Tumor Biol. (2016) 37:11691–11700
DOI 10.1007/s13277-016-5145-4

REVIEW

Deregulation of the protein phosphatase 2A, PP2A in cancer: complexity and therapeutic options

Godfrey Grech [1] · Shawn Baldacchino [1] · Christian Saliba [2] · Maria Pia Grixti [1] · Robert Gauci [1] · Vanessa Petroni [3] · Anthony G. Fenech [4] · Christian Scerri [5,6]

Received: 22 March 2016 / Accepted: 11 July 2016 / Published online: 21 July 2016
© International Society of Oncology and BioMarkers (ISOBM) 2016

Abstract The complexity of the phosphatase, PP2A, is being unravelled and current research is increasingly providing information on the association of deregulated PP2A function with cancer initiation and progression. It has been reported that decreased activity of PP2A is a recurrent observation in many types of cancer, including colorectal and breast cancer (Baldacchino et al. EPMA J. 5:3, 2014; Cristobal et al. Mol Cancer Ther. 13:938–947, 2014). Since deregulation of PP2A and its regulatory subunits is a common event in cancer, PP2A is a potential target for therapy (Baldacchino et al. EPMA J. 5:3, 2014). In this review, the structural components of the PP2A complex are described, giving an in depth overview of the diversity of regulatory subunits. Regulation of the active PP2A trimeric complex, through phosphorylation and methylation, can be targeted using known compounds, to reactivate the complex. The endogenous inhibitors of the PP2A complex are highly deregulated in cancer, representing cases that are eligible to PP2A-activating drugs. Pharmacological opportunities to target low PP2A activity are available and preclinical data support the efficacy of these drugs, but clinical trials are lacking. We highlight the importance of PP2A deregulation in cancer and the current trends in targeting the phosphatase.

Godfrey Grech, Shawn Baldacchino and Christian Saliba contributed equally to this work.

✉ Godfrey Grech
godfrey.grech@um.edu.mt

[1] Department of Pathology, Faculty of Medicine & Surgery, Medical School, University of Malta, Msida MSD2090, Malta

[2] Centre for Molecular Medicine and Biobanking, University of Malta, Msida, Malta

[3] Department of Anatomy, Faculty of Medicine & Surgery, University of Malta, Msida, Malta

[4] Department of Clinical Pharmacology & Therapeutics, Faculty of Medicine & Surgery, University of Malta, Msida, Malta

[5] Department of Physiology and Biochemistry, Faculty of Medicine & Surgery, University of Malta, Msida, Malta

[6] Molecular Genetics Clinic, Mater Dei Hospital, Msida, Malta

Keywords Molecular targets · Phosphatase activation · PP2A complex · Breast cancer · Colorectal cancer · Kinase inhibitors · Novel therapeutics

Protein phosphatase 2 (PP2 or PP2A) is a member of the PPP family which is involved in the feedback of diverse signalling pathways, affecting cell growth and proliferation, cell cycle progression, apoptosis, transcription, DNA replication and translation [1–5]. PP2A is considered to be a tumour suppressor that regulates pathways crucial for cellular transformation, supported by malignant transformation of cells following inactivation of PP2A, using a simian virus 40 small t (SV40ST) mutant that specifically inactivates the phosphatase [6].

The PP2A complex is the main serine/threonine phosphatase, regulating multiple signals in mammalian cells. PP2A is a negative regulator of several signalling pathways promoting cell growth, proliferation and survival, including the phosphoinositide-3-kinase (PI3K)/Akt/mTOR, Wnt/β-catenin, c-Myc and the Ras-Raf-mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK) family of kinases [1, 4, 6, 7]. PP2A dephosphorylates Cdc25, controlling the major DNA-responsive G2/M checkpoint, restricting entry of cells from G2 to M phase [8]. PP2A also dephosphorylates and inactivates the forkhead transcription factor, FoxM1, that controls expression of genes required for cell cycle progression through G2 phase [9]. PP2A was also shown to sustain p53-dependent apoptosis through enhanced stability of the p53 protein [10]. The apoptotic genes Bcl-2

and caspase-3 are also regulated by the PP2A complex [1, 4]. The tumour suppressor, PP2A, is important to attenuate proliferative and survival pathways, regulates cell cycle checkpoints and maintains the apoptotic capacity of the cell (Table 1). Hence, decreased activity of PP2A promotes cellular transformation.

Decreased activity of PP2A is a recurrent observation in many types of cancer, including colorectal cancer (CRC) and breast cancer [16, 17]. In fact, recent research on patients with CRC have shown that PP2A is frequently inactivated in such patients. Interestingly, the activity of PP2A is restored using FTY720, an activator of PP2A, resulting in suppressed proliferation of CRC cells, suggesting that PP2A represents a potential therapeutic target for this disease [17]. Understanding the dynamics of the PP2A complex, regulation of the endogenous inhibitors and the post-translational modifications that specifies binding of regulatory subunits will provide the means to understand the mechanisms of deregulation and targeting of the complex.

PP2A: a diversely heterogeneous complex

The predominant PP2A complex is a heterotrimer composed of an active core dimer that can exist independently, consisting of a catalytic subunit (C subunit—36 kDa), a scaffold A subunit (65 kDa) and a variable regulatory B subunit. There are two different transcripts of the A and C subunits, which are coded by different genes. The structural subunit A has two transcripts, PPP2R1A (Aα) and PPP2R1B (Aβ), that share 87 % homology and are also ubiquitously expressed and coded by genes on chromosome bands 19q13.41 and 11q23.2, respectively. Their high homology suggests similar structure and function. The α form is more abundant (90 %) than the β form (10 %), although *Xenopus* studies have shown that there are very high levels of PPP2R1B in oocytes up to fertilisation and early stages of embryogenesis. The ratio of PPP2R1B to PPP2R1A is then restored in late embryogenesis indicating a possible role in early development [18, 19]. The A subunit is composed of 15 tandem repeats of Huntington/elongation/A subunit/TOR (HEAT) making it a stable protein with an elongated architecture [20, 21].

Similarly, the α and β isoforms of the C subunit (PP2Ac) share 97 % of their sequence, and although they are both ubiquitously expressed, the α isoform is about ten times more abundant than the β isoform. The α and β isoforms are coded by the genes PPP2CA and PPP2CB on chromosome bands 5q23-q31 and 8p12-p11.2, respectively. Although the C subunit transcripts are almost identical, knockout of the PPP2CA gene leads to early embryonic death in mice, indicating that PPP2CB does not rescue this loss [22]. The C-terminus of the catalytic subunit spans residues 294–309 [18], containing two main regulated residues in this motif: the tyrosine 307 (Y307) and leucine 309 (L309), which when phosphorylated or demethylated, respectively, results in inactivation of PP2A [23–25]. Residue L309 of PP2Ac is demethylated by the phosphatase methylesterase (PME-1) and methylated by leucine carboxyl methyltransferase 1 (LCMT-1). Thus, post-translational modification of this region on PP2Ac has a role in determining the composition of the PP2A complex [26].

The B subunit is categorized into four unrelated families: B (B55/PR55), B′ (B56/PR61), B″ (PR48/72/130) and B‴ (PR93/110) which are encoded by distinct genes that give rise to various structurally related isoforms (α, β, γ, δ and ε) (Table 2). The type of B subunit bound to the core dimer determines both the substrate specificity and cellular localisation of PP2A holoenzyme complexes [30]. The B subunits of PP2A share the same binding site or have overlapping binding sites on the PP2A structural subunit A, hence explaining why the binding of one B subunit is mutually exclusive for the binding of other B subunits [20]. Substrate specificity is determined by selective recruitment of B subunits to the core dimer (A/C) [7, 31]. The variety of B subunits, encoded from all over the human genome, can form a myriad of different PP2A complexes. Different regulatory subunits are often tissue-specific. As exemplified by the B55 family, the B55α and B55β have a widespread tissue expression, whereas the B55γ and B55δ are mainly restricted to the

Table 1 Targets of PP2A and their role in carcinogenesis

Targets of PP2A	Cellular role	Reference
p53, Cdc25	Cell cycle checkpoints and regulation	[1]
PI3K/Akt	Cell proliferation and survival	[6, 11]
RAF, MAPK, ERK	Cell proliferation and survival	[4, 12, 13]
p70 S6K, mTOR	Cell proliferation, survival and translation initiation	[7, 14]
c-Myc	Tumourigenesis	[6, 15]
Wnt/β-catenin	Tumourigenesis	[1, 6]
FoxM1	Transcription factor that control expression of genes involved in the cell cycle	[9]
Bcl-2, caspase-3	Apoptosis	[4]

Table 2 The different subunits of the PP2A trimeric enzyme complex [18, 21, 27–29]

PP2A subunits					
Structural subunits, A	Regulatory subunits, B				Catalytic subunits, C
	Regulatory subunit family B	Regulatory subunit family B′	Regulatory subunit family B″	Regulatory subunit family B‴	
PPP2R1A (Aα or P65α)	PPP2R2A (B55α or PR55α)	PPP2R5A (B′56α or PR61α)	PPP2R3A (B″α or PR72α)	PPP2R4 (B‴ or PR53)	PPP2CA
PPP2R1B (Aβ or P65β)	PPP2R2B (B55β or PR55β)	PPP2R5B (B′56β or PR61β)	PPP2R3B (B″β or PR70β)	Striatin (STRN or PR110)	PPP2CB
	PPP2R2C (B55γ or PR55γ)	PPP2R5C (3 isoforms: B′56γ1, B′56γ2 and B′56γ2 or PR61γ1, PR61γ2 and PR61γ3)	PPP2R3B (PR48)	Striatin-3SG2NA (STRN3 or PR93)	
	PPP2R2D (B55δ or PR55δ)	PPP2R5D (B′56δ or PR61δ)	PPP2R3C (B″γ or PR72γ)	Striatin-4 (STRN4)	
		PPP2R5E (B′56ε or PR61ε)			

brain environment. Moreover, each distinct subunit is localised to specific areas of the brain, and furthermore shows different subcellular localisation (nuclear versus cytoplasmic).

The PPP2R5A, PPP2R5B and PPP2R5E genes of the B′56 subunit family have a nuclear export signal in the C-terminus that causes the PP2A complex to migrate to the cytoplasm. In contrast, PPP2R5C and PPP2R5D are found mainly in the nucleus as they lack the signal sequence on the C-terminus [31]. This provides evidence that compartmental PP2A activity is determined by the B subunits [21]. There may be other B subunits interacting with PP2A although some are still not adequately characterised. The S/G2 nuclear autoantigen (SG2NA) is one of these potential new members possibly in the B‴ family and is suspected to interact with the PP2A active core dimer [21].

The binding of the B subunits to the PP2A active dimer is post-translationally controlled through methylation or phosphorylation at the residues L309 and Y307, respectively. The recruitment of the B and some members of the B′ family to the active core dimer is inhibited by phosphorylation of Y307, while B″ subunit binding is unaffected. The effect of L309 methylation of the catalytic subunit of PP2A on B (B55) subunit binding is still unclear although the recruitment of B′ or B″ subunits is unaffected [31, 32]. Of interest, methylated C subunit promotes binding of the core dimer to PR55α/B [33], determining substrate specificity in vitro and the subcellular localisation of the complex in vivo [3, 6]. Suppressed methylation of the C subunit following overexpression of PME-1 or silencing of LCMT-1 results in activation of the Akt/mTOR pathway, promoting cellular transformation [34].

Aberrations of core PP2A subunits in malignancies

Scaffold subunit (PP2AA)

Genetic variations in the scaffold subunit may inhibit binding of the C and B subunits, hindering PP2A activity. PPP2R1A and PPP2R1B missense mutations or loss of heterozygosity (through homozygous deletions) has been observed in breast, lung, ovarian, colon, liver and melanoma malignancies. Loss of heterozygosity is observed in a considerable percentage of these patients and also in non-Hodgkin's lymphomas and chronic lymphocytic leukaemia although to a lesser extent [21, 35–39]. Somatic missense mutations were also detected in PPP2R1A in high-grade serous endometrial tumours [13]. A higher frequency of PPP2R1B mutations was identified in lung and breast (13 %) cancers resulting in defective attachment of the B and C subunits. In addition, low expression or loss of PPP2R1B in breast cancer contributes to transformation events [21, 40].

Regulatory subunit (PP2AB)

PPP2R2A/B55α

PPP2R2A deletions were found in 67 % of prostate cancers [41] and also reported in breast cancer and myeloma. Haploinsufficiency results in perturbed binding of B55α to the PP2A core dimer, decreasing the stability of p53, and in reduced dephosphorylation of Akt at threonine 308. In addition, inhibition of the myelocytomatosis viral oncogene (v-Myc) is suppressed, resulting in accumulation of the oncogene [42].

PPP2R5A/B'56α

Decreased expression of PPP2R5A in melanoma cells suppresses v-Myc transcriptional factor activity leading to a reduction in cellular proliferation. Dephosphorylation of v-Myc destabilizes it and leads to its rapid degradation [15].

PPP2R5C/B'56γ

Lack of adequate PP2A localisation due to the presence of a truncated form of PPP2R5C leads to an aggressive phenotype and increased metastatic potential in melanoma [15]. This is probably mediated through dephosphorylation of p70 S6K by PPP2R5C.

Catalytic subunit (PP2Ac)

Despite being very similar, the two isoforms of PP2Ac are coded by different genes and have an apparently very distinct function. Expression of the PPP2CA subunit is decreased in prostate cell lines and cancers, when compared to neighbouring normal or benign tissues [13]. Expression of a mutated PP2Ac has also been described in breast tumours positive for human epidermal growth factor 2 (HER2). This mutation, associated with phosphorylated Y307, significantly correlates with disease progression and loss of PP2A activity. Apoptosis was induced upon reactivation of PP2A activity [13].

Regulation of PP2A through binding of endogenous inhibitors

Since PP2A has an effect on a variety of cell processes, it needs to be tightly regulated. This is achieved mainly by binding of endogenously expressed inhibitory proteins. Binding of endogenous inhibitors to PP2A leads to decreased PP2A activity. Overexpression of these inhibitors is recurrently found to play a role in malignancy. The main inhibitory subunits are immunoglobin (CD79A) binding protein 1 (IGBP1; also known as α4), the nuclear oncogene SET, SET binding protein 1 (SETBP1) and cancerous inhibitor of protein phosphatase 2A (CIP2A).

IGBP1

IGBP1 is a regulatory subunit that binds and interacts with PP2A altering its activity and substrate specificity [3]. It binds to the catalytic subunit of PP2A causing its displacement from the structural A and variable B PP2A subunits. The study of Kong et al. in 2004 reported that IGBP1 deletion caused rapid cellular apoptosis, implying that IGBP1 binding attenuates PP2A activity. The interaction of IGBP1 with PP2Ac also inhibits its methylation [43].

Studies using murine erythroblast cell model resulted in identification of the key role of specific pathways to regulate the balance between proliferation and differentiation of haematopoietic progenitors. The results identified IGBP1 to be a major contributor to block erythroid differentiation. IGBP1 exerts the differentiation block by binding and suppressing the phosphatase PP2A. Interestingly, mTOR activity is maintained and, as a result, polysome recruitment of IGBP1 and hence its expression is induced [44].

SET and SETBP1

Nuclear oncogene, SET, was identified as inhibitor 2 of PP2A (I2PP2A) and has been widely implicated in leukaemogenesis. In chronic myeloid leukaemia (CML) patients, the phosphatase activity of PP2A is inhibited by the expression of the PP2A inhibitor SET. SET is overexpressed in a number of solid tumours, in CML and potentially other leukaemias. Induced suppression of SET has been reported to lead to the restoration of PP2A activity [45].

It has been shown that the reactivation of PP2A activity in these cells promoted dephosphorylation of key regulators involved in cell survival and proliferation and induced a reduction in the activity and degradation of tyrosine kinase inhibitor BCR-ABL. Hence, PP2A activation led to growth suppression, enhanced apoptosis, restored differentiation and decreased in vivo leukaemogenesis of BCR-ABL positive cells [45]. The BCR-ABL fusion is characterised by the t(9;22) translocation. BCR-ABL has been shown to enhance mTOR in an Akt-independent manner [46] and drives other pathways as MAPK and STAT5/JAK2 among other mechanisms [47] potentially through PP2A deregulation. The BCR-ABL expression is also correlated with maintenance of phosphorylation at Y307 (Y307-P) of the PP2Ac, resulting in PP2A inhibition [45]. The persistence of the PP2A Y307-P facilitates and sustains the BCR-ABL kinase activity [48].

The SET-binding protein (SETBP1 or SEB) binds to SET without hindering binding of SET to PP2A. SETBP1 protects SET from protease cleavage leading to an increase in the

availability of active full-length SET protein. SETBP1 is thus implicated in the reduction of PP2A activity and confers growth advantage in haematopoietic progenitors. It is overexpressed in acute myeloid leukaemia (AML) cases due to the t(12;18) translocation that involves the ETV6 gene [49].

SETBP1 has been found in mutated forms in colorectal carcinomas or lost in the common chromosomal aberrations at 18q21. More than 60 % of 18q21 aberrations are deletions [50] and are associated with malignant lymphoma, acute leukaemia, pancreatic carcinoma and colorectal cancer.

CIP2A

The PP2A inhibitor, CIP2A, is encoded by the KIAA1524 gene on chromosome 3q13.13. CIP2A overexpression correlates with high-grade or advanced tumour stages in breast, colon, prostate, ovarian cancers and head and neck squamous cell carcinoma [29, 51–56]. The mechanism of PP2A inhibition by CIP2A is not well annotated although some suggest that CIP2A binds to PP2Ac allosterically at its B subunit binding interface, thus altering substrate specificity and/or limiting activity [32, 36, 51, 56].

CIP2A acts by preventing PP2A-mediated dephosphorylation of the c-Myc oncoprotein leading to an increase in progenitor cell proliferation and cell renewal. PP2A dephosphorylates Myc at serine 62 which results in its ubiquitination and proteolytic degradation. CIP2A-mediated PP2A inhibition results in the stabilization of the Myc protein, resulting in increased availability of this oncoprotein [42, 51, 55, 57]. Knockdown of CIP2A led to increased PP2A activity which in turn reduces c-Myc levels.

Phosphatases as therapeutic targets in cancer

The therapeutic targeting of phosphatases exploits the cell's own mechanisms of growth suppression and promotes negative feedback mechanisms to attenuate sustained signalling. The PP2A negative feedback regulates a number of major molecules in central pathways involved in carcinogenesis (Table 1). Phosphatases are abundantly expressed in normal cells, and hence the activity of phosphatases is relatively high when compared to malignant cells with suppressed PP2A activity. Targeting malignant cells with the low PP2A activity phenotype is attractive, since the drugs have minimal effect on cells with normal phosphatase activity, providing selective tumour targeting.

Therefore, targeting a phosphatase is expected to have an effect on cellular growth and survival. This led to the development of phosphatase targets, several of which have undergone clinical trials [58–60]. However, the only FDA-approved drugs are the immunosuppressants cyclosporin A (CsA) and tacrolimus (FK506). CsA, a fungal metabolite from the fungus *Tolypocladium inflatum*, and FK506 are commonly used as immunosuppressants to prevent the rejection of organ transplants. Similar to CsA, FK506 blocks the activation of calcineurin through the formation of complexes with immunophilins [61].

The PP2A complex as a target for drug development

Suppression of PP2A activity promotes malignant transformation through various mechanisms of deregulation. Molecules that result in a decrease in the endogenous inhibitors of PP2A, inhibition of the phosphorylation and demethylation of the C-terminus of the catalytic subunit or resulting in activation of the PP2A complex in any other way are candidates for targeting tumours that are characterised with a decreased activity of PP2A (Fig. 1).

Targeting chemical modification of catalytic subunit

Inhibition of protein methylesterase 1 (PME-1) maintains methylation of the C-terminus of the catalytic subunit and hence an active PP2A heterotrimer. The aza-β-lactam, ABL127, and the sulfonyl acrylonitrile, AMZ-30, are commercially available covalent inhibitors of PME-1 [62, 63] that result in decreased cell proliferation and invasion in in vitro models of endometrial cancer [64].

The alkylating agent chloroethylnitrosourea (CENU) used as chemotherapy in various types of cancers induces PP2A methylation. CENU is a mustard gas-related β-chloro-nitrosourea compound. The compound eventually leads to Akt dephosphorylation, consequently reducing cell proliferation in melanoma [65].

Targeting endogenous inhibitors

SET

Apolipoprotein E (ApoE) is a major protein involved in lipid, vitamin and cholesterol transport. It also has anti-inflammatory effects and suppresses T-cell proliferation [66]. Recent studies on mitogen-treated lymphocytes and monocytes have shown that an ApoE mimetic peptide has an effect on PP2A activity [67]. This occurs via inhibitory binding to SET, thereby enhancing endogenous PP2A activity. This in turn reduces levels of phosphorylated kinases, signalling and inflammatory responses.

Fingolimod (FTY720) is a small molecule immunosuppressant which is being implemented for organ transplantation and multiple sclerosis [68]. It is an analogue of the natural compound myriocin, an amino fatty acid antibiotic derived from the thermophylic fungus *Mycelia sterilia*. FTY720 binds the sphingosine-1-phosphate receptors (S1PR) resulting in internalisation and potently decrease the movement of T-

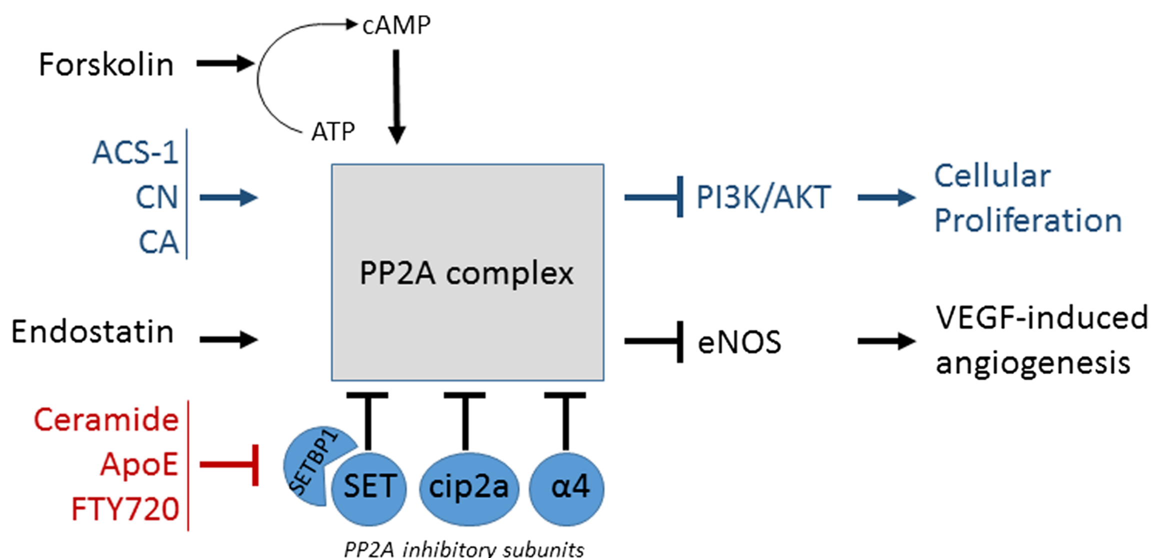

Fig. 1 Physiological inhibitory subunits suppress the activity of PP2A resulting in enhanced cellular proliferation and VEGF-induced angiogenesis. Various therapeutic options reactivate the PP2A phosphatase by acting on different subunits of the complex. *CN* chloroethylnitrosourea, *CA* carnosic acid, *eNOS* endothelial nitric oxide synthase, *ATP* adenosine triphosphate, cAMP cyclic adenosine monophosphate, *Akt* protein kinase B, *SET* Set nuclear oncogene, *CIP2A* cancerous inhibitor of protein phosphatase 2A, *α4* immunoglobin (CD79A) binding protein 1, *VEGF* vascular endothelial growth factor

lymphocytes into the freshly transplanted organs, thus preventing rejection [69]. FTY720 directly binds and disrupts repressive SET/PP2A complexes, resulting in the reactivation of the PP2A activity [70]. Studies on colorectal cancer (CRC) cell lines show that the activity of PP2A is restored using FTY720, resulting in suppressed proliferation of CRC cells [17]. Other sensitive cell lines include leukemic cell lines [71], human bladder cancer cells [72], ovarian cancer cells [73], gastric cancer cells [74] and breast cancer cells [16]. Sensitivity to FTY720 was also observed in cancer cells that are resistant to conventional chemotherapy, exemplified by sensitivity of imatinib-resitant gastrointestinal stromal tumour and myeloid cells positive for cKit mutants [75]. Of interest, OSU-2S, a non-immunosuppressive analogue of FTY720, was developed, lacking the capacity to bind to S1PR, exhibiting higher potency as an antitumour drug in hepatocellular carcinoma [76].

The phosphorylation of FTY720, a process which occurs in vivo, allows it to inhibit vascular endothelial growth factor (VEGF), consequently reducing tumour-associated angiogenesis as well as tumour cell proliferation. Of interest, FTY720 does not induce apoptosis in non-transformed cells and normal bone marrow cells. FTY720-treated leukaemia cells exhibit increased PP2A activity, leading to the dephosphorylation of various compounds, including Akt, eventually causing cell death [68].

Ceramide is known to interact with SET [77], resulting in increased PP2A activity. It is also worth considering using ceramide as a pharmacological agent, although this molecule is very quickly metabolised. However, agents that stimulate ceramide biosynthesis, such as vitamin D3, should theoretically enhance PP2A activation [78].

CIP2A

Bortezomib, a proteasome inhibitor, induced apoptosis in triple negative breast cancer (TNBC) cells and in in vivo xenograft models of TNBC, mediated by downregulation of CIP2A mRNA levels [79]. The compound celastrol directly binds and sequestrates CIP2A into a complex targeted to the ubiquitin proteasome degradation pathway, in non-small cell lung cancer cells [80]. Decreased expression of CIP2A following celastrol addition/administration induced apoptosis in cells and suppressed tumour growth in murine models. Similarly, the natural compound, ethoxysanguinarine (ESG), inhibits proliferation in lung cancer cells through the downregulation of CIP2A [81].

Molecules that upregulate expression of the catalytic and regulatory subunits

The glucocorticoid methylprednisolone has pharmacological similarity to the natural hormone produced by mammalian adrenal glands and may be used as replacement therapy in adrenal insufficiency. Its potent anti-inflammatory activity makes it useful for the management of certain forms of arthritis; skin, blood, kidney, eye, thyroid and intestinal disorders including colitis; severe allergies and asthma. Methylprednisolone is also used to treat certain types of

cancer, namely myeloid leukaemia, since it upregulates PP2A B subunits, causing cell differentiation [82].

The adenoviral E1A mediates sensitization to a variety of anticancer drug, inducing apoptosis in different cancer cell models. The mechanisms by which this takes place occur via multiple pathways. However, studies in human breast cancer cell lines have shown that E1A upregulates PP2A C subunits, eventually leading to apoptosis [83].

Activators of PP2A with unknown mechanism

The recent research on oestrogen receptor (ER) negative breast cancer has shown that nitric oxide (NO) promotes cancer progression by activating various signalling pathways, including the PI3K/Akt pathway [84] and VEGF-induced angiogenesis [85]. Of interest, endostatin activates PP2A, which is known to directly dephosphorylate endothelial NO synthase (eNOS) at Ser1177, a phosphorylation event promoted by VEGF. Hence, endostatin suppresses endothelial cell (EC) migration, acting as an anti-angiogenic factor. Such findings shed light on the possibility of targeting PP2A with therapeutic agents. Various agents are being described and illustrated in Fig. 1.

A plausible therapeutic agent for PP2A activation is forskolin, a labdane diterpene produced by the Indian coleus plant *Coleus forskohlii*. Forskolin resensitizes cell receptors by increasing the intracellular levels of cAMP. The latter is an important signal carrier necessary for the proper biological response of cells to hormones and other extracellular signals. Forskolin also has an effect on PP2A. It leads to PP2A activation, effectively antagonizing leukaemogenesis in both in vitro and in vivo models of these cancers [86].

Dithiolethiones are a well-known class of cancer chemopreventive agents, found commonly in vegetables, including broccoli. Although the clinical application of oltipraz, a typical dithiolethione, in smokers, revealed adverse side effects, anethole dithiolethione (ADT) proved to be a safer compound and is now clinically used for xerostomia [87]. A dithiolethione compound, closely related in structure to ADT, is ACS-1. The latter was found to inhibit Akt signalling in human breast and lung cancer cells by increasing PP2A activity and consequently inhibiting cell proliferation, suggesting the use of this molecule as a therapeutic agent for cancer therapy.

Carnosic acid is a natural polyphenolic diterpene found in rosemary (*Rosmarinus officinalis*) and common sage (*Salvia officinalis*). Dried leaves of rosemary or sage contain 1.5 to 2.5 % carnosic acid, which is recognised to be a potent antioxidant and exerts a protective effect on skin cells against UV-A radiation. Studies carried out in prostate cancer in vitro showed that carnosic acid also activates PP2A and consequently downregulates Akt [88].

Of interest, a novel class of small molecule activators of PP2A (SMAPs) developed by reverse engineering of tricyclic neuroleptic drugs, directly bind and activate PP2A and showed favourable antitumour properties in preclinical models of prostate cancer and lung cancer [89].

Conclusion

Inhibition of kinases has been utilised extensively as therapeutic agents in the oncology field. The occurrence of acquired resistance of tyrosine kinase inhibitors prompts for alternative drug development strategies. This review provides the knowledge base for considering the use of phosphatase activators in a subset of cancer patients classified on the basis of a suppressed activity of the PP2A phosphatase. This review focused on potential therapeutic agents that reactivate suppressed PP2A activity. Potentially therapeutic groups include a subset of tumours with haploinsufficiency due to mutations in the PP2A core complex, deactivation of the PP2A complex due to phosphorylation and demethylation of the catalytic subunit or increased expression of endogenous inhibitors of the PP2A complex. Considering the knowledge gained in modulating the activity of PP2A, the lack of clinical trials utilising PP2A activators in cancer is disappointing. Of interest, inhibition of PP2A as a potential therapeutic option is currently being investigated and a clinical trial is recruiting patients resistant to conventional treatments. The rationale towards using PP2A inhibitors stems from the function of PP2A to attenuate RAS signalling during G2 phase, promoting stable quiescence [90]. Hence, inhibition of PP2A using a small molecular inhibitor, LB-100, overcomes quiescence and sensitizes mitotic cells to combinatory treatment [91].

The future challenges include the definition of biomarker panels required to classify potential therapeutic groups, followed by well-structured clinical trials.

Compliance with ethical standards

Conflicts of interest None.

References

1. Zhang Q, Claret FX. Phosphatases: the new brakes for cancer development? Enzym Res. 2012;2012:1–11.
2. Qi Z, Yang W, Liu Y, Cui T, Gao H, et al. Loss of PINK1 function decreases PP2A activity and promotes autophagy in dopaminergic cells and a murine model. Neurochem Int. 2011;59:572–81.
3. Kloeker S, Reed R, McConnell JL, Chang D, Tran K, et al. Parallel purification of three catalytic subunits of the protein serine/threonine phosphatase 2A family (PP2AC, PP4C, and PP6C) and

analysis of the interaction of PP2AC with alpha4 protein. Protein Expr Purif. 2003;31:19–33.
4. Silverstein AM. Actions of PP2A on the MAP kinase pathway and apoptosis are mediated by distinct regulatory subunits. Proc Natl Acad Sci. 2002;99:4221–6.
5. Peyssonnaux C, Eychene A. The Raf/MEK/ERK pathway: new concepts of activation. Biol Cell. 2001;93:53–62.
6. Sablina A, Hector M, Colpaert N, Hahn WC. Identification of PP2A complexes and pathways involved in cell transformation. Cancer Res. 2010;70:10474–84.
7. Hahn K, Miranda M, Francis VA, Vendrell J, Zorzano A, et al. PP2A regulatory subunit PP2A-B′ counteracts S6K phosphorylation. Cell Metab. 2010;11:438–44.
8. Margolis SS, Perry JA, Forester CM, Nutt LK, Guo Y, et al. Role for the PP2A/B56delta phosphatase in regulating 14-3-3 release from Cdc25 to control mitosis. Cell. 2006;127:759–73.
9. Alvarez-Fernandez M, Halim VA, Aprelia M, Mohammed S, Medema RH. Protein phosphatase 2A (B55) prevents premature activation of forkhead transcription factor FoxM1 by antagonizing cyclin A/cyclin-dependent kinase-mediated phosphorylation. J Biol Chem. 2011;286:33029–36.
10. Jin Z, Wallace L, Harper SQ, Yang J. PP2A:B56{epsilon}, a substrate of caspase-3, regulates p53-dependent and p53-independent apoptosis during development. J Biol Chem. 2010;285:34493–502.
11. Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, et al. Mechanism of activation of protein kinase B by insulin and IGF-1. EMBO J. 1996;15:6541–51.
12. Puustinen P, Junttila MR, Vanhatupa S, Sablina AA, Hector ME, et al. PME-1 protects extracellular signal-regulated kinase pathway activity from protein phosphatase 2A-mediated inactivation in human malignant glioma. Cancer Res. 2009;69:2870–7.
13. Stebbing J, Lit LC, Zhang H, Darrington RS, Melaiu O, et al. (2013) The regulatory roles of phosphatases in cancer. Oncogene.
14. Ismail HMS, Myronova O, Tsuchiya Y, Niewiarowski A, Tsaneva I, et al. Identification of the general transcription factor Yin Yang 1 as a novel and specific binding partner for S6 kinase 2. Cell Signal. 2013;25:1054–63.
15. Mumby M. PP2A: unveiling a reluctant tumor suppressor. Cell. 2007;130:21–4.
16. Baldacchino S, Saliba C, Petroni V, Fenech AG, Borg N, et al. Deregulation of the phosphatase, PP2A is a common event in breast cancer, predicting sensitivity to FTY720. EPMA J. 2014;5:3.
17. Cristobal I, Manso R, Rincon R, Carames C, Senin C, et al. PP2A inhibition is a common event in colorectal cancer and its restoration using FTY720 shows promising therapeutic potential. Mol Cancer Ther. 2014;13:938–47.
18. Seshacharyulu P, Pandey P, Datta K, Batra SK. Phosphatase: PP2A structural importance, regulation and its aberrant expression in cancer. Cancer Lett. 2013;335:9–18.
19. Ruediger R, Hentz M, Fait J, Mumby M, Walter G. Molecular model of the A subunit of protein phosphatase 2A: interaction with other subunits and tumor antigens. J Virol. 1994;68:123–9.
20. Hemmings BA, Adams-Pearson C, Maurer F, Mueller P, Goris J, et al. Alpha- and beta-forms of the 65-kDa subunit of protein phosphatase 2A have a similar 39 amino acid repeating structure. Biochemistry. 1990;29:3166–73.
21. Janssens V, Goris J, Van Hoof C. PP2A: the expected tumor suppressor. Curr Opin Genet Dev. 2005;15:34–41.
22. Gu P, Qi X, Zhou Y, Wang Y, Gao X. Generation of Ppp2Ca and Ppp2Cb conditional null alleles in mouse. Genesis. 2012;50:429–36.
23. Chen J, Martin BL, Brautigan DL. Regulation of protein serine-threonine phosphatase type-2A by tyrosine phosphorylation. Science. 1992;257:1261–4.
24. Sontag J-M, Nunbhakdi-Craig V, Mitterhuber M, Ogris E, Sontag E. Regulation of protein phosphatase 2A methylation by LCMT1 and PME-1 plays a critical role in differentiation of neuroblastoma cells. J Neurochem. 2010;115:1455–65.
25. Sents W, Ivanova E, Lambrecht C, Haesen D, Janssens V. The biogenesis of active protein phosphatase 2A holoenzymes: a tightly regulated process creating phosphatase specificity. FEBS J. 2013;280:644–61.
26. Stanevich V, Jiang L, Satyshur KA, Li Y, Jeffrey PD, et al. The structural basis for tight control of PP2A methylation and function by LCMT-1. Mol Cell. 2011;41:331–42.
27. (2013) GeneCards—The human gene compendium.
28. Gray KA, Daugherty LC, Gordon SM, Seal RL, Wright MW, et al. Genenames.org: the HGNC resources in 2013. Nucleic Acids Res. 2013:41.
29. Laine A. The role of an oncoprotein cip2a in breast carcinoma. Turku: University of Turku; 2013.
30. Kong M, Fox CJ, Mu J, Solt L, Xu A, et al. The PP2A-associated protein alpha4 is an essential inhibitor of apoptosis. Science. 2004;306:695–8.
31. Slupe AM, Merrill RA, Strack S. Determinants for substrate specificity of protein phosphatase 2A. Enzym Res. 2011;2011:1–8.
32. Xu Y, Xing Y, Chen Y, Chao Y, Lin Z, et al. Structure of the protein phosphatase 2A holoenzyme. Cell. 2006;127:1239–51.
33. Yu XX, Du X, Moreno CS, Green RE, Ogris E, et al. Methylation of the protein phosphatase 2A catalytic subunit is essential for association of Balpha regulatory subunit but not SG2NA, striatin, or polyomavirus middle tumor antigen. Mol Biol Cell. 2001;12:185–99.
34. Jackson JB, Pallas DC. Circumventing cellular control of PP2A by methylation promotes transformation in an Akt-dependent manner. Neoplasia. 2012;14:585–99.
35. Calin GA, Iasio MG, Caprini E, Vorechovsky I, Natali PG, et al. Low frequency of alterations of the α (PPP2R1A) and β (PPP2R1B) isoforms of the subunit A of the serine-threonine phosphatase 2A in human neoplasms. Oncogene. 2000;19:1191–5.
36. Ruediger R, Ruiz J, Walter G. Human cancer-associated mutations in the A α subunit of protein phosphatase 2A increase lung cancer incidence in A α knock-in and knockout mice. Mol Cell Biol. 2011;31:3832–44.
37. Wang SS, Esplin ED, Li JL, Huang L, Evans GA. Alterations of the PPP2R1B gene in human lung and colon cancer. Science. 1998;282:284–7.
38. Takagi Y, Futamura M, Yamaguchi K, Aoki S, Takahashi T, et al. Alterations of the PPP2R1B gene located at 11q23 in human colorectal cancers. Gut. 2000;47:268–71.
39. Chou H-C, Chen C-H, Lee H-S, Lee C-Z, Huang G-T, et al. Alterations of tumour suppressor gene PPP2R1B in hepatocellular carcinoma. Cancer Lett. 2007;253:138–43.
40. Sablina AA, Chen W, Arroyo JD, Corral L, Hector M, et al. The tumor suppressor PP2A Aβ regulates the RalA GTPase. Cell. 2007;129:969–82.
41. Cheng Y, Liu W, Kim S-T, Sun J, Lu L, et al. Evaluation of PPP2R2A as a prostate cancer susceptibility gene: a comprehensive germline and somatic study. Cancer Genet. 2011;204:375–81.
42. Niemelä M, Kauko O, Sihto H, Mpindi JP, Nicorici D, et al. CIP2A signature reveals the MYC dependency of CIP2A-regulated phenotypes and its clinical association with breast cancer subtypes. Oncogene. 2012;31:4266–78.
43. Migueleti DLS, Smetana JHC, Nunes HF, Kobarg J, Zanchin NIT. Identification and characterization of an alternatively spliced isoform of the human protein phosphatase 2A catalytic subunit. J Biol Chem. 2011;287:4853–62.
44. Grech G, Blazquez-Domingo M, Kolbus A, Bakker WJ, Mullner EW, et al. Igbp1 is part of a positive feedback loop in stem cell factor-dependent, selective mRNA translation initiation inhibiting erythroid differentiation. Blood. 2008;112:2750–60.

45. Neviani P, Santhanam R, Trotta R, Notari M, Blaser BW, et al. The tumor suppressor PP2A is functionally inactivated in blast crisis CML through the inhibitory activity of the BCR/ABL-regulated SET protein. Cancer Cell. 2005;8:355–68.
46. Markova B, Albers C, Breitenbuecher F, Melo JV, Brümmendorf TH, et al. Novel pathway in Bcr-Abl signal transduction involves Akt-independent, PLC-γ1-driven activation of mTOR/p70S6-kinase pathway. Oncogene. 2009;29:739–51.
47. O'Hare T, Deininger MWN, Eide CA, Clackson T, Druker BJ. Targeting the BCR-ABL signaling pathway in therapy-resistant Philadelphia chromosome-positive leukemia. Clin Cancer Res. 2010;17:212–21.
48. Lucas CM, Harris RJ, Giannoudis A, Copland M, Slupsky JR, et al. Cancerous inhibitor of PP2A (CIP2A) at diagnosis of chronic myeloid leukemia is a critical determinant of disease progression. Blood. 2011;117:6660–8.
49. Barjesteh van Waalwijk van Doorn-Khosrovani S, Spensberger D, de Knegt Y, Tang M, Lowenberg B, et al. Somatic heterozygous mutations in ETV6 (TEL) and frequent absence of ETV6 protein in acute myeloid leukemia. Oncogene. 2005;24:4129–37.
50. Minakuchi M, Kakazu N, Gorrin-Rivas MJ, Abe T, Copeland TD, et al. Identification and characterization of SEB, a novel protein that binds to the acute undifferentiated leukemia-associated protein SET. Eur J Biochem. 2001;268:1340–51.
51. Junttila MR, Puustinen P, Niemelä M, Ahola R, Arnold H, et al. CIP2A inhibits PP2A in human malignancies. Cell. 2007;130:51–62.
52. Vaarala MH, Väisänen M-R, Ristimäki A. CIP2A expression is increased in prostate cancer. J Exp Clin Cancer Res. 2010;29:136.
53. Böckelman C, Lassus H, Hemmes A, Leminen A, Westermarck J, et al. Prognostic role of CIP2A expression in serous ovarian cancer. Br J Cancer. 2011;105:989–95.
54. Teng H-W, Yang S-H, Lin J-K, Chen W-S, Lin T-C, et al. CIP2A is a predictor of poor prognosis in colon cancer. J Gastrointest Surg. 2012;16:1037–47.
55. Come C, Laine A, Chanrion M, Edgren H, Mattila E, et al. CIP2A is associated with human breast cancer aggressivity. Clin Cancer Res. 2009;15:5092–100.
56. Wang J, Li W, Li L, Yu X, Jia J, et al. CIP2A is over-expressed in acute myeloid leukaemia and associated with HL60 cells proliferation and differentiation. Int J Lab Hematol. 2011;33:290–8.
57. Mannava S, Omilian AR, Wawrzyniak JA, Fink EE, Zhuang D, et al. PP2A-B56α controls oncogene-induced senescence in normal and tumor human melanocytic cells. Oncogene. 2011;31:1484–92.
58. Lazar DF, Saltiel AR. Lipid phosphatases as drug discovery targets for type 2 diabetes. Nat Rev Drug Discov. 2006;5:333–42.
59. Lyon MA, Ducruet AP, Wipf P, Lazo JS. Dual-specificity phosphatases as targets for antineoplastic agents. Nat Rev Drug Discov. 2002;1:961–76.
60. Tonks NK. Protein tyrosine phosphatases: from genes, to function, to disease. Nat Rev Mol Cell Biol. 2006;7:833–46.
61. Liu J, Farmer Jr JD, Lane WS, Friedman J, Weissman I, et al. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. Cell. 1991;66:807–15.
62. Bachovchin DA, Zuhl AM, Speers AE, Wolfe MR, Weerapana E, et al. Discovery and optimization of sulfonyl acrylonitriles as selective, covalent inhibitors of protein phosphatase methylesterase-1. J Med Chem. 2011;54:5229–36.
63. Bachovchin DA, Mohr JT, Speers AE, Wang C, Berlin JM, et al. Academic cross-fertilization by public screening yields a remarkable class of protein phosphatase methylesterase-1 inhibitors. Proc Natl Acad Sci U S A. 2011;108:6811–6.
64. Pusey M, Bail S, Xu Y, Buiakova O, Nestor M, et al. (2016) Inhibition of protein methylesterase 1 decreased cancerous phenotypes in endometrial adenocarcinoma cell lines and xenograft tumor models. Tumour Biol.
65. Madhunapantula SV, Robertson GP. Therapeutic implications of targeting AKT signaling in melanoma. Enzyme Res. 2011;2011:327923.
66. Hwang YY, Liang RH. An update in management of noncutaneous T-cell lymphomas. Adv Hematol. 2010;2010:424786.
67. Christensen DJ, Ohkubo N, Oddo J, Van Kanegan MJ, Neil J, et al. Apolipoprotein E and peptide mimetics modulate inflammation by binding the SET protein and activating protein phosphatase 2A. J Immunol. 2011;186:2535–42.
68. Neviani P, Santhanam R, Oaks JJ, Eiring AM, Notari M, et al. FTY720, a new alternative for treating blast crisis chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphocytic leukemia. J Clin Invest. 2007;117:2408–21.
69. Chiba K. FTY720, a new class of immunomodulator, inhibits lymphocyte egress from secondary lymphoid tissues and thymus by agonistic activity at sphingosine 1-phosphate receptors. Pharmacol Ther. 2005;108:308–19.
70. Pippa R, Dominguez A, Christensen DJ, Moreno-Miralles I, Blanco-Prieto MJ, et al. Effect of FTY720 on the SET-PP2A complex in acute myeloid leukemia; SET binding drugs have antagonistic activity. Leukemia. 2014;28:1915–8.
71. Cristobal I, Garcia-Orti L, Cirauqui C, Alonso MM, Calasanz MJ, et al. PP2A impaired activity is a common event in acute myeloid leukemia and its activation by forskolin has a potent anti-leukemic effect. Leukemia. 2011;25:606–14.
72. Azuma H, Takahara S, Horie S, Muto S, Otsuki Y, et al. Induction of apoptosis in human bladder cancer cells in vitro and in vivo caused by FTY720 treatment. J Urol. 2003;169:2372–7.
73. Zhang N, Qi Y, Wadham C, Wang L, Warren A, et al. FTY720 induces necrotic cell death and autophagy in ovarian cancer cells: a protective role of autophagy. Autophagy. 2010;6:1157–67.
74. Zheng T, Meng X, Wang J, Chen X, Yin D, et al. PTEN- and p53-mediated apoptosis and cell cycle arrest by FTY720 in gastric cancer cells and nude mice. J Cell Biochem. 2010;111:218–28.
75. Dumont AG, Reynoso DG, Trent JC. Essential requirement for PP2A inhibition by the oncogenic receptor c-KIT suggests PP2A reactivation as a strategy to treat c-KIT+ cancers—letter. Cancer Res. 2011;71:2403 .author reply 2404
76. Omar HA, Chou CC, Berman-Booty LD, Ma Y, Hung JH, et al. Antitumor effects of OSU-2S, a nonimmunosuppressive analogue of FTY720, in hepatocellular carcinoma. Hepatology. 2011;53:1943–58.
77. Mukhopadhyay A, Saddoughi SA, Song P, Sultan I, Ponnusamy S, et al. Direct interaction between the inhibitor 2 and ceramide via sphingolipid-protein binding is involved in the regulation of protein phosphatase 2A activity and signaling. FASEB J. 2009;23:751–63.
78. Switzer CH, Cheng RY, Vitek TM, Christensen DJ, Wink DA, et al. Targeting SET/I(2)PP2A oncoprotein functions as a multi-pathway strategy for cancer therapy. Oncogene. 2011;30:2504–13.
79. Tseng LM, Liu CY, Chang KC, Chu PY, Shiau CW, et al. CIP2A is a target of bortezomib in human triple negative breast cancer cells. Breast Cancer Res. 2012;14:R68.
80. Liu Z, Ma L, Wen ZS, Hu Z, Wu FQ, et al. Cancerous inhibitor of PP2A is targeted by natural compound celastrol for degradation in non-small-cell lung cancer. Carcinogenesis. 2014;35:905–14.
81. Liu Z, Ma L, Wen ZS, Cheng YX, Zhou GB. Ethoxysanguinarine induces inhibitory effects and downregulates CIP2A in lung cancer cells. ACS Med Chem Lett. 2014;5:113–8.
82. Uzunoglu S, Uslu R, Tobu M, Saydam G, Terzioglu E, et al. Augmentation of methylprednisolone-induced differentiation of myeloid leukemia cells by serine/threonine protein phosphatase inhibitors. Leuk Res. 1999;23:507–12.
83. Liao Y, Hung MC. A new role of protein phosphatase 2a in adenoviral E1A protein-mediated sensitization to anticancer drug-induced apoptosis in human breast cancer cells. Cancer Res. 2004;64:5938–42.

84. Switzer CH, Glynn SA, Ridnour LA, Cheng RY, Vitek MP, et al. Nitric oxide and protein phosphatase 2A provide novel therapeutic opportunities in ER-negative breast cancer. Trends Pharmacol Sci. 2011;32:644–51.
85. Urbich C, Reissner A, Chavakis E, Dernbach E, Haendeler J, et al. Dephosphorylation of endothelial nitric oxide synthase contributes to the anti-angiogenic effects of endostatin. FASEB J. 2002;16:706–8.
86. Perrotti D, Neviani P. Protein phosphatase 2A (PP2A), a drugable tumor suppressor in Ph1(+) leukemias. Cancer Metastasis Rev. 2008;27:159–68.
87. Switzer CH, Ridnour LA, Cheng RY, Sparatore A, Del Soldato P, et al. Dithiolethione compounds inhibit Akt signaling in human breast and lung cancer cells by increasing PP2A activity. Oncogene. 2009;28:3837–46.
88. Kar S, Palit S, Ball WB, Das PK. Carnosic acid modulates Akt/IKK/NF-kappaB signaling by PP2A and induces intrinsic and extrinsic pathway mediated apoptosis in human prostate carcinoma PC-3 cells. Apoptosis. 2012;17:735–47.
89. McClinch K, Avelar R, Callejas D, Kastrinsky D, Ohlmeyer M, Plymate S, et al. Therapeutic reactivation of PP2A for prostate cancer treatment; 2015 Nov 5–9; Boston, MA. Philadelphia (PA): Molecular targets and cancer therapeutics.
90. Naetar N, Soundarapandian V, Litovchick L, Goguen KL, Sablina AA, et al. PP2A-mediated regulation of Ras signaling in G2 is essential for stable quiescence and normal G1 length. Mol Cell. 2014;54:932–45.
91. Hong CS, Ho W, Zhang C, Yang C, Elder JB, et al. LB100, a small molecule inhibitor of PP2A with potent chemo- and radio-sensitizing potential. Cancer Biol Ther. 2015;16:821–33.