2A dysregulation in central insulin resistance is imaginable, and with more studies, these molecules and their participation in new pathologies could be considered as golden target molecules for future drug designing.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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