Chapter

The Key Role of the Phosphatase PP2A in the Development of Acute Myeloid Leukemia

Javier Marco, Irene Peris, Carmen Vicente and Elena Arriazu

Abstract

Acute myeloid leukemia (AML) is a heterogeneous malignant disorder of hematopoietic progenitor cells characterized by the accumulation of several genetic and epigenetic mutations. Despite the progressive understanding of the molecular heterogeneity of the disease, the survival rate of patients older than 60 years old remains poor. Therefore, it is necessary to develop an effective treatment strategy for those patients in order to beat the disease and improve life quality. Reversible phosphorylation has been widely studied over the last years, and the deregulation of kinases and phosphatase have been verified to have a huge impact in leukemogenesis. Inactivation of the tumor-suppressor protein phosphatase 2A (PP2A) is frequent in AML patients, constituting a promising target for cancer therapy. There are several PP2A inactivation mechanisms. However, overexpression of SET or cancerous inhibitors of PP2A, both endogenous inhibitors of PP2A, are recurrent events in AML patients, leading to the inactivation of the phosphatase PP2A. Preclinical studies show that PP2A reactivation using PP2A-activating drugs (PADs) manage to stop the development of the disease, and its combination with conventional chemotherapy and tyrosine kinase inhibitors have a synergistic cytotoxic effects. Recent studies have demonstrated that specifically activation of PP2A subunits, target crucial pathogenic drivers, increasing the efficacy of conventional treatments and opening new possibilities for personalized treatment in AML patients, especially in cases of PP2A deregulation. Here, we review the role of PP2A in AML as well as its drugable options.

Keywords: AML, PP2A, SET, PADs, FTY720, CM-1231

1. Introduction

Acute myeloid leukemia (AML) is a heterogeneous clonal disorder characterized by the accumulation of poorly differentiated cells, derived from the differentiation blockage of myeloid hematopoietic progenitors in the bone marrow (BM) [1]. As consequence, immature cells called "blast" displace other cell populations invading the BM and other tissues [2, 3].

AML is a malignant disorder of the bone marrow characterized by the clonal expansion and differentiation arrest of myeloid progenitor cells. Incidence increases with age, with 68 years being the median age at diagnosis. AML is the most common form of acute leukemia in adults and has the shortest survival. Effective therapies, including intensive chemotherapy and allogeneic stem cell transplantation, are

generally applicable to young patients, while treatment options for older patients (≥65 years), which are the largest group, have historically been limited to DNA methyltransferase inhibitors (i.e. azacitidine and decitabine) and low doses of cytarabine, and have only provided a modest benefit [1, 4, 5]. Besides, treatment is often ineffective in both groups due to drug resistance and relapse, particularly in patients with FMS-like tyrosine kinase 3 (FLT3)-internal tandem duplication (ITD), that represent ~25% of all AML cases, and have poor outcome, with high risk of relapse and low cure rates [1–6]. The AML treatment landscape has changed substantially since 2017. New targeted drugs have emerged, including midostaurin and gilteritinib to target FLT3, and venetoclax to target BCL-2 [1]. This has created novel treatment options, especially in older as well as in refractory/relapsed patients. The natural history of FLT3-mutated AML is changing after the approval of midostaurin for frontline therapy and gilteritinib for relapsed or refractory patients. Nevertheless, despite initial clinical responses to FLT3 kinase inhibitors (FKIs), patients eventually relapse. Mechanisms of resistance include the acquisition of secondary FLT3 mutations and protective stromal signaling within the bone marrow niche [2–4]. In the same way, venetoclax combined with hypomethylating agents or low-dose cytarabine is an effective therapy for older or unfit patients with AML, which represents most of the cases. However, it is now clear that multiple resistant sub-clones evolving contemporaneously during therapy can occur in AML and act as a barrier to the long-term success of targeted therapies. Studies about the molecular determinants of outcome with clinical relevance to patients with AML show that FLT3-ITD mutations or TP53 loss conferred cross-resistance to both venetoclax and cytotoxic-based therapies [5]. Besides, even with these and other potent targeted therapies, the disease persists within the bone marrow microenvironment, mainly due to activating parallel signaling pathways that maintain pro-survival factors. Therefore, acquired resistance to these targeted drugs remains a challenge and provides a rationale for combining either FLT3 inhibitors or venetoclax with other therapies, both conventional and investigational [6]. Reversible phosphorylation of proteins is a post-translational modification that regulates all aspect of life through the antagonistic action of kinases and phosphatases. Protein kinases are popular drug targets and are well characterized, but protein phosphatases have been relatively neglected [7]. In this chapter, we will focus on the role of protein phosphatase 2A (PP2A), inactivation of which is a recurrent event in AML, as a druggable tumor suppressor.

2. Protein phosphatase 2A

PP2A, a ubiquitously expressed protein serine/threonine phosphatase in mammalian cells, is a tumor suppressor that regulates essential cell processes and counteracts most of kinases-driven intracellular signaling pathways [7–11]. Recent evidences indicate that PP2A inactivation arises in several solid and hematological tumors causing the prolong activation of survival pathways or the inhibition of apoptotic pathways, pointing out its relevance in leukemogenesis [9, 12–14]. The use of okadaic acid (OA), a potent tumor promoter that inhibits PP2A activity, has greatly contributed to the understanding of the phosphatase functions [15].

PP2A appear in two different forms: a dimeric and a trimeric form [9, 16]. The dimer, known as the core enzyme, consists of a structural A subunit (PP2A-A) and a catalytic C subunit (PP2A-C), whereas the trimeric form, is comprised by a structural A subunit, a catalytic C subunit and a regulatory B subunit (PP2A-B). Interestingly, the function of the scaffold subunit varies depending on the PP2A complex. In the heterotrimeric form, PP2A-A mediates the interaction between the

catalytic subunit with the regulatory subunit, while in the dimeric form, it acquires a regulatory function changing the catalytic specificity. Furthermore, each subunit is encoded by different genes, which further generate distinct isoforms. PP2A-A (PPP2R1/Aα and PPPR1B/Aβ) and PP2A-C (PPP2CA/Cα and PPP2CB/Cβ) are more conserve, whereas in PP2A-B four families of genes (B/PR55/B55, B′/PR61/B56, B″/PR72, B‴/The striatins, STRN) have been recognized including 23 different alternative transcript and spliced forms, which determine the substrate specificity and intracellular localization of PP2A (**Figures 1** and **2**) [12, 14, 17, 18]. Therefore, the actual challenge is not only to identify deregulation of PP2A functions in AML patients, but also to recognize the subunit affected with the goal to develop efficient target therapies [19].

The precise mechanism of PP2A active complex assembly remains obscure, but there are evidence that determine that post-translational modifications of PP2A-C residues, such as methylation and phosphorylation, plays an essential role in modulating the formation of active PP2A holoenzym. For instance, the methylation of PP2A-C subunit in leucine 309 (L09) by leucine carboxyl methyltransferase I is crucial for PR55/B55 binding, being not an essential requisite for other B families subunits [20–22]. However, post-translational modifications not only have an activating role, but also inhibitor since phosphorylation of tyrosine 307 (Y307) impairs the interaction of PP2A-C with the PR55/B55 and PR61/B56 subunits [20]. Interestingly, both cell lines and AML patient samples show an increase of Y307 phosphorylation [23]. On the

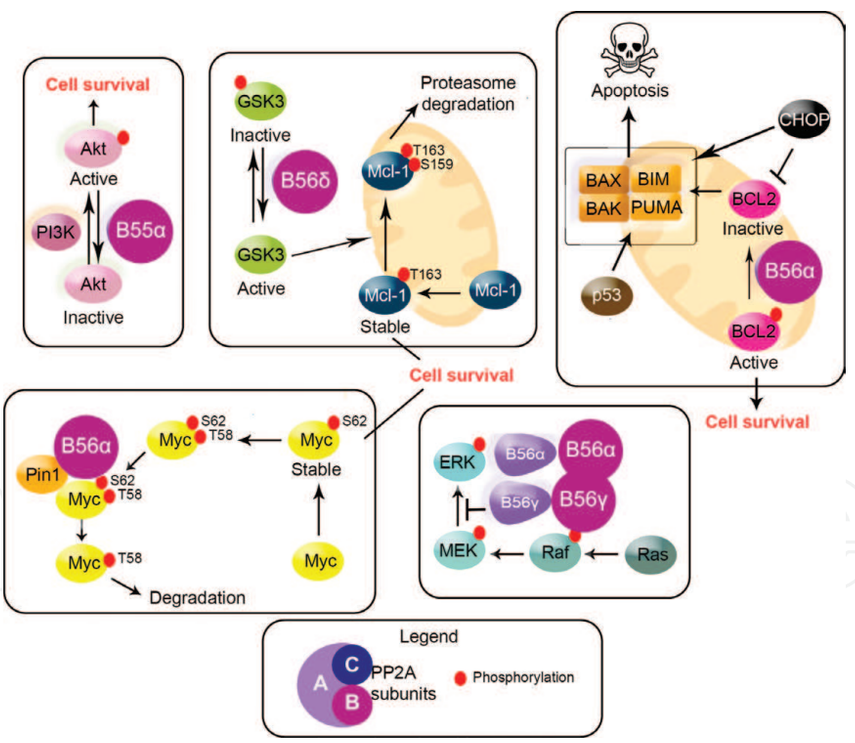
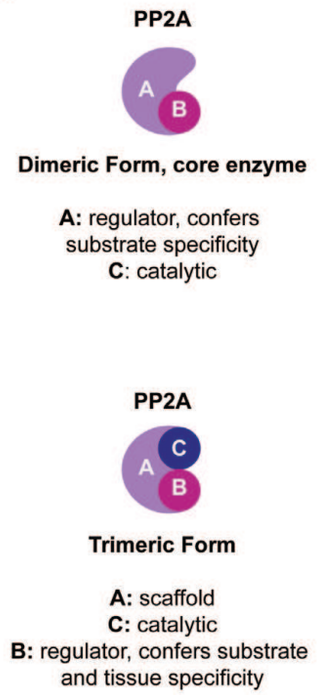


Figure 1.
*Signaling pathways involving PP2A in AML. Scheme showing some of the molecular pathways regulated by PP2A complexes. Different isoforms of PP2A regulatory subunits are shown. The regulatory subunit B55α regulates the Akt pathway by dephosphorylating and inactivating Akt, which is the responsible of GSK3 phosphorylation and inactivation. On the other hand, B56δ PP2A regulatory subunit dephosphorylates and activates GSK3. Active GSK3 can phosphorylate MCl-1 in S159 (previous phosphorylation in T163 by ERK), leading to MCL-1 proteasome degradation and contributing to apoptosis. Active GSK3 can also phosphorylate Myc in T58 (previous phosphorylation in S62 by ERK), leading to the binding of B56α PP2A regulatory subunit, which dephosphorylates Myc in S62, leaving T58 phosphorylation that generates Myc instability and proteasome degradation. B56α can also dephosphorylate and inactivate BCL-2, activating the caspase dependent apoptosis. B56γ and B56α PP2A regulatory subunits control the MEK/ERK pathway, which is responsible of MCL-1 and Myc stability. *B regulatory PP2A subunits are exemplified in representation of PP2A enzyme, which is represented in the legend. Red dots are symbolized as phosphate groups.*

A



B

Subunit	Isoform	Gene name	Other names
A	α	PPP2R1A	PR65α
	β	PPP2R1B	PR65β
C	α	PPP2CA	PPP2cα
	β	PPP2CB	PPP2Acβ
B	α	PPP2R2A	B55α, PR55α
	β	PPP2R2B	B55β, PR55β
	γ	PPP2R2C	B55γ, PR55γ
	δ	PPP2R2D	B55δ, PR55δ
B'	α	PPP2R5A	B56α, PR56/61α
	β	PPP2R5B	B56β, PR56/61β
	γ	PPP2R5C	B56γ, PR56/61γ
	δ	PPP2R5D	B56δ, PR56/61δ
	ε	PPP2R5E	B56ε, PR56/61ε
B"	α	PPP2R3A	PR72, B"α2
	β	PPP2R3B	PR70, PR48, B"β
	γ	PPP2R3C	G5PR, G4-1
	δ	PPP2R3D	PR59, B"δ
B"'		STRN	Startin, PR110
		STRN3	SG2NA
		STRN4	
		PPP2R4	PTPA, PR53

Figure 2.
PP2A subunits. (A) Schematic representation of PP2A subunits and their functions. (B) all different isoforms of every subunit of PP2A.

other hand, post-translational modifications of PP2A-B can also affect the localization of the holoenzyme, complicating its targeting [24].

We and others have determined that PP2A deregulation is a common event in AML patients, and the restoration of PP2A activity with PP2A activating drugs (PADs), such as FTY720, has potent antileukemic effects in AML cells, preventing cell growth and inducing caspase-dependent apoptosis [12, 13, 23, 26–28]. However, FTY720 induces cardio-toxicity at the anti-neoplastic dose. Hence, we develop a novel non-phosphorylable FTY720 analogue called CM-1231, which has a great antileukemic potential without inducing secondary effects [28]. Furthermore, we have shown that PADs can be used in combination with kinase inhibitors or chemotherapy agents, suggesting that PP2A activity restoration could have a huge therapy potential in AML patients [23, 25, 27, 29–32].

2.1 Mechanism of PP2A inactivation in AML

Several somatic mutations have been described in PP2A subunits in different types of tumors such as melanoma, colon, lung and breast cancers [19, 33–39]. Mutations in PP2A-Aα or PP2A-Aβ subunits cause defective binding of B and C subunits, inhibiting PP2A active holoenzyme and favoring a malignant cell transformation [36, 37]. However, the frequency of PP2A inactivation due to mutations is low, with PPP2R1A subunit owning the highest mutational percentage rate (1,17%), and it seems to be an uncommon mechanism in AML. Likewise, our analysis of the genome of 250 patients with leukemia from the Cancer Genome Atlas Research Network (https://tcga-data.nci.nih.gov/tcga), show that only one patient has somatic mutations in PPP2R2B, which encode for PR55β subunit [14, 40].

Thus, the main mechanism that employs cancer cells to evade PP2A-mediated tumor suppression is through the overexpression of proteins that

mediate PP2A post-translational modifications or molecules that inactivates the holoenzyme function [41–43].

2.2 SET/I2PP2A

The SET oncoprotein, also known as I2PP2A (Inhibitor 2 of PP2A), TAF-1β or PHAP1, is a potent endogenous PP2A inhibitor that plays an essential role in myeloid leukemias (**Figure 3**) [44]. Firstly, SET was identified as an oncogene fused with nucleoporin NUP214 (CAN) in undifferentiated leukemias [45], to later be considered as a PP2A inhibitor [46]. This protein is mostly located in the nucleus, and is implicated in a wide range of cell processes such as DNA replication, gene transcription, chromatin remodeling [47, 48], DNA repair [49], cell differentiation [50], migration [51] and cell-cycle regulation [52]. SET is up-regulated in hematological and solid tumors, including breast cancer [53] and colorectal cancer [54]. Its role has been studied in depth in chronic myeloid leukemia (CML). Interestingly, patients with BCR-ABL1 gene fusion, which constitutively activates tyrosine kinase activity, essential for CML emergence, maintenance and progression, have SET overexpression [55]. The expression of BCR-ABL1 allows recruitment and activation of JAK2, which enhance β-catenin activity and induce SET-mediated inactivation of PP2A [56].

Likewise, SET overexpression is also an important event in AML. We performed a quantification of SET expression in AML patients, observing that SET overexpression is a recurrent event (60/214, 28%) associated with poor survival in AML. Furthermore, the protein overexpression has a prognostic impact in patients with

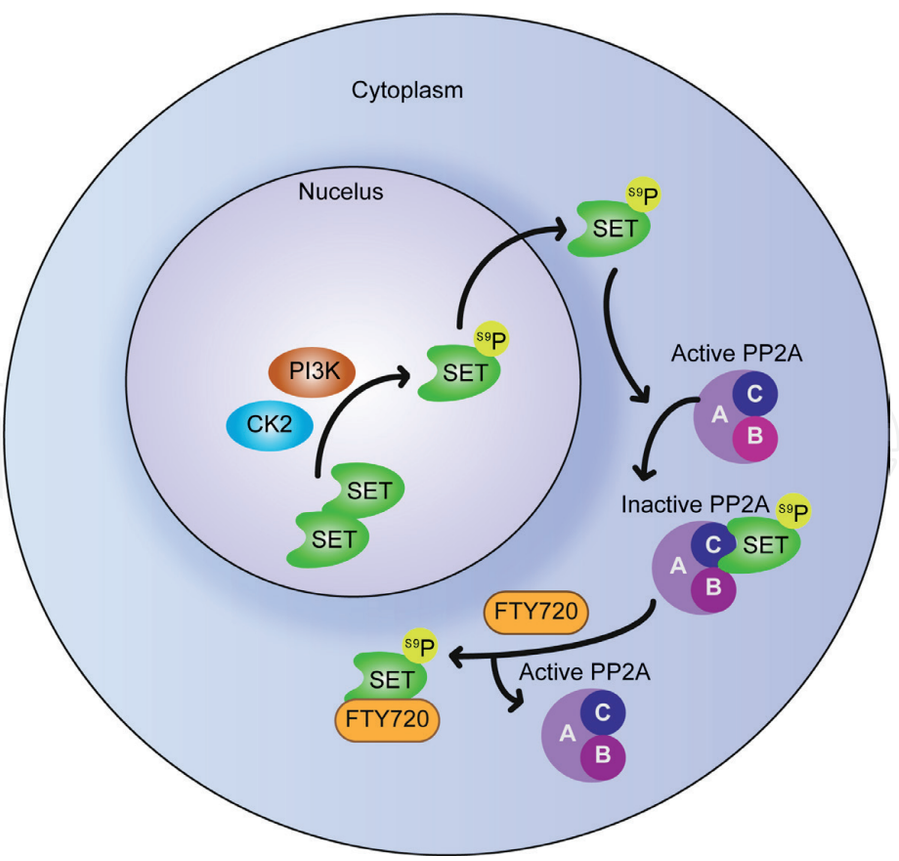


Figure 3.
PP2A inactivation by SET in AML. PI3K and CK2 can phosphorylate SET at serine 9 (S9), located in the nuclear localization signal. This phosphorylation translocates SET to the cytosol and impairs its return to the nucleus, increasing its ability to bind to the catalytic subunit of PP2A (PP2A-C), and inactivating PP2A. Treatment with FTY720 disrupts SET–PP2A interaction, allowing PP2A activation [57].

normal karyotype, defining a subgroup of patients with worse outcome. Additional observations reveals that SET overexpression is associated with other adverse prognostic markers such as monosomy 7, SET binding protein a (SETBP1) overexpression and EVI1 overexpression, suggesting that this oncoprotein could cooperate with other additional aberrations in leukemogenesis program. Our analysis by western blot confirmed that SET is overexpressed at protein levels in both AML cell lines and patients samples [29].58.

In addition, we observed that SET promote cell survival by inhibiting PP2A activity through its binding to PP2A-C, forming an inhibitory complex that prevent phosphatase activity (**Figure 3**) [30]. That is the main reason why the use of PADs such as FTY720, OP449 or its analogues, show potent antileukemic effects, since prevent the interaction between SET and PP2A, recovering the antitumoral activity of PP2A [27, 28, 30]. Nevertheless, despite the importance of SET overexpression and its prognostic impact in hematological tumors, little is known about the mechanism involved in SET regulation, constituting a barrier to the development of new PP2A activating drugs.

Recent studies have described mechanism of post-translational regulation of SET that modulate the inhibitory activity against PP2A [58, 57]. Using genetic and pharmacological approaches, we found that p38β has a dual role in SET regulation in AML. We found that p38β up-regulation, but not p38α, is a common event in AML that contributes to SET-mediated PP2A inactivation [57]. It has been reported that p38 form complexes with PP2A [59–66]. However, their connection can vary depending on the cellular context. Upon TNF-induced stress conditions in endothelium-derived cell lines, p38 positively regulates PP2A activity [63], whereas under hypoxia and survival conditions, PP2A negatively regulates p38 activity [65]. Nevertheless, the regulatory mechanism has not been discovered until now. We show for the first time that p38β contributes to PP2A inactivation via SET regulation through two mechanisms: (i) p38β promotes the phosphorylation of the casein kinase 2 (CK2) which active form phosphorylates SET on Ser9, located in a nuclear localization signal, favoring the retention of SET into the cytoplasm and consequence inhibition of PP2A. Thus, p38β is involved in SET trafficking to the cytosol and PP2A inactivation through a CK2-dependent manner. (ii) p38β also binds to SET stabilizing the oncoprotein and avoiding its degradation [57].

Similarly, it had previously described another mechanism in AML that impairs PP2A activity through the stabilization of SET in the cytoplasm. SETBP1 is a protein located in the cytoplasm that binds and stabilizes the 39 kDa full-length SET, protecting the oncoprotein from protease cleavage, and facilitating PP2A inactivation and cell proliferation. Interestingly, SETBP1 overexpression is a common event in AML, affecting the 28% of AML patients and diminishing the overall survival [29]. Later studies in other myeloid neoplasm have confirmed the crucial role of SETBP1 in leukemogenesis.

On the other hand, SET is also implicated in natural killer (NK) cell cytotoxicity. Upon cytokine stimulation (Interlukin-12, –18 and – 15), SET up-regulation impairs IFN-γ production in human NK via PP2A inactivation, limiting the anti-tumor and/or anti-inflammatory activity of the NK cells [67]. Trotta *et al*. described a model where SET/PP2A regulates granzyme B expression which leads to determine NK cytotoxicity. They observed that SET knockdown inhibited the expression of granzyme B at mRNA and protein levels, limiting NK cytotoxicity [68].

Others have reported SET as an inhibitor of the DNAse activity of the tumor-suppressor NM23-H1; a promoter of AP-1 activity; or an activator of MAPK signaling. These data suggest that SET not only induce the inactivation of PP2A but also promotes other signaling pathways ensure tumor growth.

2.3 Cancerous inhibitor of PP2A (CIP2A)

Another endogenous PP2A inhibitor is cancerous inhibitor of PP2A (CIP2A) [69], an oncoprotein that controls oncogenic cellular signals by inhibiting PP2A activity through the stabilization of c-MYC [21, 69–71], which play an important role in AML [72].

CIP2A is expressed in very few tissues in normal conditions but it is overexpressed in a wide variety of human cancers, where it is associated with an aggressive clinical behavior [70, 71, 73–76]. However, few studies have focused on AML. Wang et al. using conventional PCR found that 77.4% of AML patients [55 of 84] overexpressed CIP2A, confirming their results at protein levels, however, they did not provide quantitative data to support that [77]. Recently, our group using quantitative real-time RT-PCR studied the prevalence of this oncoprotein in a series of 203 normal karyotype AML patients. We reported that CIP2A overexpression is a recurrent event in this subgroup of the disease (51/203, 25%), and is associated with a very poor prognostic impact in the overall survival of normal karyotype AML patients. Our results indicate that CIP2A knockout downregulates c-MYC, leading to a reduction of the cell proliferation, supporting the malignant role of CIP2A and c-MYC in leukemogenesis [31].

In addition, cancerous inhibitor of PP2A has been extensively studied in CML. Similarly, high levels of CIP2A were found in CML patients at diagnosis being significantly associated with risk of progression to blast crisis. Therefore, CIP2A protein levels have been postulated as a biomarker of disease progression in Imatinib-treated CML patients [78]. Furthermore, as indicated above with SET, high levels of CIP2A are associated with an up-regulation of c-MYC and BCR-ABL1 tyrosine kinase activity [78]. However, second-generation tyrosine kinase inhibitors (TKI) manage the disruption of CIP2A/c-MYC/E2F1 loop, preventing the malignant progression and constituting a promising therapeutic strategy [79]. These data support that CIP2A inhibits PP2A activity, stabilizing E2F1, and creating a CIP2A/c-MYC/E2F1 positive feedback loop, which imatinib cannot overcome [78]. However, greater efforts are need to elucidate the exact role of CIP2A in leukemias.

2.4 PP2A-activating drugs

The increased number of studies pointing to the crucial role of PP2A inactivation in cancer growth has led to the development of drugs that favors PP2A reactivation [12, 80]. The most widely studied drugs are FTY720 and OP449, but its limitations have encouraged the search of new drugs that have greater efficacy and clinical applicability.

FTY720, an oral sphingosine analog derived from myriocin, is a metabolite isolated from fungus Isaria Sinclairii that has been approved for the treatment of patients with relapse multiple sclerosis, but recently it has been studied for its potential antitumoral properties [81]. FTY720 is administrated as a pro-drug, which needs an activation by phosphorylation through sphingosine kinase 2, binding the active form to one of the sphingosine-1-phosphate receptors (S1P1, S1P3, S1P4 or S1P5). The phosphorylated form does not prevent T-lymphocyte or B-lymphocyte activation, but does interfere with the immune cell trafficking from the lymphoid organs to the peripheral blood [82]. Likewise, FTY720 is a potent inhibitor of tumor growth and angiogenesis, being attractive its use in the treatment of both solid and hematological tumors. Interestingly, the anticancer activity of the drug depends on the ability to act as a PP2A activator [83], inducing apoptosis by interfering with Bcl-2, and suppressing mitogenic and survival signals, and inhibiting the ERK and PI3K/AKT pathways [13, 84].

Mechanistically, FTY720 binds to globular amphipathic domain of the C-terminal hydrophobic pocket of SET [85], preventing the formation of the SET/PP2A-C inhibitory complex and reactivating PP2A functionality [12, 29–32]. Our group has confirmed these results in AML, showing that FTY720 binds to SET within the last 100 amino acids of the C-terminal fragment, producing a destabilization of the SET/PP2A-C inhibitory complex, which promote PP2A reactivation and a reduction of AML cell viability [30]. Several reports back it up pointing out the efficacy of FTY720 in vitro and in vivo models of AML, suggesting that PP2A restoration decreases clonogenicity and induces a suppression of the disease [12, 29–32]. Moreover, FTY720 perturbs the sphingolipid metabolism pathway, favoring the accumulation of ceramide, a pro-apoptotic second messenger, mostly in the mitochondria, leading to the death of AML cells [86]. In the same way as in AML, the effects induced by FTY720 are well characterized in Ph positive and negative leukemias. In CML and Ph-positive B-ALL progenitors, the drug promotes the BCR-ABL1 inactivation and degradation, leading to the inhibition of survival factors such as JAK2, AKT and ERK1/2, which results in apoptosis of CD34+ progenitors in patients with TKI sensitive and TKI-resistant CML [12, 55, 84]. In addition, a recent study provide new evidences for the use of FTY720 as an oral therapeutic agent in AML, highlighting that FTY720 lipid nanoparticles were more effective in vitro and in vivo models than FTY720 solutions because are able to increase the bioavailability of the free drug [32]. However, the main problem of the usage of FTY720 continues due to the induction of cardiotoxicity at the anti-neoplastic dose by the phosphorylated form. So, it has been proposed FTY720 analogues that are not targets for phosphorylation by SPHK2 [28].

Our group has recently revealed a novel non-phosphorylable FTY720 analogue called CM-1231, which reactivates PP2A activity by preventing the formation of the SET/PP2A-C inhibitory complex, inhibiting cell proliferation and promoting apoptosis in AML cell lines and primary patient samples. Importantly, CM-1231 does not induce cardiotoxicity in zebrafish models, maintaining its anti-leukemic potential in zebrafish xenograft models [28].

Other molecules have been tested to activate PP2A in AML, such as OP449 [87]. OP449 is a small physiological stable and cell-penetrating peptide, which binds specifically to SET leading to PP2A reactivation. It has been shown that OP449 treatment suppress tumor growth, enhance apoptosis and impairs clonogenicity of CML and AML cell lines and primary samples [87, 88]. Furthermore, the combination of OP449 with chemotherapy or specific TKI in AML and CML cell lines and primary patient samples have a synergistic effect [27]. However, OP449 like others PADs are unable to activate specific PP2A complexes against the exact pathogenic driver of the disease.

The ability of PP2A to dephosphorylate hundreds of proteins is mediated by over 40 specificity-determining B subunit, which competes for the assembly and activation of PP2A heterogeneous complex [89–91]. Therefore, it is essential to identify which regulatory isoform is deregulated in order to selectively reactivate it and direct PP2A against pathogenic drivers [92–94]. DT-061, a SMAP (small molecules that activate the phosphatase PP2A), selectively binds and stabilizes a PP2A complex containing a single B-subunit, B56α, which promote the dephosphorylation of selective PP2A substrates such as c-Myc. Stabilization of the PP2A-B56α complex by DT-061 has shown potent anti-leukemic effect, and their combination with TKI have improve anti-tumor effects while provide an opportunity to decrease kinase inhibitors related toxicities in some malignancies such as lung adenocarcinoma [95]. Interestingly, Kauko *et al*. determined that PP2A inactivation is a mechanism of kinase inhibitor resistance in cancer, thus the use of DT-061 could overcome the initial therapeutic resistance [96]. These observations raise the question on the

appropriate temporal application of the drug: before the appearance of the resistance or upon its arrival. Whatever the answer, the important fact is that developing drugs against specific B regulatory subunits is a key event to face crucial pathogenic drivers [95].

Similarly, a class of small-molecules iHAPs (improved heterocyclic activators of PP2A) facilitate the assembly of the holoenzyme PP2R1A-B56ε-PPP2CA, which dephosphorylates MYBL2 transcription factor in Ser241, causing irreversible arrest of leukemic cells in the prometaphase [97]. Thus, the use of these molecules to target deregulated PP2A subunits; facilitate the activation/deactivation of specific molecular targets deregulated by PP2A inactivation in the tumoral scenario, reducing the toxicity induced by general activation of PP2A.

These findings open new possibilities to establish innovative therapeutic approach that targets PP2A in order to improve therapeutic options in AML patients.

3. Conclusion

Despite cytogenetic heterogeneity in AML was discovered 30 years ago, it was not until 15 years ago when the molecular heterogeneity of the disease began to be studied in depth. However, the general therapeutic strategy in AML patients has not changed substantially and high dose of chemotherapy continues to be the standard one. Consequently, the outcome for most patients, especially elder patients, remains poor. Thus, many new drugs targeting a variety of pathological cellular processes have been developed over the last years for the treatment of AML, although few have been translated into clinical practice. The reason is that they are used as single agents instead of following a combinatory therapy, decreasing its effectiveness. The Cancer Genome Atlas Research Network confirmed the molecular heterogeneity of the disease and organized important mutated genes in AML into a functional category, pointing out the importance of developing new compound against specific cancer pathways. In this regard, the tumor-suppressor PP2A has emerged as an important promising therapeutic target because its anti-proliferative function is inactivated in a large part of patients with AML.

PP2A inactivation is a recurrent event in AML patients. PP2A reactivation by PADs has shown important antileukemic effects in both KIT-positive and KIT-negative AML cells. Preclinical studies show that pharmacological restoration of PP2A tumor-suppressor activity by PADs (FTY720, OP499 or CM-1231) prevents the growth of tumor cells, increasing the cell death ratio. Furthermore, the combination of these drugs with both conventional chemotherapy and tyrosine kinases has synergistic cytotoxic effects in AML cells, decreasing the appearance of side effects. However, recently, have been developed small molecules that are capable of activating specific PP2A complexes that target particular disease-causing pathogenic pathways. The importance on knowing which B subunit is deregulated to applied a specific compound that reactivates this subunit opens new possibilities for personalize medicine, or personalized treatment, which improve the overall survival of patients with hematopoietic and non-hematopoietic malignancies.

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Conflict of interest

The authors declare no conflict of interest.

Abbreviations

AML	acute myeloid leukemia
BM	bone marrow
CIP2A	cancerous inhibitor of PP2A
CK2	casein kinase 2
CML	chronic myeloid leukemia
iHAPs	improved heterocyclic activators of PP2A
NK	natural killer
OA	okadiac acid
PADs	PP2A-activating drugs
PP2A	protein phosphatase 2A
SETBP1	SET binding protein 1
TKI	tyrosine kinase inhibitors

Author details

Javier Marco[1,2], Irene Peris[2,3], Carmen Vicente[1,2,3] and Elena Arriazu[1,2,4*]

1 Centro de Investigación Médica Aplicada (CIMA), University of Navarra, Pamplona, Spain

2 Biochemistry and Genetics Department, University of Navarra, Pamplona, Spain

3 IdiSNA, Instituto de Investigación Sanitaria de Navarra, Pamplona, Spain

4 CIBERONC, Instituto de Salud Carlos III, Madrid, Spain

*Address all correspondence to: earriazu@unav.es

References

[1] DiNardo CD, Wei AH. How I treat acute myeloid leukemia in the era of new drugs. Blood. 2020;**135**:85-96

[2] Perl AE. Availability of FLT3 inhibitors: how do we use them? Blood. 2019;134:741-45.

[3] Smith CC. The growing landscape of FLT3 inhibition in AML. Hematology Am Soc Hematol Educ Program. 2019;2019:539-47.

[4] Antar AI, Otrock ZK, Jabbour E, Mohty M, Bazarbachi A. FLT3 inhibitors in acute myeloid leukemia: ten frequently asked questions. Leukemia. 2020. Published 2020 Jan 9.

[5] DiNardo CD, Tiong IS, Quaglieri A, et al. Molecular patterns of response and treatment failure after frontline venetoclax combinations in older patients with AML. Blood. 2020. Published 2020 Jan 13.

[6] Daver N, Schlenk RF, Russell NH, Levis MJ. Targeting FLT3 mutations in AML: review of current knowledge and evidence. Leukemia. 2019;33:299-312.

[7] Fowle H, Zhao Z, Graña X. PP2A holoenzymes, substrate specificity driving cellular functions and deregulation incancer. Adv Cancer Res. 2019;144:55-93

[8] Bertolotti A. The split protein phosphatase system. Biochem J. 2018;475:3707-3723.

[9] Mumby M. PP2A: Unveiling a Reluctant Tumor Suppressor. Cell. 2007 Jul;130(1):21-4.

[10] Westermarck J, Hahn WC. Multiple pathways regulated by the tumor suppressor PP2A in transformation. Trends Mol Med. 2008 Apr;14(4):152-60.

[11] Low ICC, Loh T, Huang Y, Virshup DM, Pervaiz S. Ser70 phosphorylation of Bcl-2 by selective tyrosine nitration of PP2A-B56δ stabilizes its antiapoptotic activity. Blood. 2014 Oct 2;124(14):2223-34.

[12] Perrotti D, Neviani P. Protein phosphatase 2A: a target for anticancer therapy. Lancet Oncol. 2013 May;14(6):e229-38.

[13] Neviani P, Harb JG, Oaks JJ, Santhanam R, Walker CJ, Ellis JJ, et al. PP2A-activating drugs selectively eradicate TKI-resistant chronic myeloid leukemic stem cells. J Clin Invest. 2013 Oct 1;123(10):4144-57.

[14] Arriazu E, Pippa R, Odero MD. Protein Phosphatase 2A as a Therapeutic Target in Acute Myeloid Leukemia. Front Oncol. 2016 Apr 6;6.

[15] Schönthal AH. Role of serine/threonine protein phosphatase 2A in cancer. Cancer Lett. 2001 Sep;170(1):1-13.

[16] Haesen D, Sents W, Lemaire K, Hoorne Y, Janssens V. The Basic Biology of PP2A in Hematologic Cells and Malignancies. Front Oncol. 2014 Dec 11;4.

[17] Eichhorn PJA, Creyghton MP, Bernards R. Protein phosphatase 2A regulatory subunits and cancer. Biochim Biophys Acta - Rev Cancer. 2009 Jan;1795(1):1-15.

[18] Lambrecht C, Haesen D, Sents W, Ivanova E, Janssens V. Structure, Regulation, and Pharmacological Modulation of PP2A Phosphatases. In 2013. p. 283-305.

[19] Sablina AA, Hector M, Colpaert N, Hahn WC. Identification of PP2A Complexes and Pathways Involved in

Cell Transformation. Cancer Res. 2010 Dec 15;70(24):10474-84.

[20] Janssens V, Longin S, Goris J. PP2A holoenzyme assembly: in cauda venenum (the sting is in the tail). Trends Biochem Sci. 2008 Mar;33(3):113-21.

[21] Leulliot N, Quevillon-Cheruel S, Sorel I, de La Sierra-Gallay IL, Collinet B, Graille M, 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. 2004 Feb 27;279(9):8351-8.

[22] Longin S, Zwaenepoel K, Louis J V., Dilworth S, Goris J, Janssens V. Selection of Protein Phosphatase 2A Regulatory Subunits Is Mediated by the C Terminus of the Catalytic Subunit. J Biol Chem. 2007 Sep 14;282(37):26971-80.

[23] Cristóbal I, Garcia-Orti L, Cirauqui C, Alonso MM, Calasanz MJ, Odero MD. PP2A impaired activity is a common event in acute myeloid leukemia and its activation by forskolin has a potent anti-leukemic effect. Leukemia. 2011 Apr 14;25(4):606-14.

[24] Bononi A, Agnoletto C, De Marchi E, Marchi S, Patergnani S, Bonora M, et al. Protein Kinases and Phosphatases in the Control of Cell Fate. Enzyme Res. 2011;2011:1-26.

[25] Roberts KG, Smith AM, McDougall F, Carpenter H, Horan M, Neviani P, 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. 2010 Jul 1;70(13):5438-47.

[26] Ramaswamy K, Spitzer B, Kentsis A. Therapeutic Re-Activation of Protein Phosphatase 2A in Acute Myeloid Leukemia. Front Oncol. 2015 Feb 2;5.

[27] Agarwal A, MacKenzie RJ, Pippa R, Eide CA, Oddo J, Tyner JW, et al. Antagonism of SET Using OP449 Enhances the Efficacy of Tyrosine Kinase Inhibitors and Overcomes Drug Resistance in Myeloid Leukemia. Clin Cancer Res. 2014 Apr 15;20(8):2092-103.

[28] Vicente C, Arriazu E, Martínez-Balsalobre E, Peris I, Marcotegui N, García-Ramírez P, et al. A novel FTY720 analogue targets SET-PP2A interaction and inhibits growth of acute myeloid leukemia cells without inducing cardiac toxicity. Cancer Lett. 2020 Jan;468:1-13.

[29] Cristobal I, Garcia-Orti L, Cirauqui C, Cortes-Lavaud X, Garcia-Sanchez MA, Calasanz MJ, 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. 2012 Apr 1;97(4):543-50.

[30] Pippa R, Dominguez A, Christensen DJ, Moreno-Miralles I, Blanco-Prieto MJ, Vitek MP, et al. Effect of FTY720 on the SET–PP2A complex in acute myeloid leukemia; SET binding drugs have antagonistic activity. Leukemia. 2014 Sep 30;28(9):1915-8.

[31] Barragan E, Chillon MC, Castello-Cros R, Marcotegui N, Prieto MI, Hoyos M, et al. CIP2A high expression is a poor prognostic factor in normal karyotype acute myeloid leukemia. Haematologica. 2015 May 1;100(5):e183-5.

[32] de Mendoza AE-H, Castello-Cros R, Imbuluzqueta E, Cirauqui C, Pippa R, Odero MD, et al. Lipid Nanosystems Enhance the Bioavailability and the Therapeutic Efficacy of FTY720 in Acute Myeloid Leukemia. J Biomed Nanotechnol. 2015 Apr 1;11(4):691-701.

[33] Sangodkar J, Farrington CC, McClinch K, Galsky MD, Kastrinsky DB, Narla G. All roads lead

to PP2A: exploiting the therapeutic potential of this phosphatase. FEBS J. 2016 Mar;283(6):1004-24.

[34] Wang SS. Alterations of the PPP2R1B Gene in Human Lung and Colon Cancer. Science (80-). 1998 Oct 9;282(5387):284-7.

[35] Calin GA, di Iasio MG, Caprini E, Vorechovsky I, Natali PG, Sozzi G, et al. Low frequency of alterations of the α (PPP2R1A) and β (PPP2R1B) isoforms of the subunit A of the serine-threonine phosphatase 2A in human neoplasms. Oncogene. 2000 Feb 7;19(9):1191-5.

[36] Ruediger R, Pham HT, Walter G. Alterations in protein phosphatase 2A subunit interaction in human carcinomas of the lung and colon with mutations in the Aβ subunit gene. Oncogene. 2001 Apr;20(15):1892-9.

[37] Ruediger R, Pham HT, Walter G. Alterations in protein phosphatase 2A subunit interaction in human carcinomas of the lung and colon with mutations in the Aβ subunit gene. Oncogene. 2001 Apr;20(15):1892-9.

[38] Esplin ED, Ramos P, Martinez B, Tomlinson GE, Mumby MC, Evans GA. The glycine 90 to aspartate alteration in the Aβ subunit of PP2A (PPP2R1B) associates with breast cancer and causes a deficit in protein function. Genes, Chromosom Cancer. 2006 Feb;45(2):182-90.

[39] Kalla C, Scheuermann MO, Kube I, Schlotter M, Mertens D, Döhner H, et al. Analysis of 11q22–q23 deletion target genes in B-cell chronic lymphocytic leukaemia: Evidence for a pathogenic role of NPAT, CUL5, and PPP2R1B. Eur J Cancer. 2007 May;43(8):1328-35.

[40] Genomic and Epigenomic Landscapes of Adult De Novo Acute Myeloid Leukemia. N Engl J Med. 2013 May 30;368(22):2059-74.

[41] Hwang J, Lee JA, Pallas DC. Leucine Carboxyl Methyltransferase 1 (LCMT-1) Methylates Protein Phosphatase 4 (PP4) and Protein Phosphatase 6 (PP6) and Differentially Regulates the Stable Formation of Different PP4 Holoenzymes. J Biol Chem. 2016 Sep 30;291(40):21008-19.

[42] Sontag J-M, Nunbhakdi-Craig V, Sontag E. 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. 2013 Sep 20;288(38):27396-405.

[43] Tsai M-L, Cronin N, Djordjevic S. The structure of human leucine carboxyl methyltransferase 1 that regulates protein phosphatase PP2A. Acta Crystallogr Sect D Biol Crystallogr. 2011 Jan 1;67(1):14-24.

[44] Li M, Makkinje A, Damuni Z. The Myeloid Leukemia-associated Protein SET Is a Potent Inhibitor of Protein Phosphatase 2A. J Biol Chem. 1996 May 10;271(19):11059-62.

[45] Adachr Y, Pavlakis GN, Copeland TD. Identification of in vivo phosphorylation sites of SET, a nuclear phosphoprotein encoded by the translocation breakpoint in acute undifferentiated leukemia. FEBS Lett. 1994;340(3):231-5.

[46] Saito S, Miyaji-Yamaguchi M, Nagata K. Aberrant intracellular localization of SET-CAN fusion protein, associated with a leukemia, disorganizes nuclear export. Int J Cancer. 2004 Sep 10;111(4):501-7.

[47] Seo S, McNamara P, Heo S, Turner A, Lane WS, Chakravarti D. Regulation of Histone Acetylation and Transcription by INHAT, a Human Cellular Complex Containing

the Set Oncoprotein. Cell. 2001 Jan;104(1):119-30.

[48] Kutney SN, Hong R, Macfarlan T, Chakravarti D. A Signaling Role of Histone-binding Proteins and INHAT Subunits pp32 and Set/TAF-Iβ in Integrating Chromatin Hypoacetylation and Transcriptional Repression. J Biol Chem. 2004 Jul 16;279(29):30850-5.

[49] Kalousi A, Hoffbeck A-S, Selemenakis PN, Pinder J, Savage KI, Khanna KK, et al. The Nuclear Oncogene SET Controls DNA Repair by KAP1 and HP1 Retention to Chromatin. Cell Rep. 2015 Apr;11(1):149-63.

[50] Kandilci A, Mientjes E, Grosveld G. Effects of SET and SET-CAN on the differentiation of the human promonocytic cell line U937. Leukemia. 2004 Feb 4;18(2):337-40.

[51] ten Klooster JP, Leeuwen I v, Scheres N, Anthony EC, Hordijk PL. Rac1-induced cell migration requires membrane recruitment of the nuclear oncogene SET. EMBO J. 2007 Jan 24;26(2):336-45.

[52] Canela N, Rodriguez-Vilarrupla A, Estanyol JM, Díaz C, Pujol MJ, Agell N, et al. The SET Protein Regulates G 2 /M Transition by Modulating Cyclin B-Cyclin-dependent Kinase 1 Activity. J Biol Chem. 2003 Jan 10;278(2):1158-64.

[53] Cristobal I, Rincon R, Manso R, Carames C, Zazo S, Madoz-Gurpide J, 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. 2015 Jan 15;21(2):347-56.

[54] Janghorban M, Farrell AS, Allen-Petersen BL, Pelz C, Daniel CJ, Oddo J, et al. Targeting c-MYC by antagonizing PP2A inhibitors in breast cancer. Proc Natl Acad Sci. 2014 Jun 24;111(25):9157-62.

[55] Neviani P, Santhanam R, Trotta R, Notari M, Blaser BW, Liu S, et al. The tumor suppressor PP2A is functionally inactivated in blast crisis CML through the inhibitory activity of the BCR/ABL-regulated SET protein. Cancer Cell. 2005 Nov;8(5):355-68.

[56] Perrotti D, Jamieson C, Goldman J, Skorski T. Chronic myeloid leukemia: mechanisms of blastic transformation. J Clin Invest. 2010 Jul 1;120(7):2254-64.

[57] Arriazu E, Vicente C, Pippa R, Peris I, Martínez-Balsalobre E, García-Ramírez P, et al. A new regulatory mechanism of protein phosphatase 2A activity via SET in acute myeloid leukemia. Blood Cancer J. 2020 Jan 8;10(1):3.

[58] Cristóbal I, Blanco FJ, Garcia-Orti L, Marcotegui N, Vicente C, Rifon J, et al. SETBP1 overexpression is a novel leukemogenic mechanism that predicts adverse outcome in elderly patients with acute myeloid leukemia. Blood. 2010 Jan 21;115(3):615-25.

[59] Igea A, Nebreda AR. The Stress Kinase p38 as a Target for Cancer Therapy. Cancer Res. 2015 Oct 1;75(19):3997-4002.

[60] Zhong W, Zhu H, Sheng F, Tian Y, Zhou J, Chen Y, et al. Activation of the MAPK11/12/13/14 (p38 MAPK) pathway regulates the transcription of autophagy genes in response to oxidative stress induced by a novel copper complex in HeLa cells. Autophagy. 2014 Jul 29;10(7):1285-300.

[61] Beenstock J, Ben-Yehuda S, Melamed D, Admon A, Livnah O, Ahn NG, et al. The p38β Mitogen-activated Protein Kinase Possesses an Intrinsic Autophosphorylation Activity, Generated by a Short Region Composed of the α-G Helix and MAPK Insert. J Biol Chem. 2014 Aug 22;289(34):23546-56.

[62] del Barco Barrantes I, Coya JM, Maina F, Arthur JSC, Nebreda AR. Genetic analysis of specific and redundant roles for p38 and p38 MAPKs during mouse development. Proc Natl Acad Sci. 2011 Aug 2;108(31):12764-9.

[63] Grethe S, Pörn-Ares MI. p38 MAPK regulates phosphorylation of Bad via PP2A-dependent suppression of the MEK1/2-ERK1/2 survival pathway in TNF-α induced endothelial apoptosis. Cell Signal. 2006 Apr;18(4):531-40.

[64] Junttila MR, Li S, Westermarck J. Phosphatase-mediated crosstalk between MAPK signaling pathways in the regulation of cell survival. FASEB J. 2008 Apr 26;22(4):954-65.

[65] Lin S-P, Lee Y-T, Wang J-Y, Miller SA, Chiou S-H, Hung M-C, et al. Survival of Cancer Stem Cells under Hypoxia and Serum Depletion via Decrease in PP2A Activity and Activation of p38-MAPKAPK2-Hsp27. Koritzinsky M, editor. PLoS One. 2012 Nov 20;7(11):e49605.

[66] Guillonneau M, Paris F, Dutoit S, Estephan H, Bénéteau E, Huot J, et al. Oxidative stress disassembles the p38/NPM/PP2A complex, which leads to modulation of nucleophosmin-mediated signaling to DNA damage response. FASEB J. 2016 Aug 3;30(8):2899-914.

[67] Trotta R, Ciarlariello D, Col JD, Allard J, Neviani P, Santhanam R, et al. The PP2A inhibitor SET regulates natural killer cell IFN-γ production. J Exp Med. 2007 Oct 1;204(10):2397-405.

[68] Trotta R, Ciarlariello D, Dal Col J, Mao H, Chen L, Briercheck E, et al. The PP2A inhibitor SET regulates granzyme B expression in human natural killer cells. Blood. 2011 Feb 24;117(8):2378-84.

[69] Junttila MR, Puustinen P, Niemelä M, Ahola R, Arnold H, Böttzauw T, et al. CIP2A Inhibits PP2A in Human Malignancies. Cell. 2007 Jul;130(1):51-62.

[70] Niemelä M, Kauko O, Sihto H, Mpindi J-P, Nicorici D, Pernilä P, et al. CIP2A signature reveals the MYC dependency of CIP2A-regulated phenotypes and its clinical association with breast cancer subtypes. Oncogene. 2012 Sep 16;31(39):4266-78.

[71] Khanna A, Pimanda JE, Westermarck J. Cancerous Inhibitor of Protein Phosphatase 2A, an Emerging Human Oncoprotein and a Potential Cancer Therapy Target. Cancer Res. 2013 Nov 15;73(22):6548-53.

[72] Luo H, Li Q, O'Neal J, Kreisel F, Le Beau MM, Tomasson MH. c-Myc rapidly induces acute myeloid leukemia in mice without evidence of lymphoma-associated antiapoptotic mutations. Blood. 2005 Oct 1;106(7):2452-61.

[73] Come C, Laine A, Chanrion M, Edgren H, Mattila E, Liu X, et al. CIP2A Is Associated with Human Breast Cancer Aggressivity. Clin Cancer Res. 2009 Aug 15;15(16):5092-100.

[74] Khanna A, Böckelman C, Hemmes A, Junttila MR, Wiksten J-P, Lundin M, et al. MYC-Dependent Regulation and Prognostic Role of CIP2A in Gastric Cancer. JNCI J Natl Cancer Inst. 2009 Jun;101(11):793-805.

[75] Dong Q-Z, Wang Y, Dong X-J, Li Z-X, Tang Z-P, Cui Q-Z, et al. CIP2A is Overexpressed in Non-Small Cell Lung Cancer and Correlates with Poor Prognosis. Ann Surg Oncol. 2011 Mar 15;18(3):857-65.

[76] Xue Y, Wu G, Wang X, Zou X, Zhang G, Xiao R, et al. CIP2A is a predictor of survival and a novel therapeutic target in bladder urothelial cell carcinoma. Med Oncol. 2013 Mar 30;30(1):406.

[77] WANG J, LI W, LI L, YU X, JIA J, CHEN C. CIP2A is over-expressed in acute myeloid leukaemia and associated with HL60 cells proliferation and differentiation. Int J Lab Hematol. 2011 Jun;33(3):290-8.

[78] Lucas CM, Harris RJ, Giannoudis A, Copland M, Slupsky JR, Clark RE. Cancerous inhibitor of PP2A (CIP2A) at diagnosis of chronic myeloid leukemia is a critical determinant of disease progression. Blood. 2011 Jun 16;117(24):6660-8.

[79] Lucas CM, Harris RJ, Holcroft AK, Scott LJ, Carmell N, McDonald E, et al. Second generation tyrosine kinase inhibitors prevent disease progression in high-risk (high CIP2A) chronic myeloid leukaemia patients. Leukemia. 2015 Jul 13;29(7):1514-23.

[80] Ciccone M, Calin GA, Perrotti D. From the Biology of PP2A to the PADs for Therapy of Hematologic Malignancies. Front Oncol. 2015 Feb 16;5.

[81] Cohen JA, Barkhof F, Comi G, Hartung H-P, Khatri BO, Montalban X, et al. Oral Fingolimod or Intramuscular Interferon for Relapsing Multiple Sclerosis. N Engl J Med. 2010 Feb 4;362(5):402-15.

[82] Martin R. Anti-CD25 (daclizumab) monoclonal antibody therapy in relapsing–remitting multiple sclerosis. Clin Immunol. 2012 Jan;142(1):9-14.

[83] Oaks JJ, Santhanam R, Walker CJ, Roof S, Harb JG, Ferenchak G, et al. Antagonistic activities of the immunomodulator and PP2A-activating drug FTY720 (Fingolimod, Gilenya) in Jak2-driven hematologic malignancies. Blood. 2013 Sep 12;122(11):1923-34.

[84] Neviani P, Santhanam R, Oaks JJ, Eiring AM, Notari M, Blaser BW, et al. FTY720, a new alternative for treating blast crisis chronic myelogenous leukemia and Philadelphia chromosome–positive acute lymphocytic leukemia. J Clin Invest. 2007 Sep 4;117(9):2408-21.

[85] Arnaud L, Chen S, Liu F, Li B, Khatoon S, Grundke-Iqbal I, et al. Mechanism of inhibition of PP2A activity and abnormal hyperphosphorylation of tau by I 2 PP2A /SET. FEBS Lett. 2011 Sep 2;585(17):2653-9.

[86] Chen L, Luo L-F, Lu J, Li L, Liu Y-F, Wang J, et al. FTY720 Induces Apoptosis of M2 Subtype Acute Myeloid Leukemia Cells by Targeting Sphingolipid Metabolism and Increasing Endogenous Ceramide Levels. Siskind LJ, editor. PLoS One. 2014 Jul 22;9(7):e103033.

[87] Christensen DJ, Chen Y, Oddo J, Matta KM, Neil J, Davis ED, et al. SET oncoprotein overexpression in B-cell chronic lymphocytic leukemia and non-Hodgkin lymphoma: a predictor of aggressive disease and a new treatment target. Blood. 2011 Oct 13;118(15):4150-8.

[88] Switzer CH, Cheng RYS, Vitek TM, Christensen DJ, Wink DA, Vitek MP. Targeting SET/I2PP2A oncoprotein functions as a multi-pathway strategy for cancer therapy. Oncogene. 2011 Jun 7;30(22):2504-13.

[89] Wlodarchak N, Guo F, Satyshur KA, Jiang L, Jeffrey PD, Sun T, et al. Structure of the Ca2+−dependent PP2A heterotrimer and insights into Cdc6 dephosphorylation. Cell Res. 2013 Jul 11;23(7):931-46.

[90] Xu Y, Xing Y, Chen Y, Chao Y, Lin Z, Fan E, et al. Structure of the Protein Phosphatase 2A Holoenzyme. Cell. 2006 Dec;127(6):1239-51.

[91] Xu Y, Chen Y, Zhang P, Jeffrey PD, Shi Y. Structure of a Protein Phosphatase 2A Holoenzyme: Insights into

B55-Mediated Tau Dephosphorylation. Mol Cell. 2008 Sep;31(6):873-85.

[92] Arnold HK, Sears RC. A tumor suppressor role for PP2A-B56α through negative regulation of c-Myc and other key oncoproteins. Cancer Metastasis Rev. 2008 Jun 2;27(2):147-58.

[93] Letourneux C, Rocher G, Porteu F. B56-containing PP2A dephosphorylate ERK and their activity is controlled by the early gene IEX-1 and ERK. EMBO J. 2006 Feb 22;25(4):727-38.

[94] Casado P, Rodriguez-Prados J-C, Cosulich SC, Guichard S, Vanhaesebroeck B, Joel S, et al. Kinase-Substrate Enrichment Analysis Provides Insights into the Heterogeneity of Signaling Pathway Activation in Leukemia Cells. Sci Signal. 2013 Mar 26;6(268):rs6-rs6.

[95] Leonard D, Huang W, Izadmehr S, O'Connor CM, Wiredja DD, Wang Z, et al. Selective PP2A Enhancement through Biased Heterotrimer Stabilization. Cell. 2020;181(3):688-701. e16.

[96] Kauko O, O'Connor CM, Kulesskiy E, Sangodkar J, Aakula A, Izadmehr S, et al. PP2A inhibition is a druggable MEK inhibitor resistance mechanism in KRAS-mutant lung cancer cells. Sci Transl Med. 2018 Jul 18;10(450):eaaq1093.

[97] Morita K, He S, Nowak RP, Wang J, Zimmerman MW, Fu C, et al. Allosteric Activators of Protein Phosphatase 2A Display Broad Antitumor Activity Mediated by Dephosphorylation of MYBL2. Cell. 2020 Apr;181(3):702-715. e20.