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Phosphatase: PP2A structural importance, regulation and its aberrant expression in cancer

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Abstract

Protein Phosphatase 2A (PP2A) is an important and ubiquitously expressed serine threonine phosphatase and regulates the function by dephosphorylating many critical cellular molecules like Akt, p53, c-Myc and β -catenin. It plays a critical role in cellular processes, such as cell proliferation, signal transduction and apoptosis. Structurally, it is multifarious as it is composed of catalytic, scaffold and regulatory subunits. The catalytic and scaffold subunits have two isoforms and the regulatory subunit has four different families containing different isoforms. The regulatory subunit is the most diverse with temporal and spatial specificity. PP2A undergoes post-translational modifications (i.e. phosphorylation and methylation), which in turn, regulates its enzymatic activity. Aberrant expression, mutations and somatic alterations of the PP2A scaffold and regulatory subunits have been observed in various human malignancies, including lung, breast, skin and colon cancer, highlighting its role as a ‘tumor suppressor’. This review is focused on the structural complexity of serine/threonine phosphatase PP2A and summarizes its expression pattern in cancer. Additionally, the PP2A interacting and regulatory proteins and substrates are also discussed. Finally, the mouse models developed to understand the biological role of PP2A subunits in an *in vivo* model system are also reviewed in this article.

1. Introduction - Kinases and Phosphatases

In a typical cell, the functions of nearly one-third of the proteins are regulated via phosphorylation and it controls various biological functions like cell division, growth and development, survival, proliferation, and apoptosis. Depending upon the physiological necessity of the cell, proteins transiently shift from a phosphorylated to a dephosphorylated state and vice versa, and are specifically controlled by protein kinases and protein phosphatases [1]. Hence, kinases and phosphatases act as important checkpoint regulators and switches. Upon studying the various kinases and phosphatases, researchers have identified many protein kinases comprising two families - protein tyrosine kinases and protein serine threonine kinases [2], while a comparable number of phosphatases are not present in the biological system. Phosphatases are classified in two major classes, protein tyrosine phosphatases (PTPs) and protein serine/threonine phosphatases (PSPs) [3]. In general, the phosphatases may exist in active monomeric unit (having the catalytic subunit

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alone-PTP) or may contain active dimeric form (comprising of catalytic and regulatory subunit-PTP/PSP) or active holoenzyme complex of catalytic, regulatory and scaffold subunits, as observed in certain family members of protein serine phosphatase. Each subunit also has many different isoforms. Since the isoforms of a subunit for a single phosphatase can form complexes in many possible combinations with isoforms of other subunits, different variants of a specific phosphatase are present in nature. Thus, the variant based on the composition of its particular isoform of the subunits obtain specificity toward their physiological substrates. It will thus explain how phosphatases, although present in a smaller number, can balance the activity of many kinases. Thus, both the kinases and phosphatases act concurrently to control the amplitude, rate and duration of signaling response [4]. To further highlight the unique property of protein serine/threonine kinases and phosphatases, protein tyrosine kinases and protein tyrosine specific phosphatases in *S. cerevisiae*, *C. elegans*, *D. melanogaster* and humans were elaborated in **Table.1**.

2. Protein Phosphatases

Protein modification via phosphorylation mainly occurs on the hydroxyl-group containing amino acid residues, namely serine (Ser), threonine (Thr) and tyrosine (Tyr). Upon comparing the degree of phosphorylation between these three phosphorylated amino acids, phosphoserine (pSer) predominates with 86.4%, then phosphothreonine (pThr) at 11.8%, followed by phosphotyrosine (pTyr) with 1.8% [5]. Protein phosphatases nucleophilically attack the phosphate group for catalysis in the presence of a water molecule [6] and dephosphorylate these three phosphorylated amino acids. The members of the protein phosphatase super family are broadly divided into two major groups - protein serine/threonine phosphatases (PSPs) and phosphotyrosine phosphatase (PTPs). PSPs are further divided into three sub-families: phosphoprotein phosphatases (PPPs), metal dependent protein phosphatases (PPMs) and aspartate based phosphatase. PPPs are diverse and contain subfamilies known as PP1, PP2A, calcium-activated PP2B (also known as calcineurin), PP4, PP5, PP6 and PP7. The PPM family is comprised of PP2C and pyruvate dehydrogenase phosphatases; they catalyze the reaction in the presence of Mg^{2+}/Mn^{2+} . Aspartate-based phosphatase has an aspartic acid signature (DXDXT/V). Examples of this group of phosphatases are transcription initiation factor II: TFIIIF-associated C-terminal domain phosphatase/small CTD phosphatases, and the halo acid dehydrogenases (HAD) enzyme family [7]. Protein tyrosine phosphatases (PTP) specifically remove the phosphate group from post transnationally modified tyrosine residues. These PTPs have multiple functions encountering protein tyrosine kinases; one of the best examples for PTP target is the regulation of AKT signaling by dual specificity phosphatase, phosphatase and tensin homolog protein (PTEN). Furthermore, a sub group of dual specificity phosphatase family member MAP kinase phosphatases (MKPs) are the major regulator of MAP kinase pathway. The distinct complementary function of PTPs in the regulation of signal transduction and the etiology of human disease was best described in the review by Nicolas *et al*, 2006 [4].

2.1. Protein Phosphatases 2A: PP2A

PP2A is an important player in many cellular functions. It controls cell metabolism by regulating the activity of the enzymes involved in glycolysis, lipid metabolism and catecholamine synthesis [8]. It also regulates various biological processes such as the cell cycle (by mediating cdc2 kinase activation), DNA replication, transcription and translation, signal transduction, cell proliferation, cytoskeleton dynamics and cell mobility and apoptosis. It has also been shown to play a role in cell transformation and cancer [9-12]. In eukaryotes, the predicted amino acid sequence establishes a high degree of sequence conservation (78-93%) of PP2A between yeast, drosophila and mammals [13]. It is ubiquitously expressed and contributes to 0.3 - 1% of the total cellular protein in the

mammalian cell [14]. The majority of the soluble phosphatases activity at phospho serine and phospho threonine is catalyzed by PP2A. PP2A is structurally complex and exists in two different forms: dimeric form (PP2A_D) [15] and a trimeric form (PP2A_T) [16]. The dimeric form is known as the core enzyme and is composed of the catalytic and scaffold subunit, while the trimeric form is an active holoenzyme complex which consists of three subunits: catalytic (PP2Ac), scaffold (PP2AA) and regulatory subunits (PP2AB) [17](**Figure.1**).

2.2. PP2A: Catalytic subunit (PP2Ac/PPP2C)

PP2A catalytic subunit (PP2Ac) is globular in structure, ubiquitously expressed in almost every tissue, and it is most abundant in the heart and brain. The protein expression level of PP2Ac in the cell is regulated translationally to maintain a constant level [18]. The amino acid sequence of PP2Ac (37kDa) is 86% identical in human and yeast and it also shares 50% amino acid sequences with PP1 and 40% identical sequences with PP2B. PP2Ac exists in two isoforms C α and C β . They are encoded by two separate genes each comprised of seven exons and six introns. Exon 2-6 participates in substrate binding and catalysis, whereas exon 1 and 7 aid in regulation. Both isoforms consist of 309 amino acids and share 97% sequence similarity. The isoform C α is predominantly expressed in the plasma membrane and C β in the cytoplasm and nucleus. C α is expressed in higher abundance than C β due to its strong promoter activity as well as due to differences in the rate of mRNA turnover [19].

PP2Ac C-terminal tail is uniquely conserved (³⁰⁴TPDYEL³⁰⁹) and binds to the scaffold and regulatory subunits (PR61 γ). Toward the N-terminal of PP2A, C α and C β differ by eight amino acids and these amino acids are also not conserved in PP1 and PP2B, suggesting that this part of the protein may not play a role in catalysis [20]. Despite sharing 97% similarity, PP2Ac α knockout mice are not viable and die at embryonic day 6.5, indicating that PP2Ac β is unable to substitute for PP2Ac α loss, underscoring the non-redundant function of C α in embryonic development and gastrulation [21].

2.3. PP2A: Scaffold subunit (PPP2AR1/PR65/PP2A-A)

Similar to PP2Ac, the PP2A scaffold subunit is encoded by two distinct genes, PPP2R1A and PPP2R1B, resulting in two isoforms, A α and A β . Both are ubiquitously expressed and share 86% sequence similarity [22]. In about 90% of the PP2A assemblies, the core and/or holoenzyme is composed of the A α scaffold subunit and is highly abundant in all normal tissues, accounting for 0.1% of the total cell protein. On the other hand, A β is found in 10% of PP2A assembly leading to differential preference of interaction with the catalytic and regulatory subunits [23]. Both isoforms are fractionated in the cytoplasm. Uniquely, in *Xenopus* oocytes, A β is highly expressed during stage 35 of embryogenesis in the ovary for meiotic maturation and fertilization. Further, from that stage, it gradually decreases and A α expression increases [24].

In its heterotrimeric form, PP2A-A acts as a structural assembly base to escort the catalytic subunit and to facilitate interaction with the regulatory subunit and other substrates. Whereas in the dimeric form it acts as a regulator by changing the catalytic specificity [25]. Structurally, the scaffold subunit is comparatively different from other subunits. PP2A-A is composed of HEAT sequence (Huntington/elongation/A-subunit/TOR) having 15 tandem repeats of 39 amino acids each. Each repeat is composed of two α -helices and they are connected by inter- and intra-repeat loops. The catalytic subunit binds to 11-15 repeats and the regulatory subunit binds to 1-10 repeats of the HEAT sequence. The scaffold subunit escorting the catalytic subunit bends to form a base and shows a horseshoe shape-like structure. This flexible bending helps to recruit the regulatory subunit and other PP2A substrates [26].

2.4. PP2A: Regulatory subunit (PP2A-B/PR55- B/PR56or61-B'/PR72-B''/PR93or110-B''')

2.4.1. PP2A-B/PR55—The PP2A regulatory subunit is structurally diverse and has a minimum of 26 different transcript and splice variants encoded by 15 different genes in the human genome. PP2A-B is thought to be the master regulator of the PP2A holoenzyme and it is likely to act as a targeting modulator to provide temporal and spatial specificity [27]. Even though all the different family members and isoforms of PP2A-B bind to similar recognition sequences of PP2A A α , they do not possess similar gene sequences. Regulatory subunits are multifunctional and are classified into four different families: B (commonly known as B55/PR55), B' (B56/PR61), B'' (PR48/PR72/PR130), and B''' (PR93/PR110). These numbers represent the approximate molecular weight of each peptide in kDa. The protein secondary structure predicts that they form β -sheets and turns. PP2A-B family members are expressed distinctly at various developmental stages in a tissue-specific manner. B55 has four different isoforms (α , β , γ and δ). It is observed that the expression of B55 γ increases and B55 β decreases gradually after birth and are developmentally regulated [28]. Structural recognition of the B55 family is the presence of the tryptophan-aspartate repeat (40-WD repeats) involved in protein-protein interaction to facilitate substrate binding. In neurons, B55 α and B55 β are localized in the cytoplasm, whereas B55 γ is localized in the cytoskeletal fraction. It has been shown in yeast that CDC55 (B55 in human) is essential for cytokinesis. Mutation and/or deletion of CDC55 produce abnormal unbudded cells and display a partial blockage of cell separation [29]. In mammals, B55 is associated with cytoskeletal dynamics and nuclear translocation. Conditional B55 knockout in fibroblasts are unable to dephosphorylate vimentin, leading to alteration in the interphase dynamics differentiation and migration [30].

2.4.2. PP2A-B'/PR56/PR61—B56, another regulatory family has five different isoforms (α , β , γ , δ , and ϵ). The unique feature of this family is that they can be phosphorylated and are mostly α helical. They can directly bind to the core enzyme and enhance the reaction. These isoforms show 80% identical sequences in their central region but differ in their N and C terminals, leading to different expression levels in tissues. Intracellular localization of B56 isoforms vary, as B56 γ is expressed in the nucleus while B56 α , B56 β , and B56 ϵ are expressed in the cytoplasm, and PR61 δ appears to be expressed in both the nucleus and cytoplasm [20]. It is surprising to find that the nuclear localization signal (KRTVETEAVQMLKDIKK) is only present in B56 δ and B56 γ 3 and absent in B56 γ 1 and B56 γ 2, yet all these isoforms are efficiently targeted to the nucleus. In the late G1 phase of the cell cycle, B56 α regulates p53 via Cyclin G and in Wnt signaling via the APC component [31]. Studies showing differential phosphorylation, sequence alignment and spatial intracellular localization indicate that B56 governs several functions, which are yet to be understood.

2.4.3. PP2A-B'': PR72 and PP2A-B'''/PR93 or110—B'' and B''' families were discovered by yeast two hybrid screening. B'' family contains PR72 and PR130 isoforms, which differ at the N-terminal region, indicating that the origin of these variants may be due to alternate splicing. PR72 is expressed only in the heart and skeletal muscle, whereas PR130 is ubiquitously expressed in all tissues and is abundant in the heart and muscle. PR72 requires calcium binding in two EFX domains (Domain required for Nkd to interact with the basic/PDZ domains of fly Dsh or vertebrate Dvl proteins in the yeast two-hybrid assay) and this binding facilitates conformational changes in protein structure to interact with the PR65 scaffold subunit [32]. PP2A-B inhibits simian virus 40 (SV40) replication, whereas PP2A-B'' containing holoenzyme activates its replication. It was also found that B'' regulates Rb phosphorylation at p107 during UV-radiation exposure and they excite the DNA damage response gene [33]. In vitro overexpression of B'' can arrest cells at the G1 stage and inhibit cell cycle progression. B''' family is a calmodulin binding protein (CaM) and it interacts

with the core enzyme to enhance calcium-dependent signaling. It contains eight splice variants but only four are actively translated. This group also requires ATP and Mg²⁺ to activate its own activity. Striatin and S/G2 nuclear auto antigen (SG2NA) is an example of the B'' family. It is a nuclear protein expressed during the S and G2 phases of the cell cycle [34]. Different combinations of multifarious PP2A subunits (catalytic: C, scaffold: A, and regulatory: B), including their isoforms, assemble to form more than 200 types of biochemically distinct heterotrimeric PP2A holoenzymes. Comparing all of the three subunits, PP2A-A provides substantial structural flexibility (HEAT region) and can undergo various conformational changes [35]. Its three dimensional architecture allows binding of various molecules.

3. PP2A regulators, interacting proteins, substrates and inhibitors

The major hallmark of PP2A is its protein assembly or stable complex formation, through which it interacts with vital intermediate signaling molecules that are present in the cytoplasm. It also modulates their function by interacting and transforming the phosphorylation status of the partner [36]. During meiosis I, Shugoshins binds to PP2A and dephosphorylates cohesion, which prevents spindle microtubules disassembly [37]. PP2APR55 also dephosphorylates vimentin (intermediate filament) and protects cytoskeleton disassembly [38].

Various cellular proteins such as $\alpha 4$, I1_{PP2A} and I2_{PP2A} directly interact with the AC core dimer or with the free C subunit and regulate the phosphatase regulatory mechanism of PP2A [39]. Studies by McConnell *et al* 2007 have led to the identification of a novel regulator of PP2A, type 2 A interacting protein (TIP) in the mammalian system which highlights the differently regulated function of PP2A between yeast and metazoan system. The TIP protein directly interacts and retains the catalytic activity of PP2A and PP2A-like enzymes, such as PP4 and PP6, and further highlighted phosphatase as an additional therapeutic target for cancer prevention [40]. Another experimental approach by Lee *et al* 2007 led to the identification of various PP2A interacting partners that are specific to the catalytic subunit. Apart from the known interacting partners (Axin and CaMK iV), PP2Ac-specific interacting proteins, such as tuberous sclerosis complex 2 (TSC2), R-Ras, Nm23H2 and RhoB, were confirmed by pull down experiments in the presence of the Wnt3a as a ligand, a stimulus that can activate the growth regulatory signaling pathway of Wnt/beta catenin and ERK [41]. Initial studies by Seeling *et al* 1999 have shown through the yeast two hybrid system that a regulatory subunit of PP2A, B56, interacts with adenomatous polyposis coli (APC) protein, implicating the inhibition of Wnt/betacatenin signaling and an alteration in B56-specific PP2A function is essential for early xenopus development [31, 42, 43]. Recently, a novel complex assembly consisting of RAC1 kinase, PP2A and AKT in NSCLC has been shown to result in reduced phosphorylation of AKT and inhibition of cancer cell metastasis in response to Eph3 activation [44]. The phosphoprotein SET is a potent inhibitor of PP2A activity [45]. It is localized both in the nucleus and cytoplasm. SET interacts with PP2Ac via its highly acidic C-terminal domain and completely inhibits the phosphatases activity of PP2A. SET is overexpressed in various cancers like chronic myelogenous leukemia and Wilm's tumor [46]. In Alzheimer's disease, inhibition of PP2A activity by SET leads to hyper phosphorylation of the Tau protein [47].

Potent tumor promoter, Okadaic acid (a microbial toxin), inhibits the enzymatic activity of PP2Ac and thereby has facilitated various studies to understand the functional aspects of PP2A and other phosphatases [12]. Other than Okadaic acid, calyculin A, microcystin, cantharidin, nodularin, fostriecin and tautomycin are able to inhibit PP2A activity at different IC₅₀ values [48]. In addition to microbial toxin, viral protein SV40 (potent oncogene) also inactivates PP2A action by binding to the AC dimer and displacing the PR56

(PP2A-B' γ) subunit [35]. Endogenous CIP2A (cancerous inhibitor of PP2A) inhibits PP2Ac activity via interacting with c-Myc (Ser62) and stabilizes it from proteolytic degradation [49]. Recently, a major indirect activation of PP2A by inhibiting CIP2A at both the transcriptional and translational levels through the drug bortezomib was shown in triple negative breast cancer cells [50]. Further, carnosic acid, a polyphenolic diterpene, has been shown to induce apoptosis in prostate cancer cells by modulating AKT/NF κ B pathways [51]. Thus, inhibitors of CIP2A and activators of PP2A that modulate the anti-apoptotic (Both intrinsic and extrinsic) pathway may serve as potential agents to be used in the prevention and/or treatment of various cancers.

4. PP2A: Regulation and Modification

PP2A is being regulated by post translation modification, auto-regulation, subunit diversity and substrate protein interaction. Methylation [52] and phosphorylation [53] are two major modifications that have been shown to modulate PP2A catalytic efficiency. As mentioned previously TPDYFL is a conserved sequence in PP2Ac. Tyrosine (Y) and leucine (L) undergo phosphorylation and methylation, respectively. Phosphorylation of Y307 by receptor associated tyrosine kinases effectively decreases the PP2A activity by inhibiting the interaction of PP2Ac with the PP2A-PR55/PR61 subunit [54]. PP2A also possesses the auto protein tyrosine phosphatases activity, which is inhibited by auto-phosphorylated-activated protein kinases to alter PP2A efficacy [55]. Another modification is methylation of PP2A at L309 by PP2A-methyltransferase (PMT). It is also known as leucine carboxyl methyltransferase 1 or LCMT1. The demethylation occurs by PP2A-methyltransferase (PMT). The addition of a methyl group by LCMT1 at L309 enhances the binding affinity of the core dimer (A&C subunit) toward distinct regulatory subunits and provides specific activity to the holoenzyme [56]. In addition to modification, constitutive and constant PP2Ac expression is controlled by auto-regulation. PP2Ac expression is tightly regulated in the cell at the translational level but not at the transcription level [18] (**Figure 2**).

5. Regulation of PP2A in cellular pathways

PP2A activity is indispensable for every cell and takes part in the majority of the cellular pathways. Dysfunction or deregulation of PP2A will affect various physiological processes. As mentioned previously, PP2A dephosphorylation activity is essential during embryogenesis and PP2Ac α knockout mice are embryonically lethal. In *Drosophila*, Sex Combs Reduced (SCR) Hox protein determines the identity of the labial and prothoracic segments. siRNA-mediated PR56/B' knock down resulted in embryos without salivary glands and demonstrated dPP2AB' directly interacting with SCR as a positive modulator [57], indicating the importance of PP2A during cell survival and maintenance.

PP2A not only controls the cell cycle but it also controls cell death (apoptosis). Akt plays an important role in cell growth, proliferation and apoptosis. Phosphorylation at Thr-308 and Ser-473 leads to activation of Akt and it was found that deregulation of Akt is associated with various human malignancies. Co-immunoprecipitation and in-vitro pull down assay using pro-lymphoid FL5.12 cells showed a direct association of the PP2A-B55 holoenzyme with Akt, which selectively regulates phosphorylation of Akt at Thr-308 and regulates cell proliferation and survival [58]. It suggests that PP2A acts as a negative regulator for the Akt pathway. PP2A also plays a major role in the Wnt signaling pathway. In *Xenopus*, PP2A-B56 is involved in β -catenin dephosphorylation and degradation and its phosphorylation directs activation of the Wnt pathway [43]. Key players of programmed cell death like BAD (pro-apoptotic) and Bcl-2 are also regulated by PP2A. Phosphorylation of BAD suppresses, and its dephosphorylation by PP2A promotes pro-apoptotic activity [59]. Additionally, phosphorylation of Bcl-2 activates, and its dephosphorylation by PP2A suppresses anti-

apoptotic activity. In *Drosophila*, knock down or loss of either of the subunits A, C, or B56 leads to hyper phosphorylation of Bcl-2, thus initiating p53-mediated apoptosis [60].

The enzymatic activity of PP2A depends upon its stability in the cell. PP2A stability could be dependent upon the half-life of its monomeric subunits, their association with each other and interaction of the dimeric and trimeric subunits. For example, PR56/B' family members are present throughout the process of mitosis and there is a nuclear and cytoplasmic shuttling between B' α , B' β and B' ϵ during the interphase [61] with C and A subunits. It has been shown that knockdown of the PP2A-A α subunit results in a loss of C, B and B' subunits, whereas B'' and striatin remained stable. Likewise, B γ mutants are rapidly cleared, although B''/PR72 mutants are stable. These monomeric subunits are rapidly degraded via the ubiquitin/proteasome degradation pathway [62].

PP2A dephosphorylation activity maintains cell adhesion and cytoskeleton dynamics. PP2A is co-localized with β 1-integrin and is an important regulator of FAK (focal adhesion kinase) complex [63]. Inhibition of PP2A enhances FAK/Src/paxillin hyper phosphorylation, leading to disorganization of focal adhesion sites and increased cell migration in endothelial cells [64]. Its continuous disruption is linked to increased cell motility, invasiveness and loss of cell polarity. Moreover, association of increased cell motility and invasiveness due to altered PP2A activity was observed in BL6 mouse melanoma cells [65], lung carcinoma [66] and head and neck squamous carcinoma [67].

6. Tumor suppressor PP2A in transformation and cancer

Several aberrant biological and environmental factors in cancer evoke alteration in various cellular pathways during the cell cycle, cell growth and maintenance, proliferation and differentiation. Genetic instability, like mutation, amplification, rearrangement, chromosomal insertion/ deletion, frame shift, and homozygous whole gene deletion [68], favors oncogenesis by either activating constitutively certain oncogenes and/or inactivating tumor suppressor genes [69, 70]. Other ways of inactivation of tumor suppressor genes include methylation and acetylation [71].

PP2A is considered as a tumor suppressor and is thought to be functionally inactivated in cancer. Therefore, the PP2A inhibitor, okadaic acid, when injected into mice, caused tumor development [72]. Another tumor antigen-SV40 small T antigen, polyoma small T antigen replaces the B subunit of PP2A and alters the phosphatase activity of PP2A, leading to transformation [73]. These two pieces of evidence established the tumor suppressive nature of PP2A [74]. The PP2A scaffold and regulatory subunit were shown to be mutated or aberrantly expressed in many different types of cancer. Mutation in PR65 α /A α was found in lung carcinoma and melanoma [75] and PR65 β /A β mutation was seen in colon and lung cancer [76]. Other than lung and colon cancer, loss of function of scaffold subunits was also observed in cancers of the breast, skin, cervix and ovary. Mutations of scaffold PR65 α /A α , such as Glu64 to Asp in lung carcinoma, Glu64 to Gly in breast carcinoma, and Arg418 to Trp in melanoma, had been observed. Mutagenesis of Glu64 to Asp/Gly disturbed the binding ability of PR65 α /A α to PR56/B' [77]. A PR65 β /A β missense mutation, like Pro65 to Ser, Leu 101 to Pro, Lys343 to Glu, Asp504 to Gly, and Val545 to Ala, are detected in lung and colon cancers [76]. Recently, unique mutations of Arg183 to Gly/Trp and Arg182 to Trp of PPP2R1A of the regulatory subunit in ovarian cancer have been identified. In vitro mutation of these amino acids showed a defective binding ability of B, B' subunits, indicating reduced tumor suppressor function of PP2A in ovarian cancer [78]. Interestingly, about 43% of human glioma PP2A-A α levels were reduced to 10-fold, indicating a decreased expression of core and holoenzyme and high levels of unregulated catalytic C subunit [79].

In HEK293 cells, a 50% reduction in PP2A-A α levels leads to a reduction in PP2A-B' γ holoenzyme and enhanced tumor formation in immunodeficient mice due to activation of the Akt pathway [80]. Like Akt, GTPase RalA participates during transcription, migration, transport, apoptosis and cell proliferation. It was found that PP2A-A β binds and regulates the activity of RalA. Transformation associated with knockdown or loss of PP2A-A β showed hyperphosphorylation of RalA [81]. Three splice variants of PP2A-A β due to gene skipping were observed in B-cell chronic lymphocytic leukemia (B-CLL). These splice variants were unable to assemble C and B subunits and resulted in a loss of PP2A activity [82]. In breast cancer, there is an alteration of Gly90 to Asp in PP2A-A β . Mutation of Gly90 to Asp inhibits the interaction of A β with B56 γ , although the binding of the PR72/B'' subunit was not affected [83]. Very recently, Nagendra *et al* 2011 confirmed a high frequency of the PP2A-A α mutation in 32% uterine serous carcinomas. With the help of high throughput sequencing, mutation in Pro179 to Arg in the ACI-158 serous carcinoma cell line, a Pro to Leu in a primary serous carcinoma as well as a Arg to His mutation in the codon 258 of poorly differentiated endometrium cancer were also observed [84].

Like the scaffold subunit, the regulatory subunit of PP2A (mainly B55 and B56) was also mutated in many types of cancers. In acute myeloid leukemia (AML) patients, PP2AB55 α expression was found to be lower than normal. As mentioned earlier, B55 α dephosphorylates Akt at T308. When B55 α is suppressed, it leads to consistent activation of Akt to enhanced proliferation [85]. In metastatic melanoma cells, B56 γ has been found to be overexpressed as compared to normal cells [86] and hyperphosphorylation of paxillin promotes cell motility due to the truncation of B56 γ in these cells [65]. In lung cancer, B56 γ was shown to be mutated from Phe395 to Cys. They also found that as B56 γ interacts with p53 and dephosphorylates Thr55, it suppresses tumorigenicity. But due to mutations in B56 γ it is no longer able to interact with p53 causing cancer cell proliferation [87]. A study using Affymetrix SNP array in prostate cancer reported the deletion of PP2AB55/PR55 at a frequency of 67.1% in tumor samples with 2.1% homozygous deletion. Somatic copy number changes or germ line sequence variation was thought to be the reason for the deletion [88]. In various species, including humans, it was known that alteration and mutation of scaffold and regulatory subunits lead to transformation of normal cells to cancer cells, but until now there has been no report of mutation or deletion of the catalytic subunit in any type of cancer (**Table 2**).

7. Mouse models relevant to PP2A subunits

To understand the tumor suppressive function of PP2A and its biological attributes, several studies have been carried out using cell line models in various cancers, such as breast, ovary, skin, endometrium and colon. Gaining knowledge about PP2A at a molecular level requires the development of mouse models that recapitulate the human cancer progression, a valuable tool in current scientific research. As discussed previously, PP2A is a highly complex molecule made of trimeric holoenzyme which is comprised of catalytic, structural and regulatory subunits and can form around 70 different combinations of holoenzyme complex [20]. The complexity of this multi-subunit enzyme makes it difficult to identify and propose mouse models suitable for the identification of the tumor suppressor function of PP2A in cancer initiation, progression and metastasis.

The two-point mutations, E64G and E64D, in the PP2A scaffold subunit (PR65 α /A α) were identified in breast and lung cancer that leads to loss of function of the enzyme, provided the platform to study the PP2A tumor suppressive function [75]. As a result of these two mutations, the resulting mutant proteins had defective binding of the A subunit with regulatory B and catalytic C subunit. Among the B' subunits, which isoforms have a higher affinity for scaffold subunit (PP2A-A α) is a critical question to be answered. Walter *et al*

2012 have stated that the composition of the holoenzyme complex of the B' subunit varies with tissue type and is largely dependent upon the abundance of a particular isoform in that specific tissue. The second aspect to be considered is the differential binding affinity of various B subunits to the A α scaffold subunit [89]. Based on the mutations E64G and E64D, four different mouse strains were developed by Ruediger *et al* 2011: knock-in models E64D/+ and E64G/+; conditional A α knock-out F5-6/+ with exon 5 and 6 floxed for A α allele; and Δ 5-6/+ was harboring exon 5 and 6 deletion in the A α allele. The main objective for generating these strains was to determine the tumor suppressive function of PP2A. The lung cancer incidence in the heterozygous mutant mice, Δ 5-6/+, E64D/+ and Δ 5-6/E64D were 77%, 72%, and 75%, respectively. Through immunoprecipitation experiments Ruediger *et al* 2011 demonstrated that mice with the E64D/E64G mutation are found to have defective binding capacity to the B' γ subunit specifically. The authors concluded that the B' holoenzyme complex of PP2A functions as tumor suppressor, unlike the PP2A holoenzymes consisting of B and B'' subunits [90]. Thus, the *in vivo* models highlight the importance of the A α E64D mutation in lung carcinoma development through inactivation of PP2A tumor suppressor activity and pointed out the importance of b' subunit for the tumor suppressor function.

PP2A catalytic C subunits are expressed ubiquitously and they exist in two isoforms α and β with 97% homology in their amino acid sequence. However, Gotz *et al* 1998 had shown that a complete loss of Ppp2ca leads to an embryonically lethal phenotype [21] suggesting the non-redundant functions of α and β isoforms. It has been previously identified that the phosphatase activity of PP2A resides on the highly conserved histidine 118 residue of the catalytic subunit and this histidine 118 is present in the RGNHE motif of exon 3 [91, 92]. Therefore, a conditional null Ppp2ca knockout mouse was generated having replaced Ppp2ca exon 3, 4 and 5 with the Ppp2ca gene targeting vector. Overall, the loss of Ppp2ca led to abnormal embryonic development at day E6.5, whereas heterozygous Ppp2ca^{fl/ Δ} mice embryos were normal. Interestingly, Ppp2ca ^{Δ / Δ} mice embryos were found to be smaller than normal control embryos at day E7.5 and they contained both the ectoderm and endoderm but no mesodermal layer. Thus, the loss of Ppp2ca results in the absence of mesodermal germ layer, indicating defect in embryo differentiation. Interestingly, Ppp2cb knockout mice model showed no abnormal phenotype [93]. Thus, the results from these knock-out mouse models indicated that while Ppp2ca is essential for the developmental process in the mouse, Ppp2cb is not.

Despite the functional and biochemical studies in various regulatory subunits of PP2A obtained from cellular models, there are limited publications about the knock out models of the B-type subunits. The reasons for this are the complexity of the holoenzyme as a whole and a lack of characterization of the promoters and splice variants of the regulatory subunits [94]. Of the many different regulatory subunits identified so far, the transgenic mice expressing B'/PR56 γ has been initiated by Evertt *et al* in 2002. In order to produce mice that have PR56 γ overexpression in the lung, the full-length cDNA fragment of human PR56 γ was cloned into the expression vector that has the lung-specific human surfactant protein promoter and a SV40 small T-antigen poly A cassette. However, the mice obtained through this approach die neonatally. Further, it was observed that those embryos lack normal lung structure and β -catenin expression in lung tissue, suggesting a potential role of PR56 γ in association with Wnt signaling during lung development [95]. Recently, the functional consequence of another B-type specific regulatory subunit in the mice model was reported where it reveals the role of PR61/B' δ in the central nervous system [96]. The *in vivo* model systems exploring the functional consequences of the B-type specific regulatory subunit (particularly in cancer) remains unexplored. Thus the animal models described previously may set the stage for future studies on animal models relevant to the role of PP2A in cancer.

8. Conclusion

PP2A is an essential and multifarious phosphatase in the cellular system. A growing body of evidence indicates that PP2A is known to regulate the activity of more than 30 different kinases. The important kinases, like protein kinase B (AKT), PKC, p70 S6 kinase, cAMP dependent kinases, CAM-kinases, ERK/MAP kinase are the major substrates for PP2A [97]. During cell cycle progression and apoptosis, various proteins like Cdc25, Cdc6, Wee 1, DNA polymerase primase, TAU, and cyclin G2 are also controlled by PP2A [98]. In general, the biology of PP2A is more intricate to understand due to the more diverse regulatory subunits that are differentially expressed in various tissues. The tumor suppressive role of PP2A is mainly governed by these regulatory subunits that play different roles in various cells. Since the isoforms of regulatory subunits are differentially expressed in different tissues, more elaborate studies on each isoform will precisely delineate the understanding of the expression pattern and help us comprehend their role in PP2A controlled signal transduction. Further, the PP2A scaffold subunit is also deregulated in both cancer and neurodegenerative diseases.

Earlier studies have proposed that the use of PP2A as a therapeutic target for cancer was mainly based on the oncogenic potential of the microbial toxin Okadaic acid (PP2Ac Inhibitor). However, this functional loss is not the true representation of the specific holoenzyme complex comprised of the scaffold, catalytic and regulatory subunits (with respective isoform). Thus, the future prospective will be to elucidate the status of the catalytic and other individual subunits and their precise role and potential functions in cells during transformation need to be investigated further. Also, genetic manipulation of PP2A, its inhibitors and substrates will help develop therapeutic agents against cancer and other diseases. It is interesting to note that when scaffold and regulatory subunits of PP2A are deregulated in cancer cells, the cell tries to maintain a constant level of functional catalytic subunit activity (observation from our unpublished data). Further, the involvement of specific PP2A subunits in the tumor initiation, progression and metastasis with its clinical relevance will be explored using the development of genetically-engineered mouse models. In the immediate future, the genetic and epigenetic changes associated with the PP2A holoenzyme complex in cancer cells remain to be explored to find out their impact on oncogenic signaling and therapy response. How PP2A regulates various cancer signaling pathways such as PI3K/Akt, mammalian target of rapamycin (mTOR), FAK (Focal adhesion kinase) and ERK enabling cancer cells addicted for growth is still far from being completely determined for drug validation/targeting PP2A activation (**Figure 3**). The ultimate goal of this review is to significantly contribute to the understanding of the deregulation of PP2A-regulated signaling pathways contributing to cancer development. The mouse model system discussed in this review will explore the molecular mechanism behind cancer progression, which will enable us to understand PP2A and its clinical relevance and new therapies to be tested that can prevent cancer progression.

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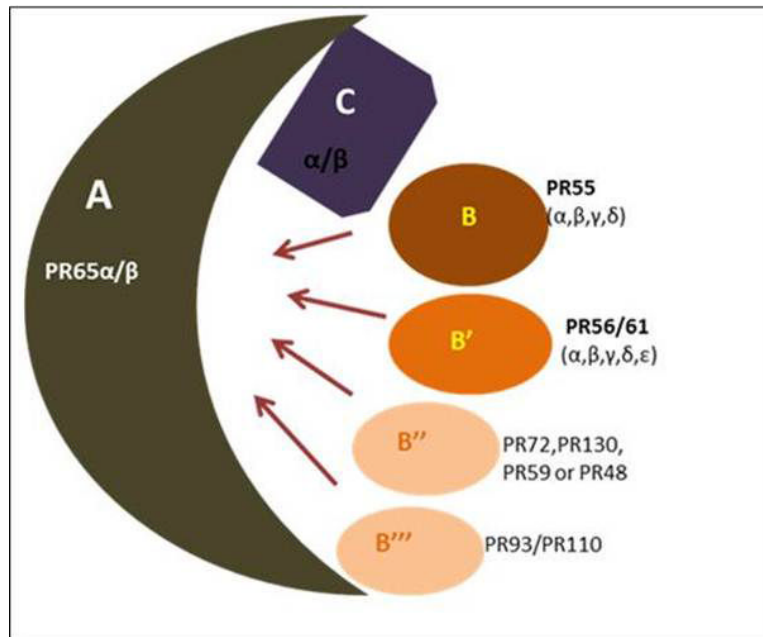


Figure 1. Schematic representation of the structural diversity of the PP2A holoenzyme complex PP2A enzymes are heterotrimers consisting of core dimer scaffold (A) and a catalytic (C) subunit that is associated with one of the regulatory (B) subunits. The scaffold A and catalytic C are encoded by two distinct genes α and β . The α and β isoforms of the catalytic subunit share 97% homology. However, in the typical cell, the catalytic α isoform predominates which is 10 times more abundant than the β isoform. Similar to catalytic subunit α and β isoforms of the scaffold subunit also share 86% identity in their primary sequence. The B-type subunits were further categorized into four unrelated families: B (PR55), B' (PR56/61), B'' (PR72/130) and B''' (PR93/110). Within each B-type family, distinct genes encode various structurally related isoforms such as α , β , γ and δ .

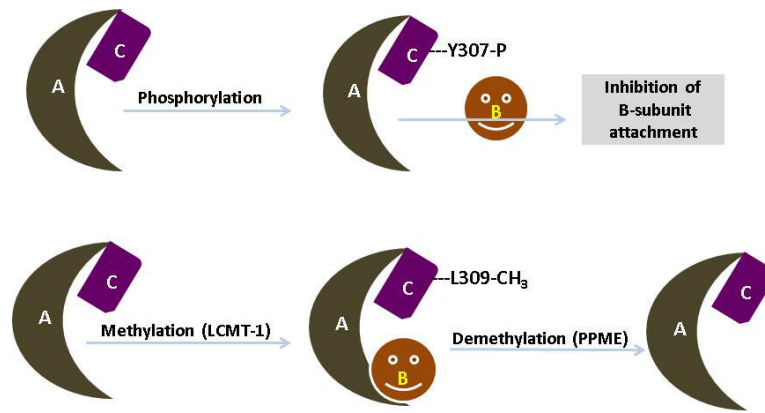


Figure 2. Post transitional modification of PP2A

PP2A is being regulated by post translation modification, auto-regulation, subunit diversity and substrate protein interaction. Phosphorylation and methylation are two major modifications that have been shown to modulate PP2A subunit associations and catalytic efficiency. Upon reversible phosphorylation of tyrosine residue 307 (located in the C-terminal part of PP2A catalytic subunit) by receptor associated tyrosine kinases (pp60^{v-src}, pp56^{lck}, EGF and insulin receptors) results in decreased phosphatase activity of PP2A. In addition to tyrosine phosphorylation, PP2A can also be auto-phosphorylated on threonine residues by activated protein kinases. Apart from the PP2A catalytic subunit, the regulatory subunits are also subjected to phosphorylation. Notably, PR61δ by PKA and PR 61α by PKR are the best evidence for the change in the PP2A activity. Similarly, methylation of PP2A occurs at leucine 309 residue by PP2A-methyltransferase and enhances the binding affinity of the AC core dimer toward a distinct regulatory subunit.

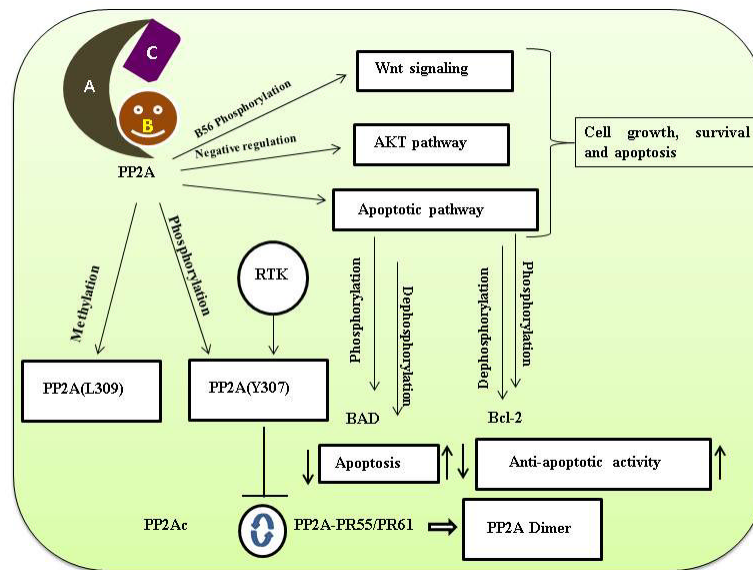


Figure 3. Representation of PP2A regulation and signaling pathways

Methylation and phosphorylation are two major post translation modifications of PP2A. The conserved sequence TPDYFL in PP2Ac undergoes phosphorylation and methylation, respectively. Phosphorylation of Y307 by receptor associated tyrosine kinases effectively decreases the PP2A activity by inhibiting the interaction of the catalytic subunit with the scaffold subunit. On the other hand, the addition of the methyl group to PP2Ac by LCMT1 at L309 by PP2A-methyltransferase (PPMT) will enhance the specific binding of the AC dimer to the distinct B regulatory subunit providing the enzymatic activity. PP2A activity is indispensable for every cell and takes part in the majority of the cellular pathways. Phosphorylation at Thr-308 and Ser-473 leads to activation of Akt and is associated with PP2A activation. PP2A also possesses a vital role in the Wnt signaling pathway and phosphorylation of PP2A-B56 directs the activation of the Wnt pathway. In addition to these pathways, PP2A activity is indispensable in apoptosis. BAD (pro-apoptotic) and Bcl-2 are also regulated by PP2A. Phosphorylation of BAD suppresses, and its dephosphorylation by PP2A promotes pro-apoptotic activity. In addition, phosphorylation of Bcl-2 activates, and its dephosphorylation by PP2A suppresses anti-apoptotic activity. Thus, this diagrammatic representation will elucidate the importance of PP2A during cell growth survival and apoptosis.

Table 1

Total number of coding proteins and number of kinases and phosphatases in the eukaryotic genome.

Proteins	S.cerevisiae	D.melanogaster	C.elegans	Human	Reference
Total number of proteins	6122	13600	18988	25000	[99]
Total Protein kinases	124	236	493	518	
			Protein Kinases-411 Protein Kinases like proteins -82	Protein serine/threonine kinase - 385 Protein tyrosine kinase (PTK) - 90 PTK like protein - 43	
Total Protein phosphatases	37	93	185	119	
				Protein serine/threonine phosphatase-21 Protein tyrosine specific phosphatase-98	

Table 2

Various isoforms of different subunits of PP2A and their nomenclature, chromosomal location and normal/aberrant expression in various cancer and normal tissues.

Subunit	Gene	Isoform	Other name	Chromosome number	Normal tissue distribution	Subcellular distribution	Aberrant tissue distribution
Scaffold (A)	PPP2R1A	α	PR65α, PP2A-Aα	19q13.33	Ubiquitously expressed in all the tissues [22].	Cytosol	Lung, breast, melanoma, endometrial and ovarian cancer [75].
	PPP2R1B	β	PR65β, PP2A-Aβ	11q23.2	Ubiquitously expressed and highly expressed in ovary (oogenesis) [22].	Cytosol	Lung, Breast, colon, ovarian, B-CLL [75].
Catalytic (C)	PPP2CA	α	PP2Aα	5q31.1	Brain and Heart (HE) [19, 100].	Cytoplasm and Nucleus	Prostate [101].
	PPP2CB	β	PP2Aβ	8p12	Brain and Heart (HE) [19, 100].	Cytoplasm and Nucleus	-
Regulatory (B)	PPP2R2A	α	PR55α, PP2ABα	8p21.1	Widely distributed in all tissues [16, 24, 30].	Membranes, cytoplasm, Microtubules Nucleus, Golgi complex, endoplasmic reticulum and neurofilaments	AML, prostate P [85, 88].
	PPP2R2B	β	PR55β, PP2ABβ	5q31-5q32	Brain and testis(HE)[16].	Cytosol	-
	PPP2R2C	γ	PR55γ, PP2ABγ	4p16.1	Brain (SE) [27].	Mainly in Cytoskeletal fraction	Lung cancer, melanoma [102].
	PPP2R2D	δ	PR55δ, PP2ABδ	10q26.3	Wide spread distribution in tissues, Testis (HE) [28].	Cytosol	-
Regulatory(B')	PPP2R5A	α	PR56/61α, PP2AB'α	1q32.2-q32.3	Cardiac tissues and skeletal muscles (HE)[103].	Cytoplasm	-
	PPP2R5B	β	PR56/61β, PP2AB'β	11q12-q13	Brain (HE) [103, 104].	Cytoplasm	-
	PPP2R5C	γ1, 2, 3	PR56/61γ, PP2AB'γ	14q32	Cardiac tissues and skeletal muscles (HE) [103-105].	Cytoplasm and Nucleus	Lung cancer (HE) reduced level in melanoma cells [86, 87].
	PPP2R5D	δ	PR56/61δ, PP2AB'δ	6p21.1	Primarily exist in brain [103, 106].	Cytoplasm, Nucleus, Mitochondria, Microsomes	-
	PPP2R5E	e	PR56/61e, PP2AB'e	14q23.1	Primarily exist in brain [103]	Cytoplasm	Breast cancer, Soft tissue sarcoma [107].
Regulatory(B'')	PPP2R3A	α	PR130, B''α1	3q22.1	Brain(HE), heart, Lung, kidney and muscle [16].	Centrosome and Golgi complex	-
	PPP2R3A	α	PR72, B''α2	3q22.1	Heart(HE) and skeletal muscle [16].	Cytosol and nucleus	-
	PPP2R3B	β	PR70, PR48, B''β	Xp22.33, Y11.3	Placenta [108].	Nucleus	-
	PPP2R3C	γ	G5PR, G4-1	14q13.2	During developmental process expressed in fetal brain [109].	Nucleus	-

Subunit	Gene	Isoform	Other name	Chromosome number	Normal tissue distribution	Subcellular distribution	Aberrant tissue distribution
	PPP2R3D	δ	PR59, B''6		Cardiac tissue, Kidney and Lungs [33].	Nucleus	-
Regulatory(B''')	STRN		Striatin, PR110	2p22.2	Brain [110].	Membrane and cytoplasm	-
	STRN3		SG2NA	14q13-q21	Neurons [110].	Nucleus	-
	PPP2R4		PTPA, PR53	9q34	Widely expressed [20, 111, 112].	Cytosol, Nucleus	-

HE-Highly expressed, SE-Specific expression