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Review

Regulation and role of the PP2A-B56 holoenzyme family in cancer

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ABSTRACT

Protein phosphatase 2A (PP2A) inactivation is common in cancer, leading to sustained activation of pro-survival and growth-promoting pathways. PP2A consists of a scaffolding A-subunit, a catalytic C-subunit, and a regulatory B-subunit. The functional complexity of PP2A holoenzymes arises mainly through the vast repertoire of regulatory B-subunits, which determine both their substrate specificity and their subcellular localization. Therefore, a major challenge for developing more effective therapeutic strategies for cancer is to identify the specific PP2A complexes to be targeted. Of note, the development of small molecules specifically directed at PP2A-B56α has opened new therapeutic avenues in both solid and hematological tumors. Here, we focus on the B56/PR61 family of PP2A regulatory subunits, which have a central role in directing PP2A tumor suppressor activity. We provide an overview of the mechanisms controlling the formation and regulation of these complexes, the pathways they control, and the mechanisms underlying their deregulation in cancer.

1. Introduction

Dynamic protein phosphorylation is a post-translational modification with an essential role in the regulation of a variety of indispensable cellular processes for the maintenance of normal homeostasis, such as proliferation, apoptosis, and differentiation [1]. Protein phosphorylation is a highly controlled and regulated process that requires the coordinated and temporal regulation of both protein kinase and phosphatase function. Dysregulation of this balance underlies the pathogenesis of many human diseases, including cancer [2]. Multiple cellular signaling cascades are regulated by phosphorylation events, and modifications in the phosphorylation state of proteins enable cells to rapidly adapt to both changes in extracellular and intracellular cues. In most cases, a phosphate group is covalently bound or removed from serine (Ser), threonine (Thr), or tyrosine (Tyr) amino acid residues and, to a lesser extent, from histidine, lysine, or arginine. Although the number of kinases is far larger than phosphatases, the structure of phosphatase complexes allows a single catalytic subunit to form hundreds of distinct holoenzymes creating a larger repertoire of protein phosphatases [3,4].

Phosphatases are divided into four main classes based on their amino acid substrate specificity: Ser/Thr phosphatases, Tyr phosphatases, dual specificity phosphatases, and histidine phosphatases [3–5]. The majority of Ser/Thr dephosphorylation is performed by ten catalytic subunits that constitute the family of Phosphoprotein Phosphatases (PPPs): PP1α/β/γ, PP2Acα/β, PP2Bc, PP4c, PP5c, PP6c, and PP7c [4]. PP1α/β/γ catalytic subunits bind to regulatory subunits to form heterodimers, while PP2Acα/β, PP4c, and PP6c form mostly heterotrimers with regulatory and scaffolding subunits [6]. Holoenzyme formation is tightly regulated through various mechanisms, including subunit post-translational modifications, to ensure that the appropriate repertoire of PPPs is present in cells to catalyze specific dephosphorylation events.

Abbreviations: AML, acute myeloid leukemia; CIP2A, cancerous inhibitor of PP2A; CLL, chronic lymphocytic leukemia; I1PP2A, inhibitor 1 of PP2A; I2PP2A, inhibitor 2 of PP2A; LCMT-1, leucine carboxyl methyltransferase-1; Leu, leucine; LSC, leukemic stem cell; MAPK, mitogen-activated protein kinase; mTORC, mammalian target of rapamycin complex; NSCLC, non-small cell lung cancer; PME-1, phosphatase methylesterase-1; Pro, proline; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; PPP, phosphoprotein phosphatase; PTPA, phosphotyrosyl phosphatase activator; RPMK, reads per kilobase of transcript per million mapped reads; Ser, serine; SLiM, short linear motif; SMAP, small molecule activator of PP2A; T-ALL, T-cell acute lymphoblastic leukemia; Thr, threonine; Tyr, tyrosine.

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The very end C-terminus of PP2Acα/β, PP4c, and PP6c are closely related and highly conserved from yeast to humans, and this region is involved in the regulation and biogenesis through distinct post-translational modifications such as phosphorylation and methylation of the C-terminal tail of the catalytic subunit [4].

Numerous studies have focused on the study of aberrant kinase activity in cancer. Although phosphatases are also essential to maintain cell homeostasis, their role in cancer has not been fully considered until recently. PP2A (protein phosphatase 2A) comprises a scaffolding A-subunit, a catalytic C-subunit, and a regulatory B-subunit. The functional complexity and specificity of PP2A mainly emerge via the existence of a repertoire of regulatory B-subunits, which determine both the substrate and subcellular localization of the heterotrimeric PP2A complex. Of importance, PP2A is inactivated in numerous solid and hematological tumors and its tumor suppressor function is mainly regulated by two of the four families of regulatory B-subunits: B55 and B56. The role and regulation of B55/PR55 family members in cancer have been recently reviewed [7,8]. Here, we will focus on the members of the B56/PR61 family of regulatory PP2A subunits. We will provide an overview of the mechanisms controlling the assembly and regulation of the PP2A-B56 complexes. We then will systematically discuss the main PP2A-B56 direct substrates, the pathways they control, and their deregulation in cancer. Of note, the recent development of small molecules targeting specific PP2A holoenzymes, such as PP2A-B56α, has emerged as a tool to determine new functions of PP2A and has opened up new therapeutic opportunities in cancer.

2. Protein phosphatase 2A (PP2A)

One of the major constituents of the total cellular Ser/Thr phosphatase pool in mammalian cells is the highly conserved and ubiquitously expressed protein PP2A. PP2A regulates a wide variety of cellular processes, such as cell cycle, proliferation, differentiation, DNA damage response, stress response, cell adhesion and mobility, and apoptosis [9]. Moreover, PP2A is a tumor suppressor that counteracts most of the kinase-driven intracellular signaling pathways underlying normal physiology as well as the pathobiology of cancer and other diseases [10,11].

2.1. Structure of the PP2A holoenzymes

PP2A is a family of holoenzymes that exist in two different forms: as dimers and trimers [12]. The dimeric form known as the core enzyme consists of a scaffold A-subunit and a catalytic C-subunit. In humans, A- and C-subunits are each encoded by two different genes, giving rise to two isoforms: *PPP2R1A*/PR65α/Aα and *PPP2R1B*/PR65β/Aβ for scaffold subunit; and *PPP2CA*/PP2Acα and *PPP2CB*/PP2Acβ for the catalytic subunit. Each heterotrimeric enzyme is composed of the core AC-dimer and a structurally distinct regulatory B-subunit, which determine the substrate specificity [13]. For the B-subunits, 15 human genes have been described, giving rise to over 40 different isoforms that are sorted into four families: B/B55/PR55, B′/B56/PR61, B″/PR72/PR70, and B‴/STRN/PR110/PR93 (Fig. 1A). From each family different isoforms have been identified: B55 (α, β, γ, δ), B56 (α, β, γ, δ, ε), PR (PR72/130, PR70/48, G5PR), and STRN (STRN, STRN3, STRN4) (Supplementary Table 1) [11,14,15]. Some of these isoforms also have different splicing variants. Although A- and C-subunits show remarkable sequence conservation among eukaryotic organisms, the distinct B-subunit genes are heterogeneous and exhibit very little sequence similarity across each family [5]; in contrast to the isoforms within each family which share significant sequence similarity. Structural studies of the B55, B56, PR72/PR70, and STRN3 regulatory subunits in the context of their trimeric holoenzymes have revealed divergent structures, which are consistent with their divergent sequences (Supplementary Fig. 1) [16–19]. Interestingly, the sequence variations in B56 family members mainly reside at the interface where the A- and B-subunits interact in the assembled PP2A complex [20]. A- and C-subunits are expressed in all tissues, with the expression of the Aα and Cα isoforms predominating in most cell types, while Aβ expression is only elevated during early development in vertebrates [11]. The expression levels of different B-subunits, on the other hand, are extremely variable depending on the cell, tissue, and developmental context (Fig. 1B). Together, more than 80 distinct heterotrimeric holoenzymes of PP2A account for 50% to 70% of the total Ser/Thr phosphatase activity in eukaryotic cells [9,11,21]. Thus, PP2A is not a single entity but a family of heterotrimeric holoenzymes with context-dependent functions.

2.2. Mechanism and regulation of the active PP2A holoenzymes assembly

PP2A is required for the appropriate function of a wide variety of biological processes; therefore, its stability and activity are regulated by multiple post-translational modifications and interacting proteins, which ensure that the appropriate repertoire of PP2A complexes is present in cells to maintain exquisitely tightly controlled and regulated enzymatic activity. Here we will focus on the aspects that regulate the formation of the PP2A-B56 complexes.

To prevent the formation of catalytically active complexes that lack the correct substrate specificity, holoenzyme assembly is tightly regulated [22]. During PP2A biogenesis, the phosphotyrosyl phosphatase activator (PTPA) interacts with the C-terminus of the monomeric C-subunit [23], inducing conformational and biochemical changes that activate the C-subunit prior to A-subunit binding and dimer formation (Fig. 2A). Thus, PTPA functions as an ATP-dependent chaperone to enhance PP2A biogenesis [6]. Unpartnered C-subunit is directed to ubiquitination and proteasome degradation. The precise function of the AC-dimer in cell signaling remains unclear, but it serves as a readily available pool for the assembly of different heterotrimers in response to both extracellular and intracellular cues. C-terminal HEAT repeats of the PP2A A-subunit contact the catalytic subunit, while N-terminal HEAT repeats mediate contacts with the various regulatory B-subunits [20]. Importantly, the phosphorylation and methylation of the C-terminal tail of the PP2A-C subunit modulate the formation of specific B-containing heterotrimers (Fig. 2B).

Phosphorylation events at this same C-terminal tail inhibit the interaction of PP2A-C with PTPA and also play an essential role in directing B-subunit binding and therefore in regulating the enzymatic activity of PP2A [24]. Phospho-mimetic mutants at Thr304 result in the disruption of B55 subunit binding to the AC-core enzyme [24,25]. In fact, this phosphorylation event is essential during mitosis, through its ability to regulate B55 subunit binding [26,27]. On the other hand, the functional implications of Tyr307 phosphorylation have not been sufficiently elucidated as a result of the lack of specific antibodies to this post-translational modification [28,29].

Reversible methylation of leucine 309 (Leu309) in the C-subunit is a critical regulator of PP2A biogenesis and modification of this residue drives biased PP2A heterotrimer formation, and changes in carboxymethylation are highly dependent on cellular context and stimuli [6,30,31]. This methylation event is catalyzed by the leucine carboxyl methyltransferase-1 (LCMT-1), while demethylation is performed by the protein phosphatase methylesterase-1 (PME-1) (Fig. 2A). Methylation removes the negative charge of the C-terminal tail of the catalytic subunit, thereby stabilizing the C-subunit and facilitating its docking into an acidic groove between the A- and B-subunits [13]. Additionally, PME-1 binding reduces PP2A activity through a rearrangement of the PP2A-C active site and displacement of the two divalent cations, which are required for the catalysis of the dephosphorylation reaction [32].

Deletion of the C-terminal leucine (ΔL309 mutation) of PP2A is frequently used as a mimetic of the unmethylated form. This approach together with CRISPR/Cas9 models knocking out LCMT-1, and the use of mass spectrometry-based proteomics approaches combined with affinity enrichments have allowed the study of the specific implications of this post-translational modification on the regulation of PP2A complex

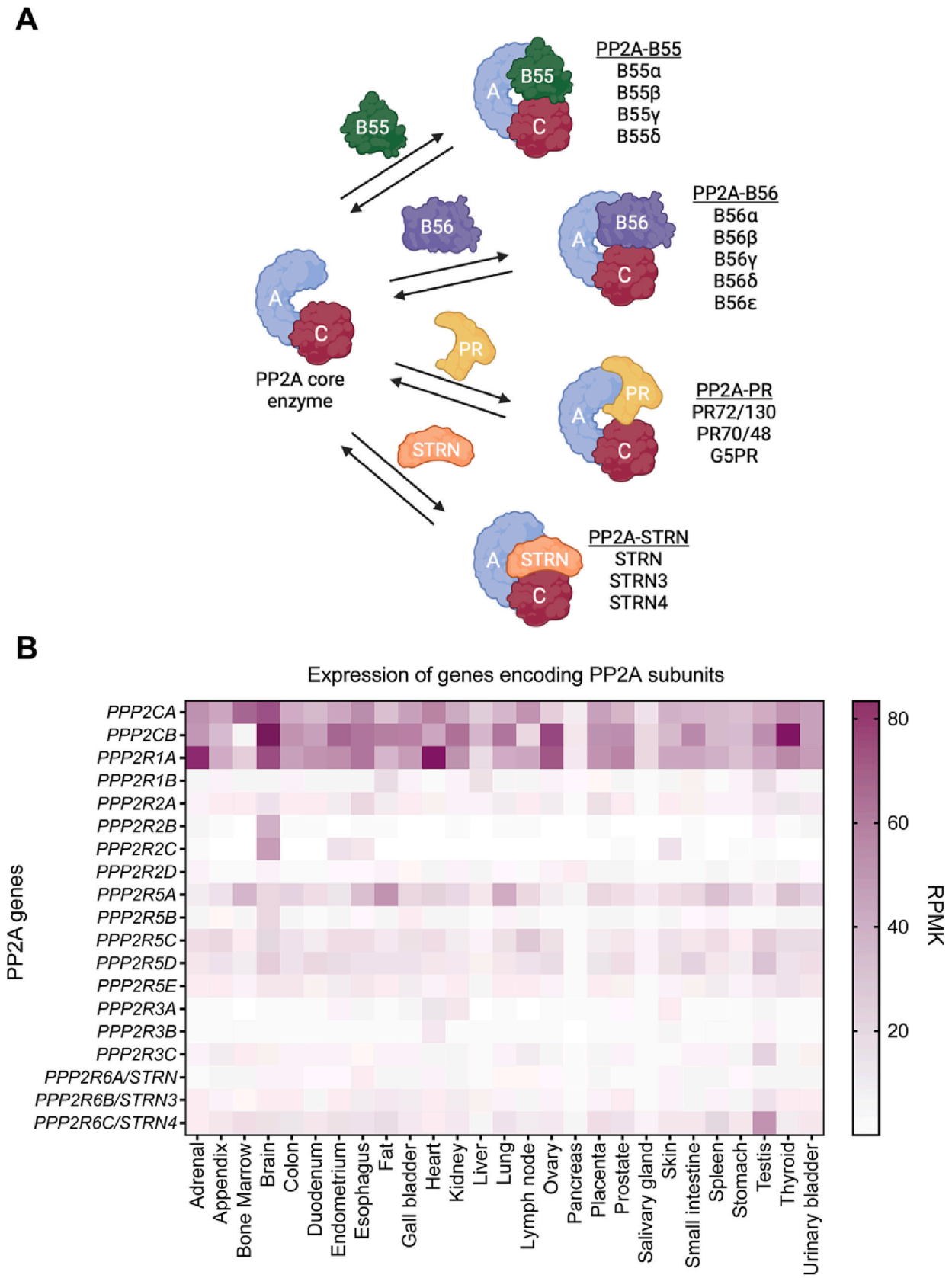

Fig. 1. PP2A holoenzymes and subunits. (A) The PP2A core enzyme is composed of the A- and C-subunits. This dimer can bind distinct PP2A regulatory subunits from the four B regulatory families (B55, B56, PR, and STRN). Between the different families of B-subunits there is very little sequence similarity. B-subunits are responsible for the substrate specificity and subcellular localization of the PP2A heterocomplex. (Figure created in BioRender.com). (B) Heatmap representation of the reads per kilobase of transcript per million mapped reads (RPMK) of genes coding for PP2A subunits in different human tissues. Data recapitulated from Gene NCBI database (2018).

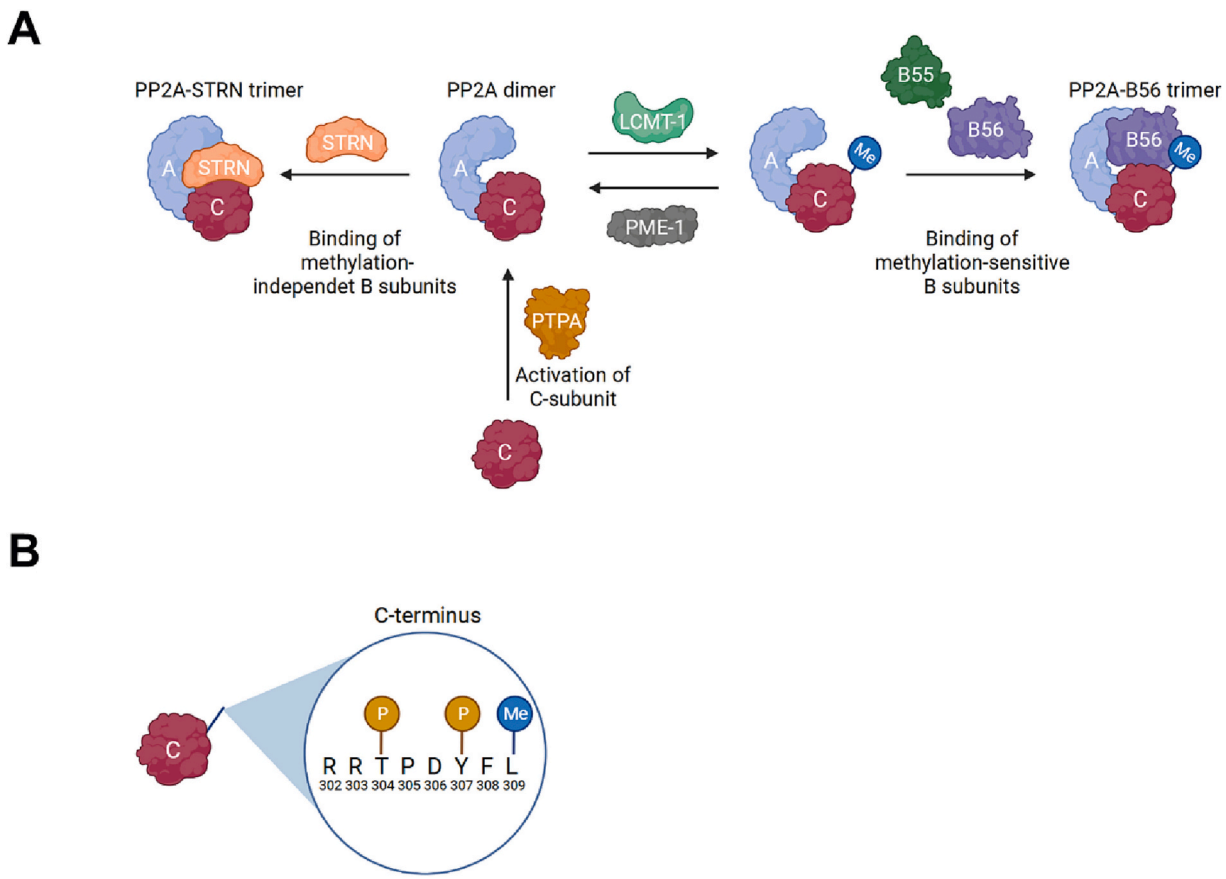

Fig. 2. PP2A assembly process and regulation. (A) Monomeric PP2A C-subunit needs to be activated by PTPA before dimerization with the scaffold A-subunit. Methylation of the C-subunit at the C-carboxyl terminus by LCMT-1 facilitates the binding of methylation-sensitive B-subunits such as B55α, B56α, B56β, and B56ε. Methylation is a reversible process and PME-1 is responsible for demethylation of the C-subunit which promotes the binding of methylation-independent B-subunits, such as those of the STRN family. (B) Detail of the very end C-terminal region of PP2A C-subunit where several residues can be modified to modulate complex activity. Thr304 and Tyr307 can be phosphorylated and Leu309 can be methylated (Figure created in BioRender.com).

assembly formation. The binding of the C-subunit to the Aα and Aβ scaffolding subunits is reduced approximately 4-fold and 2-fold, respectively, when the C-subunit is unmethylated [26]. However, the most important characteristic of this modification is its role in enhancing or diminishing the binding of specific B-subunits to the core enzyme dimer. B55 subunits strongly rely on C-terminal methylation for holoenzyme formation [26]. B56 subunits can interact with methylated and unmethylated PP2A-C, although methylation promotes their association [24]; however, recent results suggest that Leu309 methylation enhances the binding of the B56α/ε subunits more specifically. Upon ΔL309 mutation, B56α and B56ε binding were reduced by 32-fold and 11-fold, respectively, while B56γ and B56δ were reduced by only 2-fold in binding [26,33]. This difference in subunit binding is consistent with unique amino acid sequence motifs in the C-terminal tail that distinguishes the B56α/β/ε versus B56γ/δ isoforms [34], making the later ones more methyl-sensitive. Thus, specific B55 and B56 regulatory subunits are preferentially bound to methylated PP2A-C and their stability is altered when not bound to the core AC dimer [24,26]. In mammalian cells, 70-90% of PP2A-C is methylated [35]. Interestingly, PP2A-B55 holoenzymes tend to oppose proline-directed kinases while PP2A-B56 heterotrimers oppose basophilic kinases [36]. Therefore, changes in methylation affect the repertoire of holoenzymes that are formed and therefore the pathways that PP2A controls. Future studies are needed to determine which phosphorylation sites are specifically sensitive to these changes and to identify downstream signaling pathways that are distinctly regulated. Additionally, post-translational modifications of B-subunits can affect the activity and subcellular localization of PP2A, influencing which substrate proteins are targeted.

2.3. PP2A inhibitors

PP2A activity is also modulated by several endogenous inhibitors. ANP32A (I1PP2A, Inhibitor 1 of PP2A) and SET (I2PP2A, Inhibitor 2 of PP2A) bind to the C-subunit, impeding its activity, while others specifically bind and inhibit PP2A-B55 (ARPP19 and ENSA) or PP2A-B56 heterocomplexes (CIP2A and BOD1) [8]. Here we will focus on PP2A inhibitors that regulate C-subunit activity and PP2A-B56 holoenzymes.

ANP32A and SET are two potent stable PP2A inhibitors. Both proteins directly bind and inhibit the C-subunit of PP2A. Their localization, as well as their binding to PP2A and their inhibitory activity, are modulated by phosphorylation [13]. SET is usually localized to the nucleus; however, its phosphorylation by kinases such as CK2 and PI3K facilitates SET shuttling to the cytoplasm and increases its ability to bind and inactivate PP2A [37,38]. Moreover, there are cytoplasmic proteins that stabilize SET binding to the PP2A C-subunit such as SETBP1 and p38β [38,39]. ANP32A modulates the PP2A-dependent dephosphorylation of the Tau protein, whereas SET controls numerous substrates involved in cancer, including ERK, AKT, MYC, PTEN, and MCL1, through the binding and inhibition of PP2A [13]. Recent studies indicate that SET could be predominantly associated with PP2A-B56 heterocomplexes in cancer [40].

CIP2A (Cancerous Inhibitor of PP2A) directly binds to PP2A-B56α, displacing the PP2A A-subunit and thereby hijacking both the B56α and the catalytic PP2A C-subunit to form a CIP2A-B56α-PP2Ac pseudotrimer, muting the B56α substrate recognition site [41,42]. BOD1 has been identified as a specific inhibitor of PP2A-B56 holoenzyme during mitosis. PP2A-B56 regulates the phosphorylation balance at

kinetochore-microtubule attachments, and PP2A-B56 inhibition by BOD1 is required to maintain proper chromosomal alignment [43].

Aside from PP2A endogenous inhibitors, during the last decades compounds such as okadaic acid, calyculin A, or microcysteine have been essential for mimicking PP2A inhibition in diverse models. Nevertheless, these compounds have demonstrated to be poor specific, due to their ability to inhibit PP1 and PP6 catalytic subunits apart of PP2A C-subunit depending on the doses used or the substrates studied [44,45]. Currently, during the process of complex-specific PP2A activators generation, inactive analogs have also emerged. One example is the TRC-766 compound, which is structurally similar to the PP2A-B56α activator DT-061 but biologically inactive [46].

3. The B56 family of β-subunits

B56- and B55-containing PP2A complexes direct most of the tumor suppressive phosphatase activity in signaling pathways associated with cell growth, proliferation, metabolism, differentiation, and apoptosis. Furthermore, PP2A-B56 heterocomplexes regulate circadian rhythms, activation of important transcription factors, and the cell cycle. Of note, some of B55 and B56 functions overlap and therefore, some substrates can be dephosphorylated by PP2A complexes containing members from these two families. Here, we will focus on the regulation and functions of the B56 family specifically.

The B56 family is comprised of 5 members coded by 5 different genes in humans and mice: B56α (*PPP2R5A*), B56β (*PPP2R5B*), B56γ (*PPP2R5C*), B56δ (*PPP2R5D*), and B56ε (*PPP2R5E*). B56γ and B56δ have 3 alternative splicing isoforms, and B56ε has an alternative translation isoform [47,48]. Members of the B56 family show a distinct spatial distribution within the cell with B56α, B56β, and B56ε being predominantly localized to the cytoplasm, while B56γ is mainly found in the nucleus [49]. B56 subunits are structurally composed of 8 HEAT-like repeats similar to the scaffold A-subunits and show little or no similarity to any of the other B-subunit families. In PP2A-B56 complexes, the surface of B56 subunits makes extensive interactions with the scaffold A-subunit and orients the B-subunit towards the active site of the catalytic subunit [20].

Interestingly, B56 subunits work together to regulate specific functions. Knockout of the *PPP2R5C* and *PPP2R5D* genes in mice demonstrates a strong functional association between these two regulatory subunits: only mice with both genes inactivated have fetal development arrested due as a result of cardiac development problems [50]. Of note, these proteins have the most related peptide sequences among the B56 family. This suggests that there is a level of functional redundancy between members of the B56 family, although further studies are needed to fully clarify this.

3.1. Post-translational modifications of the B56-subunits

B56 proteins can be directly modified by phosphorylation and nitrosylation, two post-translational modifications that highly impact the activity of the PP2A holoenzyme. As processes in cells are bidirectional, kinases can phosphorylate B56 subunits, modulating positively or negatively the activity of PP2A-B56. As an example, ERK phosphorylates the B56γ subunit at Ser327, which is a well-conserved residue between B56 family members, resulting in the dissociation of the B-subunit from the PP2A holoenzyme thereby reducing the amount of phosphatase activity available to counteract MAPK signaling [51]. B56α can also be phosphorylated at Ser41 by PKC, reducing PP2A enzymatic activity [52]. Conversely, PKA-mediated phosphorylation of B56δ enhances the phosphatase activity of the PP2A holoenzyme containing this B-subunit [53]. Phosphorylation of B56 subunits can be also linked with their specific localization. PKR phosphorylates B56α at Ser28, promoting the mitochondrial localization of the PP2A-B56α complex [54]. Moreover, nitrosylation of B-subunits inhibits phosphatase activity and induces conformational changes transmitted between the heterotrimer subunits that affect catalytic activity [8].

3.2. PP2A-B56 substrate recognition

Regulatory B-subunits recognize specific PP2A substrates. The B56-binding motifs that determine PP2A substrate specificity are short linear motifs (SLiMs). These motifs are found in the intrinsically disordered domains of the substrates, which are long and highly conserved regions present in essential proteins. In the last few years, the identification of these SLiM motifs has been useful for the identification of new PP2A B56 substrates. LxxIxE motifs (where x is any amino acid) were suggested as preferred docking site for the B56 subunits [36,55]. SLiMs can be modified in positions 1 and 4, altering the binding affinity and biasing the substrate recognition for distinct B56 subunits. For example, the Lxx[IVL]xE sequence motif presents higher affinity for B56α, [LMFI]xx[IVL]xE for B56γ, and [LM]xx[ILV]xE for B56ε holoenzymes [56]. Additionally, the presence of phosphorylated or acidic negatively charged residues such as aspartic and glutamic acids at positions 2, 7, 8, and 9 enhance B56 binding [55,57]. A list of B56 family substrates is provided in Table 1 along with their corresponding predictive SLiMs. Interestingly, it has been reported that some PP2A-B56 substrates present no SLiM sequences but are also directly dephosphorylated by PP2A-B56 complexes. In these cases, an adaptor or mediator protein, which contains the SLiM for PP2A-B56 recognition is needed. When the PP2A-B56 complex is bound, dephosphorylation of the mediator protein and of other bound proteins might occur [55]. These scaffold proteins act to both coordinate PP2A binding to its targets and help to direct PP2A holoenzyme activity. It was initially reported that cyclin G recruited PP2A to dephosphorylate MDM2, and this mechanism was confirmed with the identification of the PP2A-B56α holoenzyme as part of the Axin complex [58,59].

3.3. Direct substrates of the PP2A-B56 holoenzymes

Next, we will describe the main substrates of the PP2A-B56 holoenzymes and discuss the role of PP2A-containing B56 complexes in the regulation of signaling pathways frequently altered in cancer.

3.3.1. The ERK signaling cascade

ERK1 and ERK2 proteins are downstream components of the mitogen-activated protein kinase (MAPK) pathway that regulates cell proliferation, differentiation, and apoptosis. MAPKs are arranged according to the stimulus that activates them: ERK1/2 are activated mainly by mitogens, while JNK and p38 are activated by stress stimuli. Notably, PP2A complexes regulate all these signal transduction cascades [60,61], and mediate the crosstalk between them [62], indicating why specificity and regulation of the distinct B-subunits are of utmost significance.

We and others have demonstrated that ERK1/2 dephosphorylation at Thr202/Tyr204 is performed by the PP2A-B56α heterocomplex [63,64]. ERK1/2 dysregulation contributes to distinct human diseases, including cancer. When active, ERK1/2 phosphorylate several proteins such as MYC and MCL1, enhancing their stability. Accordingly, PP2A reactivation in cancer cells inactivates ERK1/2, promoting cancer cell death [63,64]. Interestingly, IER3 (IEX-1) binds to ERK and B56 subunits independently, enhancing B56 phosphorylation by ERK at a conserved Ser/Pro site, and triggering B56 subunits dissociation from the PP2A catalytic subunit [51]. This creates a positive regulatory loop where ERK may inhibit PP2A-B56 family function (Fig. 3). Of note, IER3 is overexpressed in KRAS-mutant pancreatic tumor cells inhibiting PP2A activity and sustaining ERK1/2 activation [65].

Although it has not been clarified yet which specific PP2A B-subunit regulates other members of the MAPK pathway, *in vitro* and *in vivo* studies show that MEK1/2 kinases are PP2A substrates inactivated by dephosphorylation [62,66]. Reactivation of the tumor suppressor activity of PP2A efficiently inhibits RAS-driven tumorigenesis and

Table 1
Direct substrates of the PP2A-B56 holoenzymes and their predicted SLiMs.

Pathway	Substrate	Residue	B56 subunit	SLiM	References
MAPK	ERK	Thr202	B56α	45-LGYIGE-50	[63,64]
	GSK3β	Ser9	B56δ	132-LDYVPE-137	[89,90]
	JNK		B56α and B56γ	86-ITALFE-91	[70]
	MAP4K3		B56ε	375-LKSVEE-380; 567-LNELHE-572	[87]
	MEK	Ser217 and Ser221		93-LQVLHE-98; 129-LTYLRE-134	[62,66,190]
PI3K/AKT	AKT	Thr308 and Ser 473	B56β, B56γ, and B55α	223-LCFVME-228	[76–78]
	p70S6K	Thr389		145-LYLILE-150; 396-LESVKE-401	[85,86]
	PTEN	Ser380, Thr382, and Thr383		-	[191,192]
	FOXK1		B56α, B56β, B56δ, and B56ε	1-MAEVGE-6; 457-LASVPE-462	[83]
Myc regulation	MYC	Ser62	B56α	99-LEMVTE-104	[59,98,193]
	PIM-1		B56β	116-FVLILE-121; 130-FDFITE-135; 148-FWQVLE-153	[94]
Apoptosis	BCL2	Ser70	B56α	130-FATVVE-135	[64,105,106]
	BCL-XL	Ser62		27-FSDVEE-32	[107]
	BAD	Ser112		1-MFGIPE-6	[103]
	FOXO3	Ser253		281-MQTIQE-286	[103]
Wnt/ β-catenin	Fam13a	Ser322		540-LQPIIE-545; 552-FKEIKE-557; 622-IPELLE-627; 626-LEHLQE-631	[120]
	β-Catenin	Ser33, Ser37, and Thr45	B55α	566-MEEIVE-571	[115–118]
DNA damage	p53	Thr55	B56γ	341-FRELNE-346	[121,123,124]
	BRCA2		B56α	1114-LSTILE-1119	[127]
	MDM2	Ser166 and Thr216		199-LCVIRE-204	[58]
	p300			1145-LSEVFE-1150; 1652-MCMLVE-1657	[125]
Hedgehog	GLI3		B56ε	68-LSKVSE-73; 94-LPHVAE-99; 741-LSAIDE-746; 1566-LTSLAE-1571	[130]
Others	E2F1	Ser364		170-ITNVLE-175; 210-LRGLG-214; 231-LRLLSE-235	[160]

synergizes with pharmaceutical agents targeting MEK1/2 [67,68]. Indeed, PP2A A-subunit mutations confer resistance to MEK inhibitors, pointing out the importance of PP2A in MEK/ERK regulation [69].

Interestingly, studies in ovarian and endometrial cancer establish that JNK signaling is over-activated when PP2A A-subunit mutations that impair the binding of all B56 family members, except B56δ are present [70]. Regarding p38, there is evidence about both tumor suppressor and tumor promoter functions depending on cellular context [71]. We have previously reported that p38β potentiates PP2A inactivation by two mechanisms: facilitating the cytoplasmic translocation of SET through CK2 phosphorylation, and directly binding to and stabilizing the SET oncoprotein in the cytoplasm [38]. In contrast, after NOX4 activation or apoptosis induction, p38 mediates PP2A C-subunit activation, promoting MEK1/2 and ERK1/2 inactivation and contributing to the induction of apoptosis [72]. Further research is needed to determine p38 kinase cell type and cell context-specific functions.

Notably, the B55 family has also a significant role positive and negatively regulating MEK-ERK pathway [7]. PP2A-B55 dephosphorylates KSR1, the kinase suppressor of Ras 1, upon growth factor stimulation activating the MAPK cascade [73]. Contrary, PP2A-B55 can also dephosphorylate MEKK3 on its Ser526 inhibiting this kinase [74]. This points out the complex interplay between the different B-subunit families.

3.3.2. *The AKT pathway*

PI3K/AKT axis forms a key component of many signaling pathways that regulate a wide variety of cellular functions including cell proliferation, survival, metabolism, and angiogenesis in both normal and malignant cells. Mutations in the PI3K subunit genes and PTEN deletions represent some of the most common mutations in multiple types of cancers. The PI3K/AKT signaling pathway is activated in response to stimuli such as insulin, growth factors, or cytokines. Interestingly, lack of PP2A-C Leu309 methylation, and consequently, fewer B56- and B55-PP2A complexes, favor enhanced cell transformation due to AKT activation by phosphorylation of its Thr308 and Ser473 residues [75]. PP2A-B55 directly dephosphorylates Thr308 and inactivates AKT, resulting in the inhibition of cell growth and survival [76]. However, PP2A-B56 complexes can also dephosphorylate AKT at Thr308 and Ser473 (Fig. 4) [77,78]. The specific B56 subunits involved in these dephosphorylation events seem to be context-dependent: PP2A-B56γ regulates both AKT phosphorylation sites in hepatocellular carcinoma [79], while PP2A-B56β removes the phosphate groups upon insulin-induced response [80,81].

AKT proteins also participate in the regulation of the mammalian target of rapamycin complex (mTORC) signaling pathway, which is involved in the regulation of metabolism regulation and whose function is dysregulated in many cancers. AMPK, a cellular energy sensor conserved in all eukaryotic cells, is able to inhibit AKT and consequently, the mTOR pathway. AMPK phosphorylates B56γ at Ser298 and Ser336, enhancing its activity against AKT in breast cancer [82]. Moreover, activated mTORC1 induces PP2A-mediated dephosphorylation of the transcription factor FOXK1, which regulates the expression of multiple genes associated with glycolysis and downstream anabolic pathways [83]. This dephosphorylation event can be carried out by B56α, B56β, B56δ, and B56ε, but not B56γ whose phosphorylation depends on AMPK [84]. Regarding mTORC1 substrate S6K, its two isoforms presented in mammalian cells, p85S6K and p70S6K, are regulated by B56 subunits at residues Thr412 and Thr389, respectively [85,86].

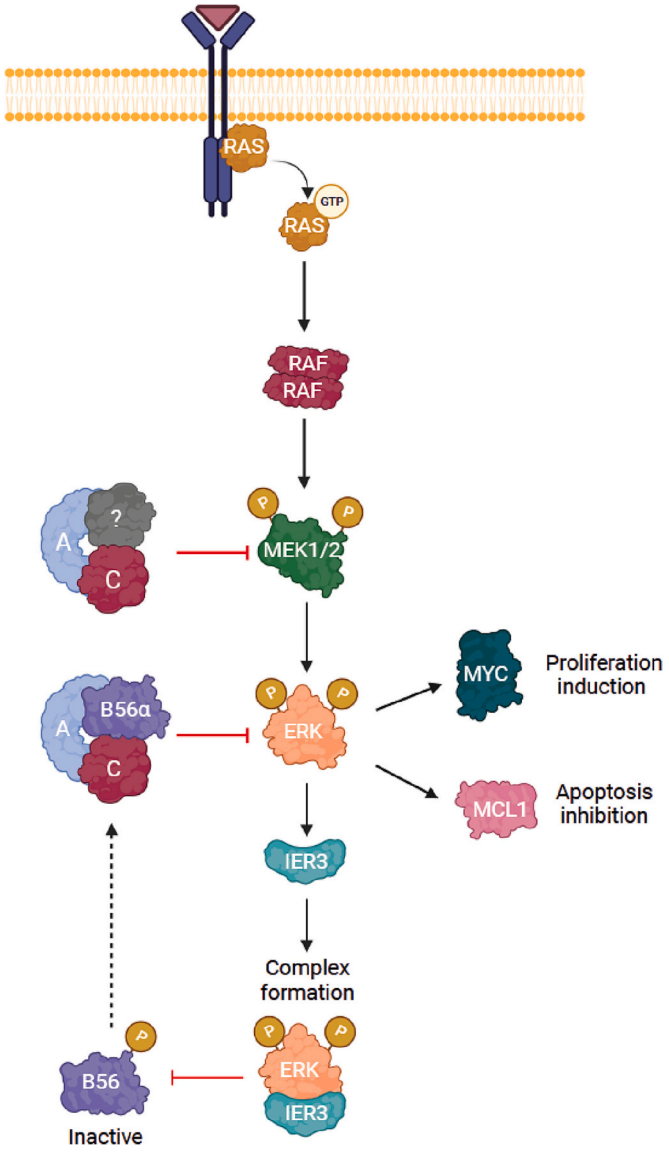

Fig. 3. Role of PP2A-B56 in the modulation of the MAPK/ERK pathway. RAS is activated by transmembrane receptors and promotes RAF dimerization and activation. Active RAF phosphorylates MEK1/2 at Ser217 and Ser221, fostering its activation. On the contrary, PP2A can inactivate MEK1/2 [62,66]. MEK1/2 is able to phosphorylate ERK at Thr202 and Tyr204 resulting in ERK activation. ERK enhances MYC and MCL1 stability by phosphorylation. ERK is a PP2A-B56α substrate and its dephosphorylation at Thr202/Tyr204 prevents its kinase activity [51,63,64]. ERK phosphorylates IER3 at Thr18 and forms a complex with it, IER3 binds B56 subunits and then ERK can phosphorylate them at Ser327 promoting the dissociation of the PP2A complex [51]. Black arrows indicate activation, red arrows denote inhibition, and dotted arrows designate a B-subunit, which when active, binds to the AC-dimer (Figure created in BioRender.com).

Nevertheless, some of these studies have been performed using *Drosophila* models. *Drosophila* has only one isoform belonging to B56 family, impeding our ability to identify which member of the B56 family carries out each dephosphorylation event. This has to be further explored in mammalian systems.

PP2A-B56 holoenzymes are also involved in the crosstalk between the PI3K/AKT/mTORC and MAPK signaling pathways. Namely, PP2A-B56ε acts as a negative regulator of MAP4K3, mediating its ability to signal to mTORC1 during amino acid withdrawal [87]. Moreover, both ERK and AKT can phosphorylate and inactivate GSK3β, while PP2A-B56δ activates it [88–90].

3.3.3. The MYC oncoprotein

The MYC protein is one of the best-characterized PP2A substrates. MYC is a master transcription factor that regulates a wide spectrum of target genes related to proliferation, differentiation, and metabolism; thus, the expression of MYC is tightly controlled in normal cells. However, the aberrant activation of MYC is one of the most common events in solid and hematopoietic neoplasias, being associated with aggressive forms of cancers, poor prognosis, and treatment resistance [91].

MYC is a short half-live protein; thus, its post-transcriptional regulation plays an essential role in its stability and function (Fig. 5). PP2A-B56 holoenzymes not only regulate MYC in a direct way but also modulate the activity of kinases involved in MYC phosphorylation. Two interdependent phosphorylation sites are critical for MYC regulation: Ser62 and Thr58 [92]. When ERK is activated by MEK phosphorylation, it forms dimers and translocates to the nucleus where it phosphorylates MYC at Ser62, stabilizing and activating it, and promoting the formation of dimers with MAX. These, p-MYC-MAX dimers bind to E-box sequences in the regulatory regions of multiple genes promoting their expression. As mentioned above, PP2A-B56α complexes inhibit ERK [51,64,77]. PIM-1 is a highly conserved Ser/Thr protein kinase that also stabilizes MYC through phosphorylation at Ser62 [93]. Interestingly, PIM-1 is a PP2A-B56β substrate and its PP2A-dependent dephosphorylation decreases its stability. Indeed, B56β knockdown increases PIM-1 protein half-life and reduces its ubiquitination [94]. Furthermore, MYC Ser62 can also be phosphorylated by CDKs.

Phosphorylation of Ser62 also primes MYC for GSK3β-mediated phosphorylation at Thr58, which initiates MYC turnover. To be active, GSK3β has to be dephosphorylated by PP2A-B56δ complex at Ser9 [95]. It has been reported that *Ppp2r5d* (B56δ) knockout mice are predisposed to spontaneous tumor development, and RNA sequencing analysis revealed MYC activation in this model [96]. This confirms that uncontrolled MYC activity due to B56δ inactivation is a tumor-predisposing factor. In fact, MYC participates in the regulation of its own half-life through direct binding and transcriptional activation of *PPP2R5D* gene [97]. Dual phosphorylation of MYC (Ser62 and Thr58) allows a PIN1-mediated Pro63 isomerization step to facilitate the direct interaction of PP2A-B56α with the N-terminal transactivation domain of MYC, which contains the Ser62 residue, thereby driving its dephosphorylation [98]. Dephosphorylation of Ser62 marks MYC for ubiquitin-mediated proteasomal degradation. To facilitate coordinated MYC degradation, the scaffold protein Axin1 mediates the formation of a complex containing MYC, GSK3β, PIN1, and PP2A-B56α (Fig. 5) [59].

Recently, several groups confirmed the role of PP2A-B56 holoenzymes in the regulation of MYC using specific pharmacological approaches in different models such as engineered MYC overexpressing lung cancer or non-small cell lung cancer (NSCLC) xenografts [23]. Interestingly, MYC also regulates PP2A function. When MYC is in an active form it can promote the expression of CIP2A and SET, two well-defined endogenous inhibitors of PP2A [41,99,100].

3.3.4. The BCL2 family of pro-apoptotic and anti-apoptotic proteins

Resisting cell death is one of the core hallmarks of cancer, tumor cells must be able to avoid apoptosis because programmed death is a natural barrier against tumorigenesis. How cancer cells evade apoptosis varies greatly by the type of cancer and even within the same kind of cancer. The mechanisms used most by tumor cells to counteract the pro-apoptotic chain of events are overexpression of anti-apoptotic proteins such as BCL2, MCL1, and BCL-XL; downregulation or inactivation of pro-apoptotic proteins such as BIM, BID, BAX, PUMA, and NOXA; or inactivation of the BAX and BAK pore-forming proteins [101–104]. The stability or activation of these proteins is regulated by post-translational modifications such as phosphorylation. PP2A-B56 complexes participate in the regulation of the mitochondrial intrinsic apoptosis pathway with a pro-apoptotic role (Fig. 6).

Regarding the anti-apoptotic proteins, BCL2 is inactivated after Ser70 dephosphorylation by the PP2A-B56α heterocomplex, increasing

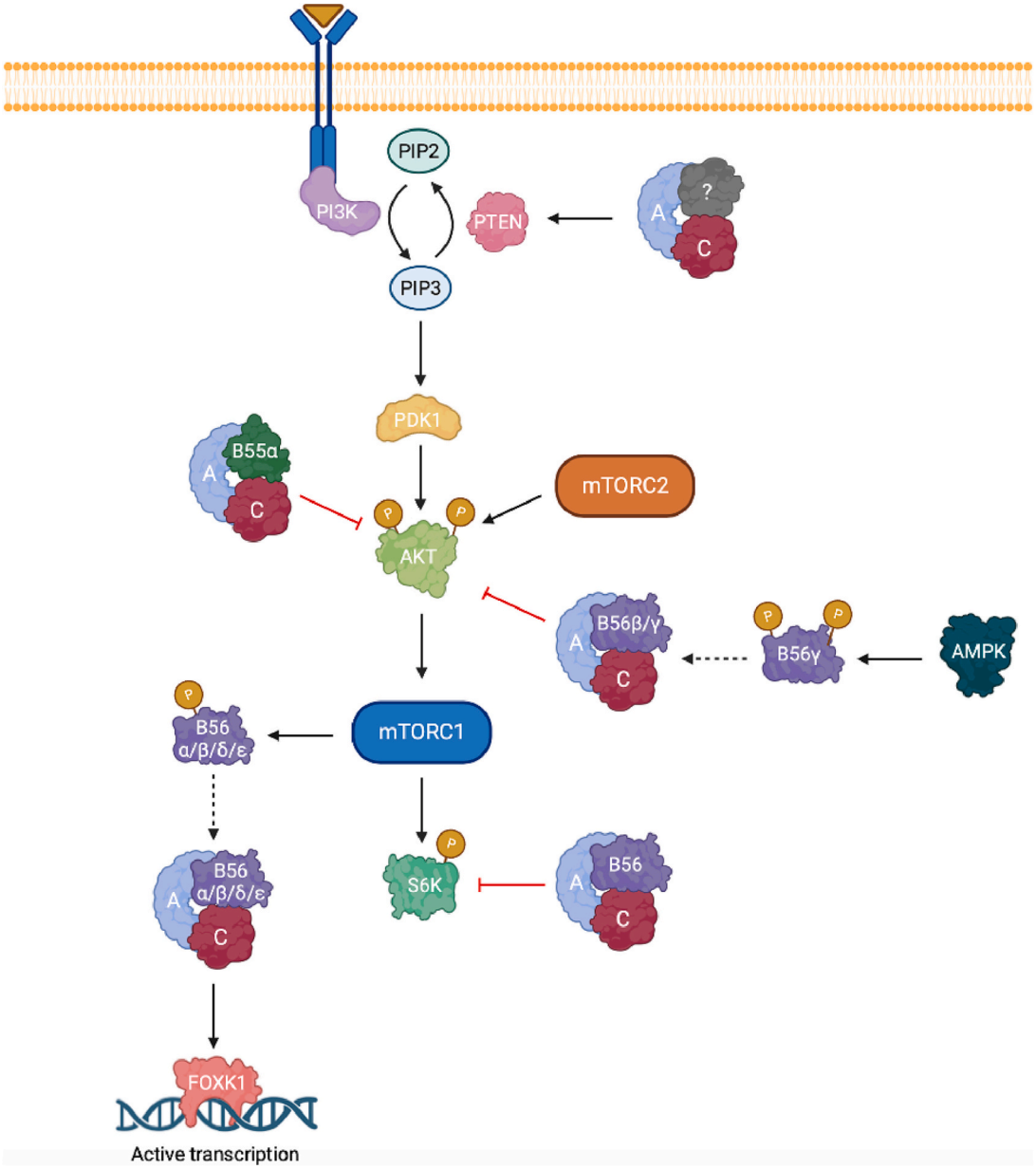

Fig. 4. PP2A-B56 regulation of PI3K/AKT/mTOR pathway. PI3K activation promotes the transition from PIP2 to PIP3 at the cell membrane. PIP3 induces the activation of PDK1 which in the last term activates AKT. The first step of negative regulation of the AKT pathway is through PTEN, a protein that catalyzes the contrary reaction of PI3K. Phosphorylation of PTEN at Ser380, Thr382, and Thr383 residues negatively regulates its activity and stability. Of importance, PTEN dephosphorylation and therefore its activation is PP2A dependent [189]. MTORC2 can also activate AKT after Thr308 and Ser473 phosphorylation. Opposing, PP2A-B55α and PP2A-B56β/γ dephosphorylate these residues [77,78]. Interestingly, the B56γ subunit is enhanced by AMPK phosphorylation [82]. AKT induces mTORC1 and subsequent S6K activation. S6K is a PP2A-B56 substrate [85,86]. Besides, mTORC1 phosphorylates B56α/β/δ/ε subunits enhancing the PP2A-mediated activation of the transcription factor FOXK1 [83,84]. Black arrows indicate activation, red arrows denote inhibition, and dotted arrows designate a B-subunit which when active binds to AC-dimer (Figure created in BioRender.com).

its association with p53 and enhancing the action of the BCL2-specific inhibitor venetoclax in hematological neoplasias [64,105,106]. Additionally, PP2A dephosphorylates BCL-XL at Ser62 enhancing its inactivation in retinal pigment epithelial cells [107], although more studies are needed to know which B-subunit is responsible for this observation.

Moreover, phosphorylation regulates MCL1 stability. MCL1 possesses many potential phosphorylation sites due to the presence of a large regulatory region with PEST motifs. Nevertheless, most of these sites are not characterized well enough to create a holistic picture of the regulation of MCL1 by phosphorylation [102]. Of importance, ERK and GSK3β, the main kinases responsible for MCL1 phosphorylation, are regulated by PP2A. ERK phosphorylates MCL1 at Thr92 and Thr163 enhancing its activation and stabilization through the avoidance of its proteasome degradation. GSK3β phosphorylates Ser155 and Ser159 inactivating the anti-apoptotic functions of MCL1 and promoting its ubiquitination and subsequent degradation [108]. Dephosphorylation of MCL1 has not been well studied yet, although Wertz, et al. demonstrated that during mitotic arrest MCL1 is associated with PP2A, and PP2A-B56 has been associated with the cell cycle protein Fam72a to modulate MCL1 phosphorylation during the G2/M phase of the cell cycle [109,110]. Taking together, PP2A-B56α and PP2A-B56δ complexes are able to inhibit MCL1 activity in cancer cells through ERK and GSK3β dephosphorylation respectively [64,89,90].

Additionally, PP2A dephosphorylates the pro-apoptotic protein BAD

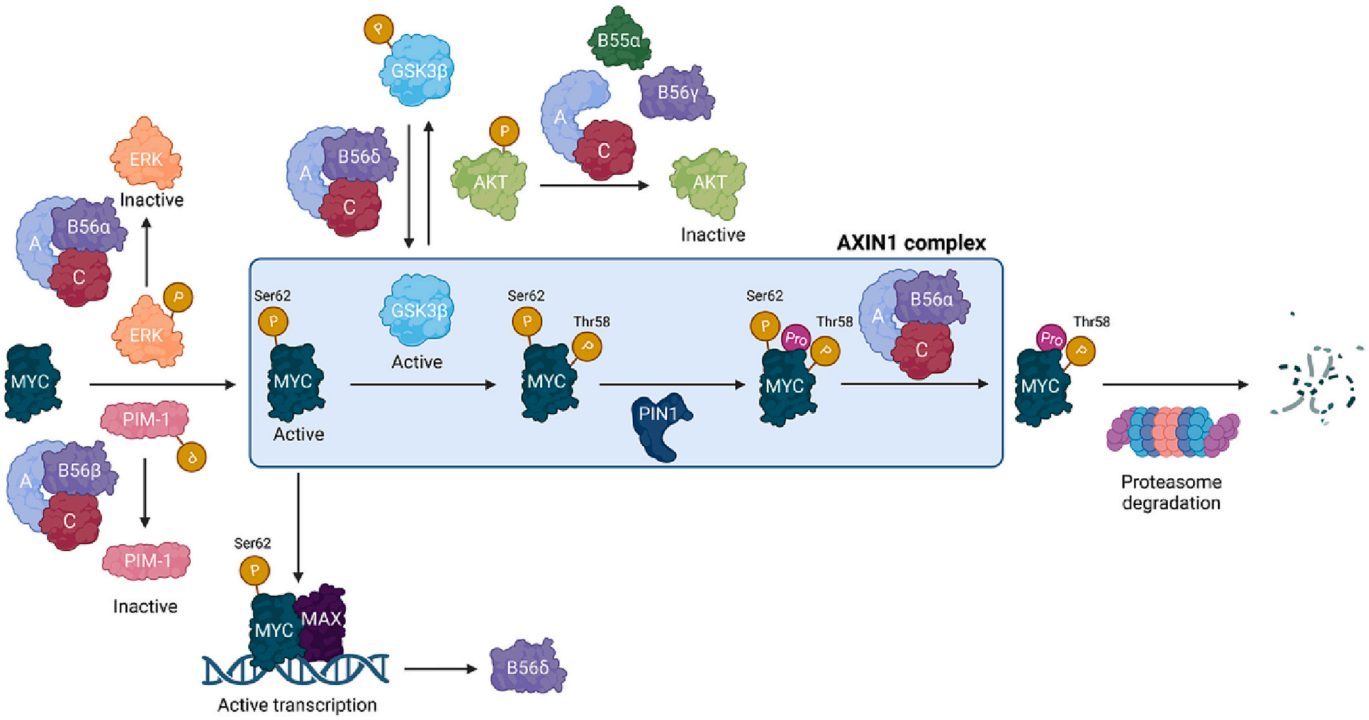

Fig. 5. Functions of PP2A-B56 in the regulation of the MYC oncoprotein. Activation of ERK or PIM-1 kinases lead to the phosphorylation of MYC at Ser62 which activates and stabilizes the protein. PP2A-B56α and PP2A-B56β complexes inhibit ERK and PIM-1, respectively, by dephosphorylation. Active MYC forms dimers with MAX and promotes the transcription of many genes, one of which is *PPP2R5D*, a gene coding for the B56δ subunit. Phosphorylation of Ser62 also primes MYC for GSK3β phosphorylation at Thr58, which initiates MYC turnover. PP2A-B56δ complex dephosphorylates and activates GSK3β, which can be inactivated by AKT, a kinase controlled by PP2A-B55α and PP2A-B56γ complexes. Dually phosphorylated MYC allows PIN1-mediated Pro63 isomerization step which enhances PP2A-B56α-mediated MYC dephosphorylation at Ser62. Thr58 p-MYC is ubiquitinated and degraded in the proteasome. The scaffold protein Axin1 coordinates this process binding MYC, GSK3β, PIN1, and PP2A-B56α (Figure created in BioRender.com).

at Ser112 and the transcription factor FoxO3A at Ser253, which enhances the expression of other pro-apoptotic proteins such as BIM [103]. Both of these proteins, when hyperphosphorylated, are sequestered in the cytoplasm by 14-3-3 proteins, resulting in the inhibition of their function. Their phosphorylation has been attributed to AKT and p38, which are kinases also regulated by the PP2A-B56 family [111,112].

3.3.5. *The β-catenin 1 protein*

The Wnt/β-catenin pathway controls differentiation, stemness, and motility of cells, and is critical for stem cell maintenance and cellular proliferation of leukemic stem cells (LSC) [113]. Importantly, Wnt/β-catenin signaling deregulation is often observed in human malignancies. PP2A-B56 complexes are important regulators of this pathway at multiple levels in a tissue-dependent manner. In the canonical Wnt/β-catenin cascade, the absence of the Wnt ligand allows phosphorylated β-catenin to form a complex composed of the scaffold protein Axin, APC, and the kinases CK1α and GSK3β. Phosphorylated β-catenin is primed for ubiquitination and proteasomal degradation [114]. However, when the Wnt ligand binds to its receptor, dephosphorylation of β-catenin is promoted and then, the PP2A holoenzymes containing B56α and B56ε form part of the complex coordinated by Axin, which furnishes necessary interactions [115,116]. Furthermore, PP2A-B56γ has also been implicated in the regulation of β-catenin during development [117,118]. PP1 also forms part of the complex dephosphorylating Axin and impairing its interaction with GSK3 when Wnt ligand is present [119]. Regarding upstream regulation, Fam13a is frequently altered in lung diseases, including chronic obstructive pulmonary disease, asthma, lung cancer, and pulmonary fibrosis. Fam13a Ser322 phosphorylation, which acts as a molecular switch to control its subcellular distribution, is carried out by AKT and removed by PP2A-B56ε. When Fam13a is dephosphorylated, it shuffles to the nucleus where activates Wnt signaling [120]. Taking together, the modulation of the Wnt/β-catenin cascade in cancer stem cells can promote their differentiation and sensitize them to cancer therapies.

3.3.6. *The TP53 tumor suppressor*

The function of PP2A-B56γ in the regulation of cell cycle progression is crucial and one mechanism through which this heterotrimer regulates these processes is through the modulation of p53 phosphorylation [121]. *TP53* encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. The encoded protein responds to diverse cellular stresses to regulate the expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. Mutations in this gene are associated with a variety of human cancers and are often associated with poor prognosis. Interestingly, the p53 transcription factor regulates the expression of several pro-apoptotic proteins such as BAX, PUMA, and NOXA [122]. DNA damage induces phosphorylation of p53 at Ser15 by ATM [121]. This modification enhances PP2A-B56γ complex assembly and its association with p53, triggering Thr55 dephosphorylation of p53 [123]. Two of the three splice variants of B56γ, B56γ1 and B56γ3, dephosphorylate p53 at Thr55, an event that stabilizes p53 promoting apoptosis [124]. B56γ3 also promotes the degradation of the transcriptional coactivator p300, which acetylates p53, increasing its transcriptional activity [125]. Moreover, ATM activity is inhibited by PP2A-B55α mediated dephosphorylation [126].

Another important substrate of PP2A-B56 phosphatase complexes involved in DNA damage response and cancer is the protein BRCA2, which plays a central role in homologous recombination. BRCA2 presents a SLiM motif that allows for its binding to the PP2A-B56α heterotrimer. Indeed, phosphorylation of surrounding sites of BRCA2 LxxlxE motif by ATM and ATR kinases stimulates the formation of the PP2A-

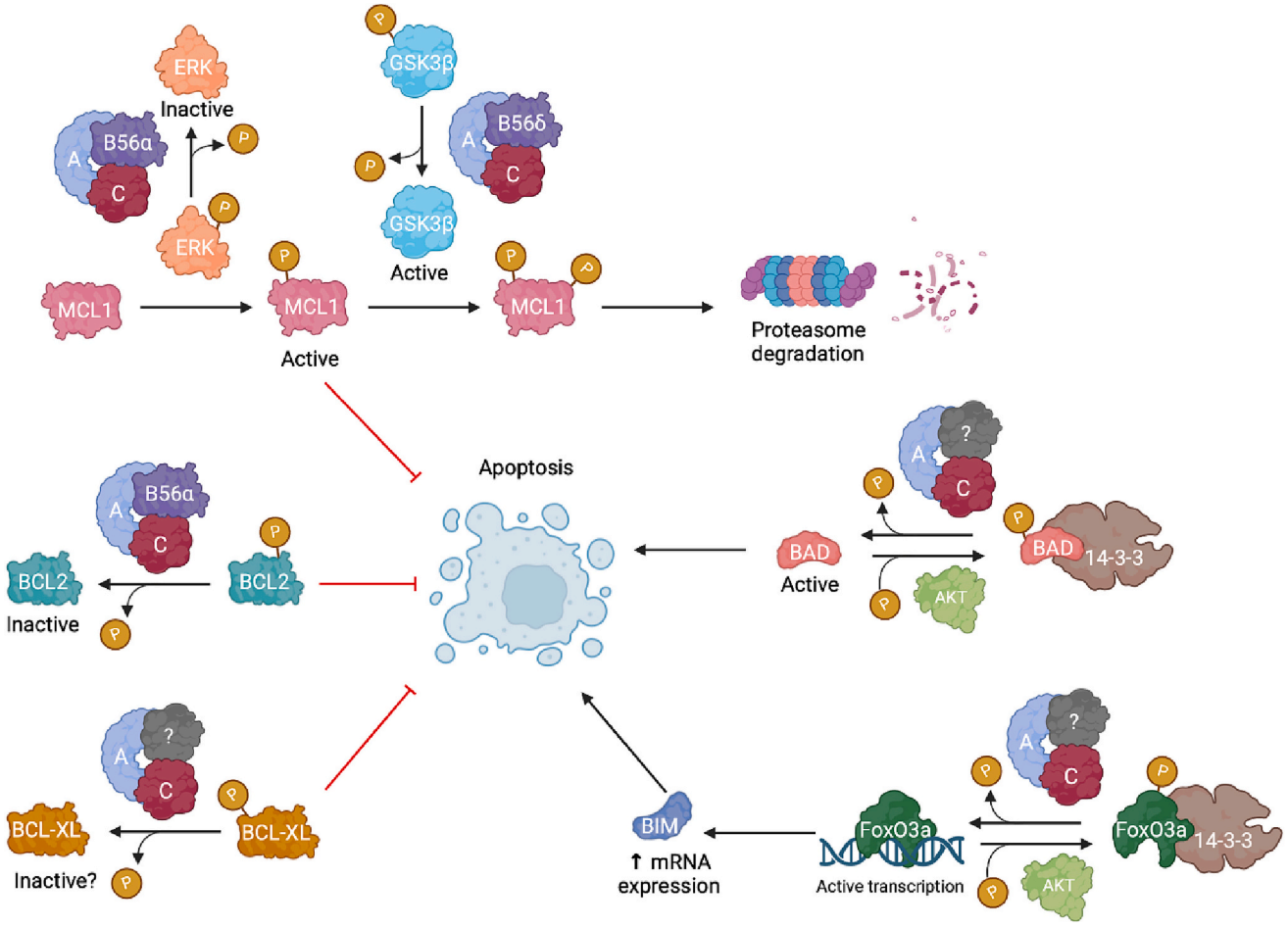

Fig. 6. Role of PP2A-B56 in triggering apoptosis. Regulation of anti-apoptotic proteins: 1) PP2A-B56α and PP2A-B56δ holoenzymes dephosphorylate ERK and GSK3β, the main kinases responsible for MCL1 phosphorylation. MCL1 phosphorylation at Thr163 by ERK enhances MCL1 protein activity and stability, while phosphorylation at Ser159 by GSK3β inactivates MCL1 and promotes its ubiquitination and subsequent degradation. Importantly, MCL1 Thr163 phosphorylation primes this protein for GSK3β phosphorylation. 2) PP2A-B56α dephosphorylates Ser70 p-BCL2 and decreases its pro-survival activity. 3) PP2A dephosphorylates Ser62 p-BCL-XL. Regulation of pro-apoptotic proteins: BAD is directly dephosphorylated by PP2A at Ser112, and BIM is expressed after activation of its transcription factor FoxO3a by PP2A-dependant dephosphorylation. When BAD and FoxO3a are phosphorylated, they are sequestered in the cytoplasm by 14-3-3 proteins. AKT phosphorylates BAD and FoxO3a (Figure created in BioRender.com).

B56α-BRCA2 complex [127]. This complex is necessary for appropriate homologous recombination repair.

3.3.7. The GLI3 protein in the Hedgehog pathway

The Hedgehog pathway is an evolutionarily conserved developmental pathway that is involved in tumorigenesis. Its main effectors are the transcription factors GLI1, GLI2, and GLI3. GLI proteins have to be phosphorylated by kinases such as Kif7, PKA, or cAMP to be translocated to the nucleus. Several studies have demonstrated an essential role for the PP2A-B56ε heterotrimer in this pathway since this complex inhibits Hedgehog signaling by dephosphorylating GLI proteins [128,129]. Furthermore, PP2A antagonizes the action of mTORC1, which activates GLI3. The inactivation of mTORC1 results in GLI3 cytosolic retention and prevents the transcription of genes involved in cell growth and proliferation. This highlights the diversity of PP2A-B56 heterocomplexes tumor suppressor roles once again [130].

All functions of PP2A-B56 holoenzymes are not mentioned here, such as their remarkable role in regulating cell cycle checkpoints and their recently described role in the regulation of Hippo-Yorkie signaling in *Drosophila* [34,131–133]. Further studies are needed to discover the role of these heterotrimers in the modulation of other pathways and substrates. Interestingly, recent phosphoproteomic analyses have revealed a large number of cancer-relevant PP2A-B55 and PP2A-B56 targets [36,134].

4. PP2A-B56 deregulation in cancer

Deregulation and dysfunction of PP2A in disorders such as cancer, neurodegenerative syndromes, and diabetes have broadened our understanding of the role of PP2A in health and disease. The first studies on the role of PP2A as a tumor suppressor came from studies demonstrating that okadaic acid-mediated inhibition of PP2A caused tumors in mice and the observation that viral proteins such as the adenovirus E4orf4, polyomavirus small and middle T antigens, and the SV40 small T antigen were oncogenic [135–137]. These viral proteins function by displacing B-subunits from the PP2A heterocomplexes, leading to altered PP2A activity [11,138]. PP2A inactivation occurs in several solid and hematological tumors, leading to the acquisition of many of cancer hallmarks such as sustained proliferative signaling and cell death resistance. In this review, we focus on the specific inhibition of complexes containing B56 subunits and how they contribute to PP2A inhibition-mediated tumorigenesis [139]. Furthermore, as indicated above, the important role of direct substrates of PP2A-B56 heterocomplexes in cancer highlights the importance of these holoenzymes in this heterogeneous disease and their potential role as biomarkers.

Current evidence based upon large-scale cancer genomic sequencing

efforts showed that PP2A inactivation in cancer is largely a result of non-genetic mechanisms [10,140]. In fact, the frequency of inactivating mutations in PP2A genes is low, with the *PPP2R1A* A-subunit gene showing the highest mutation rate: 1.17% across 9,759 samples of diverse human cancer types at diagnosis [21]. Interestingly, the recurrent pathologic mutations in the scaffold subunit occur along its B-subunit binding interface, and mutations in the residues P179, R183, S256, and R258 result in marked changes in the PP2A holoenzyme composition, impairing the binding of specific B-subunits [69,141,142].

A wide range of different non-genetic mechanisms is responsible for PP2A inactivation and holoenzyme disassembly, illustrating the complexity of PP2A regulation and signaling in each type of cancer cell. Cancer cells generally evade PP2A-mediated tumor suppression in three ways: by altering the expression of PP2A post-translational modifier proteins such as PME-1, LCMT-1, or PTPA [143–145]; by aberrant overexpression of PP2A endogenous inhibitors such as SET or CIP2A [41,146,147]; or by downregulating the expression of specific PP2A subunits [48,148,149]. Here will focus on the most common and well-studied alterations.

As indicated above, PME-1 reduces PP2A tumor suppressor activity through C-subunit demethylation [32]. PME-1 overexpression is a common event in endometrial cancer, glioblastoma, and primary T-cell acute lymphoblastic leukemia (T-ALL) cells, which prevents the binding of methyl-sensitive PP2A B-subunits to the core enzyme [150,151]. In glioma, it has been associated with therapy resistance [152]. However, the most common mechanism by which PP2A is inactivated in cancer is the overexpression of endogenous inhibitors. High expression of the SET oncoprotein has been frequently detected and associated with poor prognosis in a large variety of both solid (breast, NSCLC, pancreatic, and metastatic colorectal cancers) and hematological tumors (acute myeloid and chronic lymphocytic leukemia) [153–156]. Interestingly, SET has been associated with B56α in gastric cancer [40], indicating the possibility of specific inhibition of this heterocomplex in tumor cells. In acute myeloid leukemia (AML), SET is overexpressed in ~30% of the cases and it is associated with poor outcomes [157].

High CIP2A expression predicts poor patient prognosis in several human cancer types [158]. Specifically, CIP2A impairs PP2A-B56α activity leading to the stabilization of MYC [159]. Similarly, CIP2A stabilizes E2F1 by preventing Ser364 PP2A-B56-dependent dephosphorylation and induces hyperactivation of AKT by inhibiting the dephosphorylation of Ser473 [160,161]. CIP2A is widely overexpressed in human cancers including gastric, bladder, ovarian, tongue, hepatocellular, colon, NSCLC, AML, and chronic myeloid leukemia [158]. CIP2A overexpression in lung tumors enhances JNK activity and in AML, is a recurrent event associated with a poor prognosis [146,162].

C-KIT mutations have been associated with downregulation of B55α, B56α, B56γ, and B56δ in AML [148,163]. In these models, suppression of B56γ expression contributes to the transformation of human cells, since in G2 cell cycle stage PP2A-B56γ modulates endogenous RAS signaling and p53 function [123,133,164]. Furthermore, *PPP2R5E* mRNA and B56ε protein expressions are downregulated in ~60% of AML cases, respectively, and correlate with p53 levels, suggesting that the molecular effects of this B-subunit could occur via the modulation of p53 [149]. Moreover, recent studies have pointed out that low *PPP2R5A* (B56α) and *PPP2R5B* (B56β) expression are associated with poor prognosis in AML and hepatocellular carcinoma, respectively [64,165].

Altogether, a wide range of different mechanisms inactivates PP2A, illustrating the complexity of PP2A regulation and signaling in each type of cancer cell.

5. Perspectives of PP2A targeting in cancer

The discovery of PP2A as a tumor suppressor prompted the evaluation of the safety and efficacy of compounds which can restore PP2A activity. PP2A targeting has been difficult to achieve due to its complexity and wide range of different heterotrimers; therefore, its indirect reactivation has been proposed as the most effective strategy [14]. Several molecules targeting endogenous inhibitors of PP2A, such as FTY720 and OP449, have already been characterized [166].

FTY720 is an oral sphingosine analog approved by the FDA for the treatment of patients with relapsing multiple sclerosis and for the prophylaxis of solid organ transplantation rejection [167]. FTY720 displays anti-cancer activity by interacting with SET and consequently, indirectly reactivating PP2A [100,168–170]. Despite its proven efficacy and selectivity, FTY720 has not been re-purposed as an anti-tumor agent, partly due to its toxicity at the elevated anti-neoplastic dose required. In addition, the interaction of FTY720 with S1P receptors is sufficient to induce cardiotoxicity in mice and humans [171]. Importantly, re-activation of PP2A by FTY720 does not require its phosphorylation or S1P receptor interaction; therefore, efforts have been invested in the development of non-phosphorylable FTY720 analogs. This is the case of CM-1231, a small molecule that is safer and more effective than FTY720. CM-1231 also reactivates PP2A by disrupting the SET-PP2A interaction with a greater efficiency than FTY720, and does not demonstrate cardiotoxicity in zebrafish embryos [172]. Another non-phosphorylable analog is OSU-2S, which has also demonstrated antitumor effects in hematological neoplasias [173]. In AML, PP2A activation upon OSU-2S treatment decreases LSC population and increases leukemic blast maturation through the modulation of the PP2A/c-MYC/p21 axis [174]. Another molecule developed to activate PP2A in transformed cells is the small peptide OP449. OP449 treatment suppresses growth, enhances apoptosis, and impairs clonogenicity in AML, breast cancer, and neuroblastoma as well as in other tumor types [100,175,176].

On the other hand, the deeper knowledge about the PP2A heterocomplex structure, formation, and function, has allowed for the development of a new class of small molecule activators of PP2A (SMAPs). SMAPs are able to stabilize specific PP2A holoenzymes. These molecules hold tremendous potential within the field of cancer, not only for their translational potential but also as tools to determine new functions and substrates of specific B-subunits. DT-061, a highly optimized SMAP, specifically stabilizes the PP2A-B56α complex in an assembled and active state, whereas the binding of other regulatory subunits is either decreased or unchanged [17]. DT-061 specificity has been confirmed in AML cell lines lacking B56α subunit expression [64]. This implies that mechanistically, this class of SMAPs selectively stabilizes specific PP2A holoenzymes through their ability to bind a unique interfacial drug pocket formed where the three PP2A subunits come together [177]. DT-061 has shown its efficacy *in vitro* and *in vivo* in Burkitt lymphoma, breast cancer, AML, CLL, distinct types of lung cancer, hepatocellular carcinoma, pancreatic ductal adenocarcinoma, glioblastoma, and prostate cancer [46,63,64,68,178–182]. Of note, MYC inactivation in MYC-driven tumors can lead to faster tumor regression as a result of the dependency of these cells on MYC. Interestingly, DT-061 stabilizes the PP2A-B56α specific holoenzyme allowing MYC dephosphorylation resulting in its proteasomal degradation [178].

As PP2A holoenzymes regulate a countless variety of signaling pathways, an interesting approach to consider is the combination of PP2A activator drugs with other cancer treatments or even different strategies to activate PP2A at the same time [183]. To this end, our group showed that FTY720, CM-1231, and DT-061 combined with venetoclax and venetoclax-azacitidine treatments have synergistic effects in *in vitro* and *in vivo* AML models, confirming that PP2A activators might be used to improve the clinical effects of the standard-of-care therapy in high-risk AML patients. This combination has also been effective in diffuse large B-cell lymphoma and in T-ALL where instead of venetoclax, a BCL-XL specific inhibitor was used [64]. Moreover, combinations of either FTY720 or OP449 plus tyrosine kinases inhibitors showed promising results in T-ALL and AML models [184,185]. In addition, OSU-2S synergistically boosts the antiproliferative effects of sorafenib in hepatocellular carcinoma cells [186]. Additionally, SMAPs have been shown to have significant synergistic activity when combined with MEK inhibitors in K-RAS mutant lung cancer and with gilteritinib in

FLT3-mutated AML [68,187]. In both scenarios, the synergy mechanism results in MYC degradation and AKT inactivation. These small PP2A modulators have also been combined with CDK9 inhibitors in MLL-rearranged AML and solid tumors, revealing an important synergistic relationship as a result of PP2A's interactions with the INTAC complex [188].

Collectively, these findings open many new avenues to translate these novel PP2A activation strategies to the clinic and improve the therapeutic options available to cancer patients.

6. Concluding remarks

Reversible phosphorylation of proteins is a post-translational modification that regulates all aspects of life through the antagonistic action of kinases and phosphatases. Although the number of genes codifying for kinases (>500) is far larger than phosphatases (<200) [3,4], the structural complexity of the phosphatase families allows for a single catalytic subunit to be part of hundreds of structurally distinct holoenzymes and dephosphorylate target substrates with exquisite selectivity. The key to the accuracy of substrate recognition by PP2A is provided by the different regulatory B subunits, which determine the substrate specificity and the subcellular localization of the heterotrimers. Therefore, it is essential to understand the function and regulation of individual PP2A B-subunits [7,8,42]. In this review we have summarized the regulation and known effects of specific PP2A-B56 holoenzymes and their roles in cancer.

PP2A-B56 heterotrimers are tumor suppressors that play essential roles in cellular homeostasis by controlling the regulation of major signaling pathways. Through the upregulation of several protein kinases involved in mitogenic and survival signaling (e.g. ERK and AKT), the stabilization of oncoproteins (e.g. MYC), the destabilization of tumor suppressors (e.g. p53), or the regulation of anti-apoptotic proteins (e.g. MCL1), dysregulation of specific PP2A-B56 holoenzymes are critical determinants and drivers of cell transformation. Furthermore, since PP2A is a major antagonist of kinase activity and its deregulation in solid and hematological tumors is very common, a deeper understanding of the function and regulation of individual PP2A heterocomplexes has facilitated the development of new therapeutic approaches in cancer [54]. In this regard, we must highlight the advances in the development of SMAPs, small molecules capable of selectively stabilizing individual PP2A heterotrimers in a rational and context-dependent manner [17,46,63,64,68,177–181]. As noted above, several drug combinations that include PP2A modulators have already been successful *in vitro* and *in vivo* models of cancer. Furthermore, the synergistic effects of these combinations are associated with the suppression of key pathways not only for cancer cell survival but also for therapeutic resistance, supporting the possible near-term clinical translation of these approaches for the treatment of a wide range of human cancers [64,69].

Ethics approval and consent to participate

Not applicable.

Consent of publication

All listed authors have read and approved this manuscript.

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Authors' contributions

I.P. and M.D.O. conceived the review and wrote the manuscript. I.P and S.R-M. prepared the figures and tables. I.P., S.R-M., C.V., G.N., and M.D.O. revised the manuscript. All authors approved this manuscript.

Declaration of Competing Interest

G. Narla is chief scientific officer at RAPPTA Therapeutics, is a SAB member at Hera BioLabs, reports receiving commercial research support from RAPPTA Therapeutics, and has ownership interest (including patents) in RAPPTA Therapeutics, an asset development company developing small molecule modulators of PP2A.

Data availability

No data was used for the research described in the article.

Acknowledgments

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbcan.2023.188953.

References

[1] A. Bononi, C. Agnoletto, E. De Marchi, S. Marchi, S. Patergnani, M. Bonora, et al., Protein kinases and phosphatases in the control of cell fate, Enzyme Res. 2011 (2011), https://doi.org/10.4061/2011/329098.
[2] T. Hunter, Protein kinases and phosphatases: the Yin and Yang of protein phosphorylation and signaling, Cell 80 (1995) 225–236.
[3] E.D.G. Fleuren, L. Zhang, J. Wu, R.J. Daly, The kinome 'at large' in cancer, Nat. Rev. Cancer 16 (2016) 83–98.
[4] M.J. Chen, J.E. Dixon, G. Manning, Genomics and evolution of protein phosphatases, Sci. Signal. 10 (2017) 1–18.
[5] Y. Shi, Serine/threonine phosphatases: mechanism through structure, Cell 139 (2009) 468–484.
[6] D.L. Brautigan, S. Shenolikar, Protein serine/threonine phosphatases: keys to unlocking regulators and substrates, Annu. Rev. Biochem. 87 (2018) 921–964.
[7] P. Amin, S. Awal, S. Vigneron, S. Roque, F. Mechali, J.C. Labbé, et al., PP2A-B55: substrates and regulators in the control of cellular functions, Oncogene 41 (2022) 1–14.
[8] P. Goguet-Rubio, P. Amin, S. Awal, S. Vigneron, S. Charrasse, F. Mechali, et al., PP2A-B55 holoenzyme regulation and cancer, Biomolecules 10 (2020) 1–17.
[9] V. Janssens, J. Goris, Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling, Biochem. J. 353 (2001) 417–439.
[10] H. Fowle, Z. Zhao, X. Graña, PP2A holoenzymes, substrate specificity driving cellular functions and deregulation in cancer, Adv. Cancer Res. 144 (2019) 55–93.
[11] P.J.A. Eichhorn, M.P. Creyghton, R. Bernards, Protein phosphatase 2A regulatory subunits and cancer, Biochim. Biophys. Acta 1795 (2009) 1–15.
[12] D. Haesen, W. Sents, K. Lemaire, Y. Hoorne, V. Janssens, The basic biology of PP2A in hematologic cells and malignancies, Front. Oncol. 4 (2014) 1–11.
[13] Y. Xu, Y. Xing, Y. Chen, Y. Chao, Z. Lin, E. Fan, et al., Structure of the Protein Phosphatase 2A Holoenzyme, Cell 127 (2006) 1239–1251.
[14] D. Perrotti, P. Neviani, Protein phosphatase 2A: a target for anticancer therapy, Lancet Oncol. 14 (2013) e229–e238.
[15] C. Lambrecht, D. Haesen, W. Sents, E. Ivanova, V. Janssens, Structure, regulation, and pharmacological modulation of PP2A phosphatases, Methods Mol. Biol. 1053 (2013) 283–305.
[16] Y. Xu, Y. Chen, P. Zhang, P.D. Jeffrey, Y. Shi, Structure of a protein phosphatase 2A holoenzyme: insights into B55-mediated Tau dephosphorylation, Mol. Cell 31 (2008) 873–885.
[17] D. Leonard, W. Huang, S. Izadmehr, C.M. O'Connor, D.D. Wiredja, Z. Wang, et al., Selective PP2A enhancement through biased heterotrimer stabilization, Cell 181 (2020) 688–701.e16.

[18] N. Wlodarchak, F. Guo, K.A. Satyshur, L. Jiang, P.D. Jeffrey, T. Sun, et al., Structure of the Ca2+-dependent PP2A heterotrimer and insights into Cdc6 dephosphorylation, Cell Res. 23 (2013) 931–946.

[19] Y. Tang, G. Fang, F. Guo, H. Zhang, X. Chen, L. An, et al., Selective Inhibition of STRN3-Containing PP2A phosphatase restores hippo tumor-suppressor activity in gastric cancer, Cancer Cell 38 (2020) 115–128.e9.

[20] U.S. Cho, W. Xu, Crystal structure of a protein phosphatase 2A heterotrimeric holoenzyme, Nature 445 (2007) 53–57.

[21] J. Sangodkar, C.C. Farrington, K. McClinch, M.D. Galsky, D.B. Kastrinsky, G. Narla, All roads lead to PP2A: exploiting the therapeutic potential of this phosphatase, FEBS J. 283 (2016) 1004–1024.

[22] W. Sents, E. Ivanova, C. Lambrecht, D. Haesen, V. Janssens, The biogenesis of active protein phosphatase 2A holoenzymes: a tightly regulated process creating phosphatase specificity, FEBS J. 280 (2013) 644–661.

[23] F. Guo, V. Stanevich, N. Wlodarchak, R. Sengupta, L. Jiang, K.A. Satyshur, et al., Structural basis of PP2A activation by PTPA, an ATP-dependent activation chaperone, Cell Res. 24 (2014) 190–203.

[24] S. Longin, K. Zwaenepoel, J.V. Louis, S. Dilworth, J. Goris, V. Janssens, Selection of protein phosphatase 2A regulatory subunits is mediated by the C terminus of the catalytic Subunit, J. Biol. Chem. 282 (2007) 26971–26980.

[25] V. Janssens, S. Longin, J. Goris, PP2A holoenzyme assembly: in cauda venenum (the sting is in the tail), Trends Biochem. Sci. 33 (2008) 113–121.

[26] S.P. Lyons, E.C. Greiner, L.E. Cressey, M.E. Adamo, A.N. Kettenbach, Regulation of PP2A, PP4, and PP6 holoenzyme assembly by carboxyl-terminal methylation, Sci. Rep. (2021) 11, https://doi.org/10.1038/s41598-021-02456-z.

[27] I. Nasa, L.E. Cressey, T. Kruse, E.P.T. Hertz, J. Gui, L.M. Graves, et al., Quantitative kinase and phosphatase profiling reveal that CDK1 phosphorylates PP2Ac to promote mitotic entry, Sci. Signal. (2020) 13, https://doi.org/10.1126/scisignal.aba7823.

[28] I.E. Frohner, I. Mudrak, S. Schüchner, D. Anrather, M. Hartl, J.-M. Sontag, et al., PP2AC Phospho-Tyr307 antibodies are not specific for this modification but are sensitive to other PP2AC modifications including Leu309 methylation, Cell Rep. 30 (2020) 3171–3182.e6.

[29] S. Mazhar, D. Leonard, A. Sosa, D. Schlatzer, D. Thomas, G. Narla, et al., Challenges and reinterpretation of antibody-based research on Phosphorylation of Tyr 307 on PP2Ac challenges and reinterpretation, Cell Rep. 30 (2020) 3164–3170, e3.

[30] S. Longin, K. Zwaenepoel, E. Martens, J.V. Louis, E. Rondelez, J. Goris, et al., Spatial control of protein phosphatase 2A (de)methylation, Exp. Cell Res. 314 (2008) 68–81.

[31] V. Stanevich, L. Jiang, K.A. Satyshur, Y. Li, P.D. Jeffrey, Z. Li, et al., The structural basis for tight control of PP2A methylation and function by LCMT-1, Mol. Cell 41 (2011) 331–342.

[32] Y. Xing, Z. Li, Y. Chen, J.B. Stock, P.D. Jeffrey, Y. Shi, Structural mechanism of demethylation and inactivation of protein phosphatase 2A, Cell 133 (2008) 154–163.

[33] T.J. Haanen, C.M. O'Connor, G. Narla, Biased holoenzyme assembly of protein phosphatase 2A (PP2A): From cancer to small molecules, J. Biol. Chem. 298 (2022), 102656.

[34] G. Vallardi, L.A. Allan, L. Crozier, A.T. Saurin, Division of labour between pp2a-b56 isoforms at the centromere and kinetochore, Elife 8 (2019) 1–25.

[35] J.A. Lee, D.C. Pallas, Leucine carboxyl methyltransferase-1 is necessary for normal progression through mitosis in mammalian cells, J. Biol. Chem. 282 (2007) 30974–30984.

[36] T. Kruse, S.P. Gnosa, I. Nasa, D.H. Garvanska, J.B. Hein, H. Nguyen, et al., Mechanisms of site-specific dephosphorylation and kinase opposition imposed by PP2A regulatory subunits, EMBO J. 39 (2020), e103695.

[37] N.T. Vasudevan, M.L. Mohan, M.K. Gupta, A.K. Hussain, S.V. Naga Prasad, Inhibition of protein phosphatase 2A activity by PI3Kγ regulates β-Adrenergic receptor function, Mol. Cell 41 (2011) 636–648.

[38] E. Arriazu, C. Vicente, R. Pippa, I. Peris, E. Martínez-Balsalobre, P. García-Ramírez, et al., A new regulatory mechanism of protein phosphatase 2A activity via SET in acute myeloid leukemia, Blood Cancer J 10 (2020) 3.

[39] I. Cristóbal, F.J. Blanco, L. Garcia-Orti, N. Marcotegui, C. Vicente, J. Rifon, et al., SETBP1 overexpression is a novel leukemogenic mechanism that predicts adverse outcome in elderly patients with acute myeloid leukemia, Blood 115 (2010) 615–625.

[40] S. Enjoji, R. Yabe, S. Tsuji, K. Yoshimura, H. Kawasaki, M. Sakurai, et al., Stemness is enhanced in gastric cancer by a SET/PP2A/E2F1 axis, Mol. Cancer Res. 16 (2018) 554–563.

[41] J. Wang, J. Okkeri, K. Pavic, Z. Wang, O. Kauko, T. Halonen, et al., Oncoprotein CIP 2A is stabilized via interaction with tumor suppressor PP 2A/B56, EMBO Rep. 18 (2017) 437–450.

[42] K. Pavic, N. Gupta, J.D. Omella, R. Derua, A. Aakula, R. Huhtaniemi, et al., Structural mechanism for inhibition of PP2A-B56α and oncogenicity by CIP2A, Nat. Commun. 14 (2023) 1143.

[43] I.M. Porter, K. Schleicher, M. Porter, J.R. Swedlow, Bod1 regulates protein phosphatase 2A at mitotic kinetochores, Nat. Commun. 4 (2013) 2677.

[44] A. Takai, K. Sasaki, H. Nagai, G. Mieskes, M. Isobe, K. Isono, et al., Inhibition of specific binding of okadaic acid to protein phosphatase 2A by microcystin-LR, calyculin-A and tautomycin: method of analysis of interactions of tight-binding ligands with target protein, Biochem. J. 306 (Pt 3) (1995) 657–665.

[45] T.D. Prickett, D.L. Brautigan, The alpha4 regulatory subunit exerts opposing allosteric effects on protein phosphatases PP6 and PP2A, J. Biol. Chem. 281 (2006) 30503–30511.

[46] K. McClinch, R.A. Avelar, D. Callejas, S. Izadmehr, D. Wiredja, A. Perl, et al., Small-molecule activators of protein phosphatase 2A for the treatment of castration-resistant prostate cancer, Cancer Res. 78 (2018) 2065–2080.

[47] J. Yang, C. Phiel, Functions of B56-containing PP2As in major developmental and cancer signaling pathways, Life Sci. 87 (2010) 659–666.

[48] E. Arriazu, R. Pippa, M.D. Odero, Protein phosphatase 2A as a therapeutic target in acute Myeloid Leukemia, Front. Oncol. 6 (2016) 78.

[49] E. Martens, I. Stevens, V. Janssens, J. Vermeesch, J. Götz, J. Goris, et al., Genomic organisation, chromosomal localisation tissue distribution and developmental regulation of the PR61/B′ regulatory subunits of protein phosphatase 2A in mice, J. Mol. Biol. 336 (2004) 971–986.

[50] J.J. Dyson, F. Abbasi, P. Varadkar, B. McCright, Growth arrest of PPP2R5C and PPP2R5D double knockout mice indicates a genetic interaction and conserved function for these PP2A B subunits, FASEB BioAdv. 4 (2022) 273–282.

[51] C. Letourneux, G. Rocher, F. Porteu, B56-containing PP2A dephosphorylate ERK and their activity is controlled by the early gene IEX-1 and ERK, EMBO J. 25 (2006) 727–738.

[52] U. Kirchhefer, A. Heinick, S. König, T. Kristensen, F.U. Müller, M.D. Seidl, et al., Protein phosphatase 2A is regulated by protein kinase Cα (PKCα)-dependent phosphorylation of its targeting subunit B56α at Ser41, J. Biol. Chem. 289 (2014) 163–176.

[53] J.H. Ahn, T. McAvoy, S.V. Rakhilin, A. Nishi, P. Greengard, A.C. Nairn, Protein kinase A activates protein phosphatase 2A by phosphorylation of the B56δ subunit, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 2979–2984.

[54] V.R. Ruvolo, S.M. Kurinna, K.B. Karanjeet, T.F. Schuster, A.M. Martelli, J. A. McCubrey, et al., PKR regulates B56α-mediated BCL2 phosphatase activity in acute lymphoblastic leukemia-derived REH cells, J. Biol. Chem. 283 (2008) 35474–35485.

[55] E.P.T. Hertz, T. Kruse, N.E. Davey, B. López-Méndez, J.O. Sigurðsson, G. Montoya, et al., A conserved motif provides binding specificity to the PP2A-B56 phosphatase, Mol. Cell 63 (2016) 686–695.

[56] C.G. Wu, H. Chen, F. Guo, V.K. Yadav, S.J. McIlwain, M. Rowse, et al., PP2A-B′ holoenzyme substrate recognition, regulation and role in cytokinesis, Cell Discov. 3 (2017) 1–19.

[57] X. Wang, R. Bajaj, M. Bollen, W. Peti, R. Page, Expanding the PP2A interactome by defining a B56-Specific SLiM, Structure 24 (2016) 2174–2181.

[58] K. Okamoto, H. Li, M.R. Jensen, T. Zhang, Y. Taya, S.S. Thorgeirsson, et al., Cyclin G recruits PP2A to dephosphorylate Mdm2, Mol. Cell 9 (2002) 761–771.

[59] H.K. Arnold, X. Zhang, C.J. Daniel, D. Tibbitts, J. Escamilla-Powers, A. Farrell, et al., The Axin1 scaffold protein promotes formation of a degradation complex for c-Myc, EMBO J. 28 (2009) 500–512.

[60] Zheng H. Yun, Shen F. Jin, Tong Y. Qing, Y. Li, PP2A inhibits cervical cancer cell migration by Dephosphorylation of p-JNK, p-p38 and the p-ERK/MAPK signaling pathway, Curr. Med. Sci. 38 (2018) 115–123.

[61] C. Xu, X. Wang, Y. Zhu, X. Dong, C. Liu, H. Zhang, et al., Rapamycin ameliorates cadmium-induced activation of MAPK pathway and neuronal apoptosis by preventing mitochondrial ROS inactivation of PP2A, Neuropharmacology 105 (2016) 270–284.

[62] M.R. Junttila, S.-P. Li, J. Westermarck, Phosphatase-mediated crosstalk between MAPK signaling pathways in the regulation of cell survival, FASEB J. 22 (2008) 954–965.

[63] J. Sangodkar, A. Perl, R. Tohme, J. Kiselar, D.B. Kastrinsky, N. Zaware, et al., Activation of tumor suppressor protein PP2A inhibits KRAS-driven tumor growth, J. Clin. Invest. 127 (2017) 2081–2090.

[64] I. Peris, S. Romero-Murillo, E. Martínez-Balsalobre, C.C. Farrington, E. Arriazu, N. Marcotegui, et al., Activation of the PP2A-B56α heterocomplex synergizes with venetoclax therapies in AML through BCL2 and MCL1 modulation, Blood 141 (2023) 1047–1059.

[65] M.N. Garcia, D. Grasso, M.B. Lopez-Millan, T. Hamidi, C. Loncle, R. Tomasini, et al., IER3 supports KRASG12D-dependent pancreatic cancer development by sustaining ERK1/2 phosphorylation, J. Clin. Invest. 124 (2014) 4709–4722.

[66] J. Westermarck, S.-P. Li, T. Kallunki, J. Han, V.-M. Kähäri, p38 mitogen-activated protein kinase-dependent activation of protein phosphatases 1 and 2A inhibits MEK1 and MEK2 activity and Collagenase 1 (MMP-1) gene expression, Mol. Cell. Biol. 21 (2001) 2373–2383.

[67] D.D. Wiredja, M. Ayati, S. Mazhar, J. Sangodkar, S. Maxwell, D. Schlatzer, et al., Phosphoproteomics profiling of nonsmall cell lung cancer cells treated with a Novel phosphatase activator, Proteomics 17 (2017), e1870214.

[68] O. Kauko, C.M. O'Connor, E. Kulesskiy, J. Sangodkar, A. Aakula, S. Izadmehr, et al., PP2A inhibition is a druggable MEK inhibitor resistance mechanism in KRAS-mutant lung cancer cells, Sci. Transl. Med. (2018) 10, https://doi.org/10.1126/scitranslmed.aaq1093.

[69] C.M. O'Connor, D. Leonard, D. Wiredja, R.A. Avelar, Z. Wang, D. Schlatzer, et al., Inactivation of PP2A by a recurrent mutation drives resistance to MEK inhibitors, Oncogene 39 (2020) 703–717.

[70] A.L. Jeong, S. Han, S. Lee, J. Su Park, Y. Lu, S. Yu, et al., Patient derived mutation W257G of PPP2R1A enhances cancer cell migration through SRC-JNK-c-Jun pathway, Sci. Rep. 6 (2016) 1–12.

[71] A. Igea, A.R. Nebreda, The stress Kinase p38α as a target for cancer therapy, Cancer Res. 75 (2015) 3997–4002.

[72] J.T. Chiou, Y.J. Shi, Y.C. Lee, L.J. Wang, Y.J. Chen, Chang L. Sen, Carboxyl group-modified α-lactalbumin induces TNF-α-mediated apoptosis in leukemia and breast cancer cells through the NOX4/p38 MAPK/PP2A axis, Int. J. Biol. Macromol. 187 (2021) 513–527.

[73] S. Ory, M. Zhou, T.P. Conrads, T.D. Veenstra, D.K. Morrison, Protein phosphatase 2A positively regulates Ras signaling by dephosphorylating KSR1 and Raf-1 on critical 14-3-3 binding sites, Curr. Biol. 13 (2003) 1356–1364.

[74] A.M. Silverstein, C.A. Barrow, A.J. Davis, M.C. Mumby, Actions of PP2A on the MAP kinase pathway and apoptosis are mediated by distinct regulatory subunits, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 4221–4226.

[75] J.B. Jackson, D.C. Pallas, Circumventing cellular control of PP2A by methylation promotes transformation in an Akt-dependent manner, Neoplasia 14 (2012) 585–599.

[76] Y.-C. Kuo, K.-Y. Huang, C.-H. Yang, Y.-S. Yang, W.-Y. Lee, C.-W. Chiang, Regulation of phosphorylation of Thr-308 of Akt, cell proliferation, and survival by the B55alpha regulatory subunit targeting of the protein phosphatase 2A holoenzyme to Akt, J. Biol. Chem. 283 (2008) 1882–1892.

[77] G. Rocher, C. Letourneux, P. Lenormand, F. Porteu, Inhibition of B56-containing protein phosphatase 2As by the early response gene IEX-1 leads to control of Akt activity, J. Biol. Chem. 282 (2007) 5468–5477.

[78] N. Vereshchagina, M.-C. Ramel, E. Bitoun, C. Wilson, The protein phosphatase PP2A-B' subunit Widerborst is a negative regulator of cytoplasmic activated Akt and lipid metabolism in Drosophila, J. Cell Sci. 121 (2008) 3383–3392.

[79] L. Che, Z.-B. Du, W.-H. Wang, J.-S. Wu, T. Han, Y.-Y. Chen, et al., Intracellular antibody targeting HBx suppresses invasion and metastasis in hepatitis B virus-related hepatocarcinogenesis via protein phosphatase 2A-B56γ-mediated dephosphorylation of protein kinase B, Cell Prolif 55 (11) (2022), e13304.

[80] S. Padmanabhan, A. Mukhopadhyay, S.D. Narasimhan, G. Tesz, M.P. Czech, H. A. Tissenbaum, A PP2A regulatory subunit regulates C. elegans insulin/IGF-1 signaling by modulating AKT-1 phosphorylation, Cell 136 (2009) 939–951.

[81] J.T. Rodgers, R.O. Vogel, P. Puigserver, Clk2 and B56β mediate insulin-regulated assembly of the PP2A phosphatase holoenzyme complex on Akt, Mol. Cell 41 (2011) 471–479.

[82] K. Kim, A. Baek, J.-E. Hwang, Y.A. Choi, J. Jeong, M.-S. Lee, et al., Adiponectin-activated AMPK stimulates dephosphorylation of AKT through protein phosphatase 2A activation, Cancer Res. 69 (2009) 4018–4026.

[83] L. He, A.P. Gomes, X. Wang, S.O. Yoon, G. Lee, M.J. Nagiec, et al., mTORC1 Promotes Metabolic Reprogramming by the Suppression of GSK3-Dependent Foxk1 Phosphorylation, Mol. Cell 70 (2018) 949–960.e4.

[84] H. Nakatsumi, T. Oka, T. Higa, M. Shirane, K.I. Nakayama, Nuclear–cytoplasmic shuttling protein PP2AB56 contributes to mTORC1-dependent dephosphorylation of FOXK1, Genes Cells 23 (2018) 599–605.

[85] J.P.L. Sim, W. Ziyin, A.H. Basil, S. Lin, Z. Chen, C. Zhang, et al., Identification of PP2A and S6 kinase as modifiers of Leucine-Rich Repeat Kinase-induced neurotoxicity, NeuroMolecular Med. 22 (2020) 218–226.

[86] K. Hahn, M. Miranda, V.A. Francis, J. Vendrell, A. Zorzano, A.A. Teleman, PP2A regulatory subunit PP2A-B' counteracts S6K phosphorylation, Cell Metab. 11 (2010) 438–444.

[87] L. Yan, V. Mieulet, D. Burgess, G.M. Findlay, K. Sully, J. Procter, et al., PP2A T61 epsilon is an inhibitor of MAP4K3 in nutrient signaling to mTOR, Mol. Cell 37 (2010) 633–642.

[88] M.A. Hermida, J. Dinesh Kumar, N.R. Leslie, GSK3 and its interactions with the PI3K/AKT/mTOR signalling network, Adv. Biol. Regul. 65 (2017) 5–15.

[89] M. Elgendy, M. Cirò, A. Hosseini, J. Weiszmann, L. Mazzarella, E. Ferrari, et al., Combination of hypoglycemia and metformin impairs tumor metabolic plasticity and growth by modulating the PP2A-GSK3β-MCL-1 axis, Cancer Cell 35 (2019) 798–815.e5.

[90] R. Pan, V. Ruvolo, H. Mu, J.D. Leverson, G. Nichols, J.C. Reed, et al., Synthetic lethality of combined Bcl-2 Inhibition and p53 activation in AML: mechanisms and superior antileukemic efficacy, Cancer Cell 32 (2017) 748–760.e6.

[91] R. Dhanasekaran, A. Deutzmann, W.D. Mahauad-Fernandez, A.S. Hansen, A. M. Gouw, D.W. Felsher, The MYC oncogene - the grand orchestrator of cancer growth and immune evasion, Nat. Rev. Clin. Oncol. 19 (2022) 23–36.

[92] A.S. Farrell, R.C. Sears, MYC degradation, Cold Spring Harb. Perspect Med. 4 (2014) 1–16.

[93] Y. Zhang, Z. Wang, X. Li, N.S. Magnuson, Pim kinase-dependent inhibition of c-Myc degradation, Oncogene 27 (2008) 4809–4819.

[94] J. Ma, H.K. Arnold, M.B. Lilly, R.C. Sears, A.S. Kraft, Negative regulation of Pim-1 protein kinase levels by the B56beta subunit of PP2A, Oncogene 26 (2007) 5145–5153.

[95] M.A. Gregory, Y. Qi, S.R. Hann, Phosphorylation by glycogen synthase kinase-3 controls c-myc proteolysis and subnuclear localization, J. Biol. Chem. 278 (2003) 51606–51612.

[96] C. Lambrecht, L. Libbrecht, X. Sagaert, P. Pauwels, Y. Hoorne, J. Crowther, et al., Loss of protein phosphatase 2A regulatory subunit B56δ promotes spontaneous tumorigenesis in vivo, Oncogene 37 (2018) 544–552.

[97] L. Liu, R.N. Eisenman, Regulation of c-Myc protein abundance by a protein Phosphatase 2A-Glycogen synthase Kinase 3β-Negative feedback pathway, Genes Cancer 3 (2012) 23–36.

[98] A.S. Farrell, C. Pelz, X. Wang, C.J. Daniel, Z. Wang, Y. Su, et al., Pin1 regulates the dynamics of c-Myc DNA binding to facilitate target gene regulation and oncogenesis, Mol. Cell. Biol. 33 (2013) 2930–2949.

[99] R. Pippa, M.D. Odero, The role of MYC and PP2A in the initiation and progression of Myeloid Leukemias, Cells 9 (2020) 1–16.

[100] R. Pippa, A. Dominguez, D.J. Christensen, I. Moreno-Miralles, M.J. Blanco-Prieto, M.P. Vitek, et al., Effect of FTY720 on the SET-PP2A complex in acute myeloid leukemia; SET binding drugs have antagonistic activity, Leukemia 28 (2014) 1915–1918.

[101] O. Kutuk, A. Letai, Regulation of Bcl-2 family proteins by posttranslational modifications, Curr. Mol. Med. 8 (2008) 102–118.

[102] V.V. Senichkin, A.Y. Streletskaia, A.S. Gorbunova, B. Zhivotovsky, G.S. Kopeina, Saga of Mcl-1: regulation from transcription to degradation, Cell Death Differ. 27 (2020) 405–419.

[103] M.A. Pagano, E. Tibaldi, P. Molino, F. Frezzato, V. Trimarco, M. Facco, et al., Mitochondrial apoptosis is induced by Alkoxy phenyl-1-propanone derivatives through PP2A-mediated dephosphorylation of Bad and Foxo3A in CLL, Leukemia 33 (2019) 1148–1160.

[104] S.J. Gardai, D.A. Hildeman, S.K. Frankel, B.B. Whitlock, S.C. Frasch, N. Borregaard, et al., Phosphorylation of Bax Ser184 by Akt regulates its activity and apoptosis in neutrophils, J. Biol. Chem. 279 (2004) 21085–21095.

[105] P. Ruvolo, X. Deng, W. May, Phosphorylation of Bcl2 and regulation of apoptosis, Leukemia 15 (2001) 515–522.

[106] P.P. Ruvolo, W. Clark, M. Mumby, F. Gao, W. Stratford May, W.S. May, A functional role for the B56 α-Subunit of protein Phosphatase 2A in Ceramide-mediated Regulation of Bcl2 Phosphorylation Status and Function, J. Biol. Chem. 277 (2002) 22847–22852.

[107] R. Antony, W.J. Lukiw, N.G. Bazan, Neuroprotectin D1 induces dephosphorylation of Bcl-xL in a PP2A-dependent manner during oxidative stress and promotes retinal pigment epithelial cell survival, J. Biol. Chem. 285 (2010) 18301–18308.

[108] L.W. Thomas, C. Lam, S.W. Edwards, Mcl-1; the molecular regulation of protein function, FEBS Lett. 584 (2010) 2981–2989.

[109] I.E. Wertz, S. Kusam, C. Lam, T. Okamoto, W. Sandoval, D.J. Anderson, et al., Sensitivity to antitubulin chemotherapeutics is regulated by MCL1 and FBW7, Nature 471 (2011) 110–114.

[110] Y. Fu, X. Jia, J. Yuan, Y. Yang, T. Zhang, Q. Yu, et al., Fam72a functions as a cell-cycle-controlled gene during proliferation and antagonizes apoptosis through reprogramming PP2A substrates, Dev. Cell 58 (2023) 398–415.e7.

[111] S. Grethe, M.I. Pörn-Ares, p38 MAPK regulates phosphorylation of Bad via PP2A-dependent suppression of the MEK1/2-ERK1/2 survival pathway in TNF-alpha induced endothelial apoptosis, Cell. Signal. 18 (2006) 531–540.

[112] J. Zhuang, S.F. Hawkins, M.A. Glenn, K. Lin, G.G. Johnson, A. Carter, et al., Akt is activated in chronic lymphocytic leukemia cells and delivers a pro-survival signal: the therapeutic potential of Akt inhibition, Haematologica 95 (2010) 110–118.

[113] N. van Gils, F. Denkers, L. Smit, Escape from treatment; the different faces of Leukemic stem cells and therapy resistance in acute Myeloid Leukemia, Front. Oncol. 11 (2021), 659253.

[114] Y. Zhang, X. Wang, Targeting the Wnt/β-catenin signaling pathway in cancer, J. Hematol. Oncol. 13 (2020) 165.

[115] J. Yang, J. Wu, C. Tan, P.S. Klein, PP2A:B56epsilon is required for Wnt/beta-catenin signaling during embryonic development, Development 130 (2003) 5569–5578.

[116] M.J. Ratcliffe, K. Itoh, S.Y. Sokol, A positive role for the PP2A catalytic subunit in Wnt signal transduction, J. Biol. Chem. 275 (2000) 35680–35683.

[117] S. Baek, J.M. Seeling, Identification of a novel conserved mixed-isoform B56 regulatory subunit and spatiotemporal regulation of protein phosphatase 2A during Xenopus laevis development, BMC Dev. Biol. 7 (2007) 139.

[118] A.D. Everett, C. Kamibayashi, D.L. Brautigan, Transgenic expression of protein phosphatase 2A regulatory subunit B56gamma disrupts distal lung differentiation, Am. J. Phys. Lung Cell. Mol. Phys. 282 (2002) L1266–L1271.

[119] W. Luo, A. Peterson, B.A. Garcia, G. Coombs, B. Kofahl, R. Heinrich, et al., Protein phosphatase 1 regulates assembly and function of the beta-catenin degradation complex, EMBO J. 26 (2007) 1511–1521.

[120] Z. Jin, J.W. Chung, W. Mei, S. Strack, C. He, G.W. Lau, et al., Regulation of nuclear-cytoplasmic shuttling and function of Family with sequence similarity 13, member A (Fam13a), by B56-containing PP2As and Akt, Mol. Biol. Cell 26 (2015) 1160–1173.

[121] G.P. Shouse, X. Cai, X. Liu, Serine 15 phosphorylation of p53 directs its interaction with B56gamma and the tumor suppressor activity of B56gamma-specific protein phosphatase 2A, Mol. Cell. Biol. 28 (2008) 448–456.

[122] J. Wang, H.R. Thomas, Z. Li, N.C.F. Yeo, H.E. Scott, N. Dang, et al., Puma, noxa, p53, and p63 differentially mediate stress pathway induced apoptosis, Cell Death Dis. 12 (2021) 659.

[123] G.P. Shouse, Y. Nobumori, M.J. Panowicz, X. Liu, ATM-mediated phosphorylation activates the tumor-suppressive function of B56γ-PP2A, Oncogene 30 (2011) 3755–3765.

[124] H.-H. Li, X. Cai, G.P. Shouse, L.G. Piluso, X. Liu, A specific PP2A regulatory subunit, B56gamma, mediates DNA damage-induced dephosphorylation of p53 at Thr55, EMBO J. 26 (2007) 402–411.

[125] J. Chen, J.R. St-Germain, Q. Li, B56 regulatory subunit of protein phosphatase 2A mediates valproic acid-induced p300 degradation, Mol. Cell. Biol. 25 (2005) 525–532.

[126] P. Kalev, M. Simicek, I. Vazquez, S. Munck, L. Chen, T. Soin, et al., Loss of PPP2R2A inhibits homologous recombination DNA repair and predicts tumor sensitivity to PARP inhibition, Cancer Res. 72 (2012) 6414–6424.

[127] S.M. Ambjørn, J.P. Duxin, E.P.T. Hertz, I. Nasa, J. Duro, T. Kruse, et al., A complex of BRCA2 and PP2A-B56 is required for DNA repair by homologous recombination, Nat. Commun. (2021) 12, https://doi.org/10.1038/s41467-021-26079-0.

[128] H. Jia, Y. Liu, W. Yan, J. Jia, PP4 and PP2A regulate Hedgehog signaling by controlling Smo and Ci phosphorylation, Development 136 (2009) 307–316.

[129] A.M. Rorick, W. Mei, N.L. Liette, C. Phiel, H.M. El-Hodiri, J. Yang, PP2A: B56epsilon is required for eye induction and eye field separation, Dev. Biol. 302 (2007) 477–493.

[130] S. Krauss, J. Foerster, R. Schneider, S. Schweiger, Protein phosphatase 2A and rapamycin regulate the nuclear localization and activity of the transcription factor GLI3, Cancer Res. 68 (2008) 4658–4665.
[131] S.J. Neal, Q. Zhou, F. Pignoni, Protein phosphatase 2A with B' specificity subunits regulates the Hippo-Yorkie signaling axis in the Drosophila eye disc, J. Cell Sci. (2022), https://doi.org/10.1242/jcs.259558.
[132] R.J. Davis, J. Swanger, B.T. Hughes, B.E. Clurman, The PP2A-B56 phosphatase opposes Cyclin E autocatalytic degradation via site-specific Dephosphorylation, Mol. Cell. Biol. 37 (2017) 1–13.
[133] N. Naetar, V. Soundarapandian, L. Litovchick, K.L. Goguen, A.A. Sablina, C. Bowman-Colin, et al., PP2A-mediated regulation of ras signaling in G2 is essential for stable quiescence and normal G1 length, Mol. Cell 54 (2014) 932–945.
[134] O. Kauko, S.Y. Imanishi, E. Kulesskiy, L. Yetukuri, T.D. Laajala, M. Sharma, et al., Phosphoproteome and drug-response effects mediated by the three protein phosphatase 2A inhibitor proteins CIP2A, SET, and PME-1, J. Biol. Chem. 295 (2020) 4194–4211.
[135] M. Suganuma, H. Fujiki, H. Suguri, S. Yoshizawa, M. Hirota, M. Nakayasu, et al., Okadaic acid: an additional non-phorbol-12-tetradecanoate-13-acetate-type tumor promoter, Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 1768–1771.
[136] D.C. Pallas, L.K. Shahrik, B.L. Martin, S. Jaspers, T.B. Miller, D.L. Brautigan, et al., Polyoma small and middle T antigens and SV40 small t antigen form stable complexes with protein phosphatase 2A, Cell 60 (1990) 167–176.
[137] R. Shtrichman, R. Sharf, H. Barr, T. Dobner, T. Kleinberger, Induction of apoptosis by adenovirus E4orf4 protein is specific to transformed cells and requires an interaction with protein phosphatase 2A, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 10080–10085.
[138] J. Westermarck, W.C. Hahn, Multiple pathways regulated by the tumor suppressor PP2A in transformation, Trends Mol. Med. 14 (2008) 152–160.
[139] A.A. Sablina, M. Hector, N. Colpaert, W.C. Hahn, Identification of PP2A complexes and pathways involved in cell transformation, Cancer Res. 70 (2010) 10474–10484.
[140] O. Kauko, J. Westermarck, Non-genomic mechanisms of protein phosphatase 2A (PP2A) regulation in cancer, Int. J. Biochem. Cell Biol. 96 (2018) 157–164.
[141] S.E. Taylor, C.M. O'Connor, Z. Wang, G. Shen, H. Song, D. Leonard, et al., The highly recurrent PP2A Aa-subunit mutation P179R alters protein structure and impairs PP2A enzyme function to promote endometrial tumorigenesis, Cancer Res. 79 (2019) 4242–4257.
[142] D. Haesen, L.A. Asbagh, R. Derua, A. Hubert, S. Schrauwen, Y. Hoorne, et al., Recurrent PPP2R1A mutations in uterine cancer act through a dominant-negative mechanism to promote malignant cell growth, Cancer Res. 76 (2016) 5719–5731.
[143] N. Leulliot, S. Quevillon-cheruel, I. Sorel, Sierra-gallay I.L.D. La, B. Collinet, M. Graille, et al., Structure of protein phosphatase Methyltransferase 1 (PPM1), a Leucine Carboxyl methyltransferase involved in the regulation of protein Phosphatase 2A activity *, J. Biol. Chem. 279 (2004) 8351–8358.
[144] M.L. Tsai, N. Cronin, S. Djordjevic, The structure of human leucine carboxyl methyltransferase 1 that regulates protein phosphatase PP2A, Acta Crystallogr. D Biol. Crystallogr. 67 (2011) 14–24.
[145] J.M. Sontag, V. Nunbhakdi-Craig, E. Sontag, Leucine carboxyl methyltransferase 1 (LCMT1)-dependent methylation regulates the association of protein phosphatase 2A and Tau protein with plasma membrane microdomains in neuroblastoma cells, J. Biol. Chem. 288 (2013) 27396–27405.
[146] E. Barragan, M.C. Chillon, R. Castello-Cros, N. Marcotegui, M.I. Prieto, M. Hoyos, et al., CIP2A high expression is a poor prognostic factor in normal karyotype acute myeloid leukemia, Haematologica 100 (2015) e183–e185.
[147] E. Mäkelä, E. Löyttyniemi, U. Salmenniemi, O. Kauko, T. Varila, V. Kairisto, et al., Arpp19 promotes Myc and Cip2a expression and associates with patient relapse in acute Myeloid Leukemia, Cancers (Basel) 11 (2019) 1774.
[148] K.G. Roberts, A.M. Smith, F. McDougall, H. Carpenter, M. Horan, P. Neviani, et al., Essential requirement for PP2A inhibition by the oncogenic receptor c-KIT suggests PP2A reactivation as a strategy to treat c-KIT+ cancers, Cancer Res. 70 (2010) 5438–5447.
[149] I. Cristobal, C. Cirauqui, R. Castello-Cros, L. Garcia-Orti, M.J. Calasanz, M. D. Odero, Downregulation of PPP2R5E is a common event in acute myeloid leukemia that affects the oncogenic potential of leukemic cells, Haematologica 98 (2013) e103–e104.
[150] E. Wandzioch, M. Pusey, A. Werda, S. Bail, A. Bhaskar, M. Nestor, et al., PME-1 modulates protein phosphatase 2A activity to promote the malignant phenotype of endometrial cancer cells, Cancer Res. 74 (2014) 4295–4305.
[151] P. Puustinen, M.R. Junttila, S. Vanhatupa, A.A. Sablina, M.E. Hector, K. Teittinen, et al., PME-1 protects extracellular signal-regulated kinase pathway activity from protein phosphatase 2A-mediated inactivation in human malignant glioma, Cancer Res. 69 (2009) 2870–2877.
[152] A. Kaur, O.V. Denisova, X. Qiao, M. Jumppanen, E. Peuhu, S.U. Ahmed, et al., PP2A inhibitor PME-1 drives kinase inhibitor resistance in glioma cells, Cancer Res. 76 (2016) 7001–7011.
[153] I. Cristóbal, R. Rincón, R. Manso, C. Caramés, S. Zazo, J. Madoz-Gúrpide, et al., Deregulation of the PP2A inhibitor SET shows promising therapeutic implications and determines poor clinical outcome in patients with metastatic colorectal cancer, Clin. Cancer Res. 21 (2015) 347–356.
[154] Y.H. Huang, P.Y. Chu, J.L. Chen, C.T. Huang, C.H. Lee, K.Y. Lau, et al., SET overexpression is associated with worse recurrence-free survival in patients with primary breast cancer receiving adjuvant tamoxifen treatment, J. Clin. Med. (2018) 7, https://doi.org/10.3390/jcm7090245.
[155] H. Liu, Y. Gu, H. Wang, J. Yin, G. Zheng, Z. Zhang, et al., Overexpression of PP2A inhibitor SET oncoprotein is associated with tumor progression and poor prognosis in human non-small cell lung cancer, Oncotarget 6 (2015) 14913–14925.
[156] H.R. Mody, S.W. Hung, K. Naidu, H. Lee, C.A. Gilbert, T.T. Hoang, et al., SET contributes to the epithelial-mesenchymal transition of pancreatic cancer, Oncotarget 8 (2017) 67966–67979.
[157] I. Cristobal, L. Garcia-Orti, C. Cirauqui, X. Cortes-Lavaud, M.A. Garcia-Sanchez, M.J. Calasanz, et al., Overexpression of SET is a recurrent event associated with poor outcome and contributes to protein phosphatase 2A inhibition in acute myeloid leukemia, Haematologica 97 (2012) 543–550.
[158] A. Khanna, J.E. Pimanda, J. Westermarck, Cancerous inhibitor of protein Phosphatase 2A, an emerging human oncoprotein and a potential cancer therapy target, Cancer Res. 73 (2013) 6548–6553.
[159] M. Niemelä, O. Kauko, H. Sihto, J.-P. Mpindi, D. Nicorici, P. Pernilä, et al., CIP2A signature reveals the MYC dependency of CIP2A-regulated phenotypes and its clinical association with breast cancer subtypes, Oncogene 31 (2012) 4266–4278.
[160] A. Laine, H. Sihto, C. Come, M.T. Rosenfeldt, A. Zwolinska, M. Niemelä, et al., Senescence sensitivity of breast cancer cells is defined by positive feedback loop between CIP2A and E2F1, Cancer Discov. 3 (2013) 182–197.
[161] K.-F. Chen, C.-Y. Liu, Y.-C. Lin, H.-C. Yu, T.-H. Liu, D.-R. Hou, et al., CIP2A mediates effects of bortezomib on phospho-Akt and apoptosis in hepatocellular carcinoma cells, Oncogene 29 (2010) 6257–6266.
[162] B. Peng, Y. Chai, Y. Li, X. Liu, J. Zhang, CIP2A overexpression induces autoimmune response and enhances JNK signaling pathway in human lung cancer, BMC Cancer 15 (2015) 895.
[163] I. Cristóbal, L. Garcia-Orti, C. Cirauqui, M.M. Alonso, M.J. Calasanz, M.D. Odero, PP2A impaired activity is a common event in acute myeloid leukemia and its activation by forskolin has a potent anti-leukemic effect, Leukemia 25 (2011) 606–614.
[164] W. Chen, R. Possemato, K.T. Campbell, C.A. Plattner, D.C. Pallas, W.C. Hahn, Identification of specific PP2A complexes involved in human cell transformation, Cancer Cell 5 (2004) 127–136.
[165] P. Liu, J. Wei, F. Mao, Z. Xin, H. Duan, Y. Du, et al., Establishment of a prognostic model for hepatocellular carcinoma based on endoplasmic reticulum stress-related gene analysis, Front. Oncol. 11 (2021) 1–15.
[166] S. Mazhar, S.E. Taylor, J. Sangodkar, G. Narla, Targeting PP2A in cancer: combination therapies, Biochim. Biophys. Acta, Mol. Cell Res. 1866 (2019) 51–63.
[167] J.A. Cohen, F. Barkhof, G. Comi, H.-P. Hartung, B.O. Khatri, X. Montalban, et al., Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis, N. Engl. J. Med. 362 (2010) 402–415.
[168] Q. Liu, X. Zhao, F. Frissora, Y. Ma, R. Santhanam, D. Jarjoura, et al., FTY720 demonstrates promising preclinical activity for chronic lymphocytic leukemia and lymphoblastic leukemia/lymphoma, Blood 111 (2008) 275–284.
[169] P. Neviani, J.G. Harb, J.J. Oaks, R. Santhanam, C.J. Walker, J.J. Ellis, et al., PP2A-activating drugs selectively eradicate TKI-resistant chronic myeloid leukemic stem cells, J. Clin. Invest. 123 (2013) 4144–4157.
[170] A. Estella-Hermoso de Mendoza, R. Castello-Cros, E. Imbuluzqueta, C. Cirauqui, R. Pippa, M.D. Odero, et al., Lipid nanosystems enhance the bioavailability and the therapeutic efficacy of FTY720 in acute Myeloid Leukemia, J. Biomed. Nanotechnol. 11 (2015) 691–701.
[171] L. Koyrakh, M.I. Roman, V. Brinkmann, K. Wickman, The heart rate decrease caused by acute FTY720 administration is mediated by the G protein-gated potassium channel IKACh, Am. J. Transplant. 5 (2005) 529–536.
[172] C. Vicente, E. Arriazu, E. Martínez-Balsalobre, I. Peris, N. Marcotegui, P. García-Ramírez, et al., A novel FTY720 analogue targets SET-PP2A interaction and inhibits growth of acute myeloid leukemia cells without inducing cardiac toxicity, Cancer Lett. 468 (2020) 1–13.
[173] S. Goswami, C.-L. Chiang, K. Zapolnik, J. Nunes, A. Ventura, X. Mo, et al., ROR1 targeted immunoliposomal delivery of OSU-2S shows selective cytotoxicity in t(1;19)(q23;p13) translocated B-cell acute lymphoblastic leukemia, Leuk. Res. 118 (2022), 106872.
[174] S. Goswami, R. Mani, J. Nunes, C.-L. Chiang, K. Zapolnik, E. Hu, et al., PP2A is a therapeutically targetable driver of cell fate decisions via a c-Myc/p21 axis in human and murine acute myeloid leukemia, Blood 139 (2022) 1340–1358.
[175] G. Shlomai, Z. Zelenko, I.M. Antoniou, M. Stasinopoulos, A. Tobin-Hess, M. P. Vitek, et al., OP449 inhibits breast cancer growth without adverse metabolic effects, Endocr. Relat. Cancer 24 (2017) 519–529.
[176] C. Galiger, M. Dahlhaus, M.P. Vitek, K.-M. Debatin, C. Beltinger, PPP2CA is a novel therapeutic target in neuroblastoma cells that can be activated by the SET inhibitor OP449, Front. Oncol. 12 (2022), 744984.
[177] D.L. Brautigan, C. Farrington, G. Narla, Targeting protein phosphatase PP2A for cancer therapy: development of allosteric pharmaceutical agents, Clin. Sci. 135 (2021) 155–1556.
[178] C.C. Farrington, E. Yuan, S. Mazhar, S. Izadmehr, L. Hurst, B.L. Allen-Petersen, et al., Protein phosphatase 2A activation as a therapeutic strategy for managing MYC-driven cancers, J. Biol. Chem. 295 (2020) 757–770.
[179] B.L. Allen-Petersen, T. Risom, Z. Feng, Z. Wang, Z.P. Jenny, M.C. Thoma, et al., Activation of PP2A and inhibition of mTOR synergistically reduce MYC signaling and decrease tumor growth in pancreatic ductal adenocarcinoma, Cancer Res. 79 (2019) 209–219.
[180] J. Merisaari, O.V. Denisova, M. Doroszko, V. Le Joncour, P. Johansson, W.P. J. Leenders, et al., Monotherapy efficacy of blood-brain barrier permeable small molecule reactivators of protein phosphatase 2A in glioblastoma, Brain Commun. 2 (2020) fcaa002.

[181] X. He, M. Li, H. Yu, G. Liu, N. Wang, C. Yin, et al., Loss of hepatic aldolase B activates Akt and promotes hepatocellular carcinogenesis by destabilizing the Aldob/Akt/PP2A protein complex, PLoS Biol. 18 (2020), e3000803.
[182] K.D. Jayappa, B. Tran, V.L. Gordon, C.G. Morris, S. Saha, C.C. Farrington, et al., PP2A modulation overcomes multidrug resistance in chronic lymphocytic leukemia via mPTP-dependent apoptosis, J. Clin. Invest. (2023), https://doi.org/10.1172/JCI155938.
[183] R.N. Goto, L.M. Sobral, K. Stringhetta-Padovani, C.B. Garcia, G. da Silva, M. P. Vitek, et al., Synergic effect of OP449 and FTY720 on oral squamous cell carcinoma, Eur. J. Pharmacol. 882 (2020), 173268.
[184] N.P. Richard, R. Pippa, M.M. Cleary, A. Puri, D. Tibbitts, S. Mahmood, et al., Combined targeting of SET and tyrosine kinases provides an effective therapeutic approach in human T-cell acute lymphoblastic leukemia, Oncotarget 7 (2016) 84214–84227.
[185] A. Agarwal, R.J. MacKenzie, R. Pippa, C.A. Eide, J. Oddo, J.W. Tyner, et al., Antagonism of SET using OP449 enhances the efficacy of tyrosine kinase inhibitors and overcomes drug resistance in myeloid leukemia, Clin. Cancer Res. 20 (2014) 2092–2103.
[186] H.A. Omar, M.F. Tolba, J.-H. Hung, T.H. Al-Tel, OSU-2S/Sorafenib synergistic antitumor combination against hepatocellular carcinoma: the role of PKCδ/p53, Front. Pharmacol. 7 (2016) 463.
[187] M. Scarpa, P. Singh, C.M. Bailey, J.K. Lee, S. Kapoor, R.G. Lapidus, et al., PP2A-activating drugs enhance FLT3 inhibitor efficacy through AKT inhibition–dependent GSK-3b–mediated c-Myc and Pim-1 proteasomal degradation, Mol. Cancer Ther. 20 (2021) 676–690.
[188] S.J. Vervoort, S.A. Welsh, J.R. Devlin, E. Barbieri, D.A. Knight, S. Offley, et al., The PP2A-Integrator-CDK9 axis fine-tunes transcription and can be targeted therapeutically in cancer, Cell 184 (2021) 3143–3162.e32.
[189] Y. Tang, J. Berlind, N. Mavila, Inhibition of CREB binding protein-beta-catenin signaling down regulates CD133 expression and activates PP2A-PTEN signaling in tumor initiating liver cancer cells, Cell Commun. Signal 16 (2018) 9.
[190] S. Kins, P. Kurosinski, R.M. Nitsch, J. Götz, Activation of the ERK and JNK signaling pathways caused by neuron-specific inhibition of PP2A in transgenic mice, Am. J. Pathol. 163 (2003) 833–843.
[191] S. Das, J.E. Dixon, W. Cho, Membrane-binding and activation mechanism of PTEN, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 7491–7496.
[192] M. Rahdar, T. Inoue, T. Meyer, J. Zhang, F. Vazquez, P.N. Devreotes, A phosphorylation-dependent intramolecular interaction regulates the membrane association and activity of the tumor suppressor PTEN, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 480–485.
[193] H.K. Arnold, R.C. Sears, Protein Phosphatase 2A regulatory subunit B56α Associates with c-Myc and negatively regulates c-Myc accumulation, Mol. Cell. Biol. 26 (2006) 2832–2844.