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Targeting A Tumor Suppressor To Suppress Tumor Growth: News and Views on Protein Phosphatase 2A (PP2A) as a Target for Anti-cancer Therapy

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Abstract

Protein phosphatase 2A (PP2A), one of the major serine-threonine phosphatases in mammalian cells, maintains cell homeostasis by counteracting most of the kinase-driven intracellular signaling pathways. Unrestrained activation of oncogenic kinases together with inhibition of tumor suppressors is frequently required for the development of cancer. Because it has been found genetically altered or functionally inactivated in many solid cancers and leukemias, PP2A is indeed a *bona fide* tumor suppressor. For example, the phosphatase activity of PP2A is suppressed in chronic myelogenous leukemia and other malignancies characterized by the aberrant activity of oncogenic kinases. Notably, preclinical studies indicate that pharmacologic restoration of PP2A tumor suppressor activity by PP2A activating drugs (PADs, e.g. FTY720) effectively antagonizes cancer development and progression. Herein, we systematically discuss the importance of PP2A as a druggable tumor suppressor in light of the possible introduction of PADs into anti-cancer therapeutic protocols.

1. Introduction

The life and death of a cell is controlled by a tightly regulated network of complex signaling pathways. To maintain cell homeostasis, cell-cell and cell-microenvironment interactions result in the transduction of signals that promote survival and growth but also prevent unrestrained proliferation by inducing cell senescence and apoptosis in response to DNA damage, oxidative stress or aging. Cancer cells are characterized by alterations that lead to loss of tumor suppressor gene function, and uncontrolled proto-oncogene expression and/or activity. These oncoproteins (e.g. BCR-ABL1, FLT3-ITD, RAS) aberrantly enhance cell proliferation and survival, and/or impair differentiation thereby allowing the expansion of tumor cell clone(s)(1).

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Search strategy and selection criteria

Data for this Review were identified by searches of Medline, PubMed, and references from relevant articles using the search terms “PP2A” “leukemia” “cancer” “chronic myeloid leukemia” “I2PP2A” “CIP2A” “tumor suppressor”. Abstracts and reports from meetings were included only when they related directly to previously published work. Only articles published in English between 1980 and 2012 were included.

Conflict of Interest

The authors declared no conflicts of interest.

Contributors.

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Common oncogenes are protein kinases (e.g. SRC, c-ABL)(1); molecules (e.g. RAS) that constitutively activate downstream kinases (e.g. RAF); transcription factors (e.g. AP-1, MYC, RUNX1, HOX genes)(2); and anti-apoptotic genes (e.g. Bcl-2)(3). Equally, tumor suppressor genes counterbalance the activity of proto-oncogenes by limiting cell growth, promoting apoptosis and/or DNA repair (e.g. p53, Rb, APC, ATM) and their loss of function is frequently observed in cancer development and progression(4).

Many cancer promoting signals involve serine, threonine or tyrosine phosphorylation that usually acts as the on-off switch of target proteins(5). In normal cells, phosphoregulation is under the control of protein kinases and phosphatases(5). Deregulation of this balance is a central mechanism by which cells escape external and internal self-limiting signals, resulting in malignant transformation. In fact, a large proportion of cancers is characterized by aberrant activation of protein kinases whose activity is often necessary and, sometimes, sufficient to induce cancer.

1a. Chronic Myelogenous Leukemia (CML): A kinase-driven malignancy

The best-characterized example of a malignancy initiated and driven by the unrestrained activity of a protein kinase is Chronic Myelogenous Leukemia (CML)(6). In hematopoietic stem cells (HSCs) the reciprocal translocation between chromosome 9 and chromosome 22 [t(9;22)(q34;q11)] generates the Philadelphia Chromosome (Ph+), the hallmark of CML.(6) This rearrangement between the 5' portion of the breakpoint cluster region (BCR) gene and the 3' portion of the c-Abl proto-oncogene 1 (ABL1) (6) encodes the constitutively active BCR-ABL1 oncogenic tyrosine kinase (6).

CML is a myeloproliferative disorder characterized by accumulation of apparently normal myeloid cells. CML is clinically defined by two phases: a protracted chronic phase (CML-CP, median duration of 5 years) that, if left untreated, progresses into a rapidly fatal blast crisis (CML-BC, median survival of 6 months) with either myeloid or lymphoid features(6, 7). In CML-CP, BCR-ABL1 expression is sufficient to increase survival and induce an expansion of hematopoietic leukemic stem/progenitor cells still able to terminally differentiate(6). Specifically, BCR-ABL1 exerts its oncogenic activity through a complex network of pathways (e.g. RAS/RAF/MAPK; PI-3K/AKT; and the STAT5 pathway) that promote proliferation/survival independently from the bone marrow (BM) microenvironment(6). Conversely, CML-BC is characterized by additional genetic/genomic aberrations and by increased BCR-ABL1 expression/activity which is responsible for enhanced survival and proliferation, suppression of differentiation, and induction of self-renewal of leukemic progenitors.(6)

Given the central role of BCR-ABL1 kinase activity in CML-CP, patients are treated with BCR-ABL1 tyrosine kinase inhibitors (TKIs). Introduction of imatinib mesylate, the first successfully developed TKI, as first-line therapy for CML has greatly improved the treatment of CML-CP patients; in fact, 80% of patients quickly achieve and maintain complete cytogenetic response (CCyR) 6 years after initiating imatinib treatment, and progression to CML-BC is extremely low beyond the first two years of treatment(7). However, a few patients may fail to respond or develop resistance to imatinib treatment due to intrinsic heterogeneity of the disease, BCR-ABL1 amplification or overexpression, or the presence/acquisition of mutations in the ATP-binding domain, catalytic domain, activation loop, and amino acids that make direct contact with imatinib BCR-ABL1 kinase domain (e.g. T315I point-mutation)(8). To overcome TKI resistance, second- (dasatinib and nilotinib) and third-generation (ponatinib or AP24534) TKIs have been developed. These are more potent than imatinib and exert beneficial effects in some but not all cases of primary and advanced CML, although nearly 20% of CML-CP patients still do not respond to the newly developed drugs and long-term response is not achieved in CML-BC(8).

The mechanisms driving CML progression into the blastic phase are still controversial. Increased BCR-ABL1 activity/expression undoubtedly plays an essential role in almost all CML-BC patients, but also BCR-ABL1-induced genetic/chromosomal abnormalities can predispose to transformation and/or influence the aggressiveness of the advanced stages(7). Indeed, high levels of BCR-ABL1 activity sustain activation of proliferation and survival pathways, increase genomic instability, induce a block in differentiation and promote self-renewal, because of the concurrent inhibition of tumor suppressors (e.g. p53, PP2A)(7).

1b. Tumor Suppressor phosphatases

While aberrant global phosphorylation leads to aberrant activation of signaling pathways linked to neoplastic transformation, protein phosphatases invest a major role in negatively regulating such molecular networks. Indeed, the loss of function of different tumor suppressor phosphatases has been detected in many cancers.

The first phosphatase described as such was PTEN, a dual-specificity phosphatase that has been found genetically or functionally inactivated in glioblastomas, prostate and breast cancer, endometrial neoplasms and hematological malignancies. PTEN functions by inducing apoptosis and/or inhibiting cell growth, adhesion and migration(9).

The protein phosphatase 2A (PP2A) has a well established role as a regulator of cell cycle and apoptosis(10). Because inhibition of its activity or loss of some of its functional subunits is a characteristic of neoplastic transformation, PP2A is now widely designated as a tumor suppressor(10). Importantly, many groups, including ours, have reported that PP2A activity can be pharmacologically restored both *in vitro* and in animal models of cancer(11). Indeed, restoration of PP2A activity in leukemic cells kills cancer cells while sparing normal cells, through the simultaneous targeting of the oncogenic kinase, its downstream effectors, and other important mediators of cancer cell survival, proliferation and self-renewal that act in an oncogene independent manner(7, 11).

The SH2-containing protein tyrosine phosphatase 1 (SHP-1) has also been described as a potential tumor suppressor in myeloid and lymphoid malignancies(12). Abnormal cancer cell growth is, in fact, also dependent on the inhibitory effect of the activity of different oncogenic tyrosine kinases (e.g. FLT3/ITD and JAK) on SHP-1 expression (12, 13). In fact, downregulation of SHP-1 expression and function was observed in leukemias, including CML and lymphomas (12, 13). Interestingly, a potential cross talk seems to exist between PP2A and SHP-1 phosphatases; SHP-1 activity was found essential for PP2A-induced BCR-ABL1 inactivation/degradation(14) and Lyn kinase-dependent proliferative arrest in Ph⁺ leukemias (13). Interestingly, SHP-1 associates with BCR-ABL1 and PP2A, and its phosphatase activity counteracts BCR-ABL1 leukemogenic potential(14).

2. Protein Phosphatase 2A (PP2A): An Essential Tumor Suppressor

2.1 PP2A Structure and regulation

PP2A represents a major cellular serine-threonine phosphatase. Its core structure is a dimeric holoenzyme (PP2A_D) composed of a 36 kDa catalytic C subunit (PPP2CA/B, α and β isoforms) and a 65 kDa structural/scaffold A subunit (PR65 or PPP2R1A/B, α and β isoforms) (Table 1). The subunits A and C are ubiquitously expressed and are frequently associated to a regulatory B subunit that dictates subcellular localization and substrate specificity(5). As 20 different regulatory subunits have been identified, this may potentially lead to over 75 distinct PP2A holoenzymes(10). There are three unrelated families of B regulatory subunits: B (B55/PR55/PPP2R2), B' (B56/PR61/PPP2R5), B''(PR72/PPP2R3), and B'''(PR93/PR110) (Table 1)(10). Additionally, the Phosphatase Two A Phosphatase

Activator (PTPA/PPP2R4) interacts with AC dimers and is required for converting PP2A from inactive to an active phosphatase (Table 1)(10).

Some of the B subunits are also tissue and development stage specific. For example, B56 α and B56 γ are highly expressed in heart and skeletal muscle, and B56 β is highly expressed in brain(15). Moreover, B55 β , B55 γ , and B56 γ expression is induced during neuronal differentiation, and the B56 γ subunit is involved in lung growth and development(16, 17). Interestingly, the correct subcellular compartmentalization of PP2A, which depends also on its trimeric composition, may be of critical importance for regulation of various cellular processes (reviewed in(5)). In fact, cytoplasmic PP2A may control cell growth and survival, while at the mitochondrial membrane it can induce apoptosis and, in the nucleus, it might affect chromosome stability and chromatid segregation(5).

Given the complexity of functions of PP2A enzymes, the assembly of each specific PP2A complex is tightly and precisely regulated, in part through specific post-translational modifications (e.g. methylation and phosphorylation) of the C-terminal tail of the catalytic subunit. For example, carboxymethylation of Leu309 by LCMT-1 (Leucine Carboxyl MethylTransferase 1) and PME-1 (Phosphatase MethylEsterase 1) enhances the affinity of PP2A for the PR55/B subunits but not others(18).

The quaternary structure of PP2A can also be modulated by phosphorylation of PP2A_C at Tyr307 and on Thr304. Phosphorylation of Tyr307 prevents methylation from occurring, therefore preventing formation of PR55/B-containing PP2A complexes(18). Conversely, Tyr307 phosphorylation inhibits interaction of PP2A_C with PR61/B' $\alpha\beta\gamma\epsilon$ subunits; similarly, phosphorylation of Thr304 inhibits the recruitment of PR55/B subunits.(18) Overall, both phosphorylation events result in inactivation of PP2A. Phosphorylation of B subunits may also play a critical role in the regulation of PP2A activity. In response to survival and mitogenic signals, ERK phosphorylates B56 γ on Ser337 thereby leading to disassembly and inactivation of PP2A that, otherwise, would dephosphorylate ERK and inhibit the MAPK pathway(18). By contrast, DNA damage promotes ATM-dependent B56 γ 3 phosphorylation at Ser510, a step essential to promote formation of B56 γ 3-PP2A complexes capable of directly activating p53 tumor-suppressive functions(19).

In addition to the trimeric structure of PP2A, various factors bind to PP2A complexes and affect its phosphatase activity(10). In particular, viral proteins (e.g. the polyoma small T and middle T, and the SV40 small T antigens) express their transforming potential by directly inhibiting PP2A through displacement of regulatory subunits(10). Likewise, PP2A endogenous inhibitors, which are often found overexpressed in cancer, can also suppress PP2A activity (14, 20–22). To date four specific inhibitors have been identified in mammals: I1PP2A/PHAP1/PP32, I2PP2A/SET, CIP2A/p90, and TIP (type 2A-interacting protein) (Table 1)(23–25).

2.2 Physiological Functions of PP2A

While most signaling pathways require a cascade of events that are usually carried out by activation of specific kinases, such signals are for the most part counteracted by a limited number of phosphatases. Among these, PP2A has a major role in the maintenance of normal cell division (reviewed in (11, 26, 27)). Indeed, PP2A controls the G1/S transition; in this cell cycle phase a B56 γ 3-containing PP2A complex accumulates in the nucleus(28), and, its methylation levels gradually change from the G0 throughout the G1 and S phases of cell cycle. Furthermore, a B55 α -containing PP2A prevents transition from the G2 to the M phase by inhibiting the maturation-promoting factor MPF (Cdk1/cyclin B complex) through dephosphorylation and inhibition CAK (Cdk-activating kinase) and Wee1 kinases. This B55 α -containing complex also controls the mitotic spindle breakdown, chromatin

decondensing, and the post-mitotic reassembly of the nuclear envelope and Golgi apparatus. Unsurprisingly, PP2A also positively regulates mitotic exit through the PP2A/B56 δ dependent dephosphorylation/inactivation of the phosphatase Cdc25, which results in the hyperphosphorylation (inactivation) of Cdk1. PP2A is also involved in the negative regulation of cell proliferation and survival. Reportedly, specific PP2A complexes inhibit mitogenic and anti-apoptotic signals by dephosphorylating and inactivating MEK1 and ERK-family kinases, decreasing the stability and function of transcription factors (c-Myc and STAT5). (23, 29) Similarly, PP2A suppresses cap-dependent translation of oncogenes such as Mcl-1 and c-Myc through direct and indirect (via inhibition of Mnk1/2 kinases) dephosphorylation of eIF4E (eukaryotic initiation factor 4E) (Fig. 1)(30).

Consistent with its role as a tumor suppressor, PP2A possesses pro-apoptotic activity (reviewed in(31)) in virtue of its ability to negatively regulate the PI3K/Akt pathway upon direct Akt dephosphorylation, inactivate the antiapoptotic Bcl-2, and activate the proapoptotic factors BAD and Bim. Notably, PP2A-dependent direct Akt inactivation and BAD dephosphorylation result in its activation and translocation to the mitochondrial membrane where it binds and inhibits Bcl-2. Moreover, during endoplasmic reticulum stress-induced apoptosis, the proapoptotic BH3-only protein Bim becomes active and contributes to apoptosis upon dephosphorylation by PP2A, which can also dephosphorylate Ser70 of Bcl-2 through direct binding to its BH4 domain thereby resulting in enhanced Bcl-2/p53 interaction and overall inhibition of Bcl-2's antiapoptotic function (Fig. 1).

PP2A also has a role in the regulation of the Wnt pathway and, therefore, to embryonic development, cell growth and stem cell survival/self-renewal (reviewed in (10)). The main downstream effector of the Wnt pathway is β -catenin which, when stabilized, accumulates in the nucleus and induces TCF-dependent transcription. Although individual PP2A complexes have been shown to have both positive and negative effects on this pathway, overall PP2A is considered a negative regulator of the Wnt pathway as PP2A inhibition results in enhancement of β -catenin-dependent transcription. Furthermore, PP2A is also a major regulator of β -catenin-driven signals because a) a B56 γ -PP2A complex directly destabilizes β -catenin; b) GSK-3 β , the β -catenin negative regulator, is activated both directly and through Akt inhibition by PP2A; and c) the B subunit PR55 α directly binds β -catenin making PP2A part of the β -catenin destruction complex where it induces its degradation upon dephosphorylation (Fig. 1).

3. PP2A in disease and cancer

Based on the considerations made above, it is not surprising that alterations of PP2A subunits or loss of its phosphatase activity have been linked to cancer development and to other non-neoplastic diseases. For example, Alzheimer's disease is, at least in part, caused by hyperphosphorylation and accumulation of the protein tau(32). PP2A is not only the major tau phosphatase in human brain, but several abnormalities of PP2A have been reported in Alzheimer's disease including decreased PP2A_C mRNA and protein levels; downregulation of PR65/A and B55 α protein expression; reduced PP2A_C methylation; increased PP2A_C phosphorylation; and, up-regulation of the PP2A inhibitors SET and I1PP2A (reviewed in(32)) (Table 2). Thus, inhibition of PP2A seems essential for the pathophysiology of Alzheimer's disease and it also represents an attractive therapeutic target.

The potential role of PP2A in cancer has been hypothesized two decades ago when it was reported that okadaic acid, a selective inhibitor of PP2A had potent tumor-promoting activity, and that the transforming viral antigens (e.g. SV40 small T) exert their tumorigenic potential through displacement of multiple PP2A regulatory subunits(10). Given the

complexity of PP2A structure, function and regulation, it is clear that the role of PP2A as tumor suppressor may be largely cell context and subunit dependent. Moreover, loss of a specific PP2A activity may represent both a major event contributing to cancer development and progression, and a dismal prognostic factor(11, 33–38) because of the key role played by PP2A as mediator of anti-proliferative and pro-apoptotic signals (e.g. Akt). Thus, a careful evaluation of PP2A enzymatic activity, genetic integrity of its relevant subunits, and expression/activity of endogenous PP2A inhibitors and activators, is mandatory before knighting PP2A as a tumor suppressor in a specific cancer. In this regard, Sablina et al. used an shRNA approach to assess the contribution of PP2A to cell transformation by systematically knocking down specific PP2A regulatory subunits(39). Individual suppression of at least four subunits (i.e. B56 α , B56 γ , PR72/130 and PTPA) induced cell transformation, partially recapitulating the transformed phenotype induced by the SV40 small T antigen(39). However, the molecular mechanism used by each PP2A complex was not identical. In fact, suppression of B56 α , PR72/130 or PTPA induced a significant increase in c-Myc expression; instead, downregulation of B56 γ and PTPA levels resulted in a marked increase in β -catenin dependent transcription and Akt activation(39). To complicate this scenario, shRNA-mediated suppression of PTPA expression dramatically interfered with the assembly of PP2A heterotrimeric complexes, most likely by abolishing PP2A_C methylation and reducing PP2A_C binding to the structural A subunit. Interestingly, PTPA shRNA-expressing cells showed the strongest transformed phenotype, suggesting that this regulator of PP2A may, per se, act as a tumor suppressor(39).

Consistent with a role of the B regulatory subunits in cancer development and metastasis, expression of B56 α and B56 γ was reduced in melanomas, with their lowest expression detected in metastatic tissues in which low PP2A activity correlated with c-Myc overexpression, sustained phosphorylation of paxillin and its recruitment to focal adhesions(35, 40). In a similar study, B55 α protein expression was inhibited in a large cohort of AML patient samples and this correlated with increased Akt phosphorylation (Thr308) and loss of complete hematologic remission(38). Interestingly B55 α was also found deleted in a subset of luminal B breast cancers(41).

The scaffold PR65/A has also been found altered in numerous malignancies (Table 2). Reportedly, mutations of the PR65 β isoform have been detected in 15% of primary lung tumors, 6% of lung tumor-derived cell lines, 15% of primary colorectal carcinomas, and 13% of breast cancers(10). Aberrant transcripts were also found in 29% of hepatocellular carcinomas, and loss of heterozygosity at the PR65 β locus (11q23) was detected in breast, ovary, cervix, stomach, bladder carcinomas and melanoma(42, 43). Interestingly, PR65 β gene deletion or alternative splicing were associated with reduced PP2A activity in aggressive B-cell chronic lymphocytic leukemia (B-CLL) (44), in which the importance of PR65 β loss-of-function might rest in its non-random correlation with hyperphosphorylation of RalA, a factor strongly involved in the regulation of apoptosis, proliferation and cell migration (10).

Similarly, PR65 α alterations were detected in 9.1% of type I ovarian tumors; 6.7% of type I uterine carcinomas; 19.2% of type II uterine (serous) carcinomas; and, 43% of primary human gliomas(45). Accordingly, animal studies revealed that PR65 α point mutations and deletion increase incidence of lung cancer (46), most likely because of the inability of the mutated scaffold subunit to complex with PP2A_C and/or with individual B subunits (47). In this regard, suppression of PP2A activity in AML carrying Kit^{D816V} and Kit^{V560G} mutations depends on reduced expression of PP2A A- and B-subunits (B55 α , B56 α , B56 γ , B56 δ) (Table 2); and pharmacologic PP2A reactivation induced apoptosis and inhibited proliferation both in vitro and in animal models of AML.(48)

Loss of PP2A tumor suppressor ability can also be achieved through the aberrant expression of other factors that interact with PP2A (i.e. CIP2A, SET, SETBP1). CIP2A (Cancerous inhibitor of PP2A) interacts and inhibits PP2A activity(23), was found overexpressed in various malignancies (i.e. hepatocellular carcinoma, triple negative breast cancer, in head and neck squamous cell carcinoma, colorectal cancer, serous ovarian cancer, non-small-cell lung cancer, prostate cancer, chronic myeloid leukemia, and acute myeloid leukemia), and its expression generally correlates with poor prognosis(20–22, 33, 34, 49, 50) (Table 2). Interestingly, CIP2A stabilizes c-Myc oncogene by preventing its PP2A-dependent dephosphorylation on Ser62 (23). This can be antagonized by treatment with the proteasome inhibitor bortezomib; in fact,, ex vivo treatment of solid tumor cells with bortezomib suppresses CIP2A expression thereby leading to reactivation of PP2A activation and, consequently, Akt dephosphorylation/inactivation(49, 50). Given the importance of PP2A inhibition in maintaining the activation c-Myc- and Akt-driven oncogenic survival and mitogenic signals, CIP2A is quickly becoming an attractive therapeutic target.

SET (I2PP2A, inhibitor 2 of PP2A) is another binding partner of PP2A with inhibitory function (Table 2). SET was identified as an oncogene found fused to CAN in t(6;9) acute undifferentiated non-lymphocytic leukemia and also fused with the nucleoporin Nup-214a in adult and pediatric T-ALL patients carrying the cryptic and recurrent deletion, del (9)(q34.11q34.13)(51, 52). Furthermore, SET is overexpressed in BCR-ABL1⁺ leukemias (CML-CP and -BC, and Ph⁺ B-ALL)(14, 53). Interestingly overexpression of SET is a poor prognostic factor in AML patients, in primary B-CLL cells, and B-cell non-Hodgkin lymphoma cell lines(37, 54). Physiologically, SET is a specific inhibitor of PP2A and its deregulation in leukemia suggested that impairment of PP2A might contribute to leukemogenesis(24). Moreover, SET is able to suppress the DNase activity of NM23-H1 tumor suppressor, increase AP-1 activity, activate MAPK signaling, and regulate granzyme B and interferon- γ production in human NK cells(55–59). In this regard, it was shown that natural killer (NK) cell activation correlates with increased SET expression and inhibition of PP2A activity(59). Pharmacologic inhibition of PP2A activity or ectopic SET expression in primary human NK cells enhances monokine-induced IFN- γ production, cytotoxicity and Granzyme B expression(58, 59). Thus, it is plausible that the SET-PP2A interplay is important in the control of NK anti-tumor activity.

The molecular mechanism by which SET inhibits PP2A phosphatase activity is not limited to its overexpression. In CD34⁺ progenitors from Jak2^{V617F} myeloproliferative (MPD) disorders like Polycythemia Vera (PV), PP2A is inactivated in a Jak2^{V617F} kinase- and SET-dependent manner, but this does not correlate to increased SET levels, but to SET Ser9 phosphorylation and subcellular localization, both of which depend on Jak2^{V617F}-induced PI-3K γ -PKC-driven signals (*Oaks J.J. and Perrotti D., manuscript in preparation 2012*) (60). Notably, PI-3K γ -dependent SET serine phosphorylation and its cytoplasmic localization, prevents nitric oxide synthase (NOS2)/peroxynitrite (PN)-mediate PP2A activation in Jak2^{V617F} MPDs(60). Moreover, Jak2, PI-3K, and PKC kinase inhibitors as well as expression of specific SET phosphomutants or shRNA restored PP2A activity and induced apoptosis of Jak2^{V617F} cell lines and/or CD34⁺ PV progenitors(60).

SETBP1 (SET binding protein 1) is a SET regulator found fused in frame with a nucleoporin, Nup-98, in a pediatric case of T-ALL with t(11;18)(61). SETBP1 was also found overexpressed in 27.6% of AML patients at diagnosis and linked to poor prognosis especially in elderly patients (Table 2)(36). Mechanistically, SETBP1 protects SET from protease cleavage, increasing the amount of full-length SET protein and leading to the formation of the PP2A inhibitory SETBP1-SET-PP2A complex(36).

4. Restoring PP2A in Chronic Myelogenous Leukemia

Suppression of PP2A activity has a central role in the pathogenesis of CML. PP2A activity is barely detectable in CML-BC and significantly impaired in CML-CP as a consequence of SET overexpression in leukemic progenitors(14); SET levels increase during CML progression in a BCR-ABL1 kinase- and expression-dependent manner(14). Remarkably, several targets are shared between BCR-ABL1 and PP2A, most of them essential for BCR-ABL1 leukemogenesis. Induction of PP2A activity by downmodulating SET or overexpressing PP2A resulted in dephosphorylation of MAPK, STAT5, Jak2, Akt, and Lyn; decreased Myc expression; and, increased levels of the pro-apoptotic BAD and the hypophosphorylated form of Rb(13, 14) (Fig. 2). Importantly, restoration of a functional PP2A by treatment with PP2A-activating drugs (PADs; e.g. Forskolin, 1,9-dideoxy forskolin, FTY720) promoted SHP-1 tyrosine phosphatase-dependent BCR-ABL1 inactivation/degradation(14, 53) (Fig. 2) thereby resulting in killing of leukemic progenitors, carrying either BCR-ABL1 wild-type or Y253H and T315I mutants, both *in vitro* and in animal models of CML-BC and Ph⁺ B-ALL(14, 53).

Recently, a role for CIP2A in the regulation of PP2A in CML has emerged. Lucas et al. reported that patients that progressed to CML-BC had significantly higher levels of CIP2A protein and increased levels of Tyr307-phosphorylated PP2Ac (inactive) at diagnosis than patients who do not progress, further correlating lower PP2A activity with worse prognosis(62). Importantly, CIP2A knockdown resulted in increased PP2A activity, decreased c-Myc and SET levels, and decreased BCR-ABL1 activity, suggesting that SET and CIP2A might be part of the same PP2A-containing complex and might cooperate in inhibiting PP2A function toward specific oncogenic substrates(62). Interestingly, the cellular microenvironment seems to play a role in suppression of PP2A activity in cancer cells. Salas et al reported that upregulation of sphingosine kinase-1 (SK-1) and overproduction of sphingosine-1-phosphate (S1P) is a mechanism that contributes to imatinib resistance in K562 cells.(63) The upregulation of SK-1/S1P was dependent on enhanced triggering of S1P receptor 2 signaling (S1P2), resulting in stabilization of BCR-ABL1 through inhibition of PP2A. Importantly, interference with the SK-1/S1P2 signaling enhanced PP2A activity and resulted in BCR-ABL1 inhibition/degradation in cells harboring the T315I BCR-ABL1 mutant(63). PP2A activity was also induced upon co-treatment with imatinib and the proteasome inhibitors Bortezomib or PSI (Proteasome Inhibitor I), where inhibition of PP2A proteasomal degradation led to its reactivation and potentiated the effect of imatinib on BCR-ABL1(64), consistent with the effect (see above) of bortezomib on CIP2A expression. Another challenge in the eradication of CML is the resistance of BCR-ABL1⁺ quiescent stem cells to all known TKIs, and their persistence in TKI-responsive CML patients in a durable (more than 3 years) major or complete molecular response, supporting the concept that CML stem cells are not BCR-ABL1 kinase addicted(65). These leukemic stem cells (LSCs) can potentially reinitiate the disease upon TKI therapy interruption. LSCs in CML are characterized by aberrant activation of pathways that control the survival and self-renewal of normal stem cells, such as the wnt pathway(7). There is emerging evidence that PP2A is also inactivated in the LSC compartment in a BCR-ABL1-independent, Jak2-dependent manner and that treatment with PADs can impair their survival and self-renewal and weaken their ability to initiate and propagate CML *in vivo* without harming normal hematopoiesis(65, 66).

5. Protein Phosphatase 2A Activating Drugs (PADs)

5.1 Forskolin and 1,9-dideoxy forskolin

Forskolin, a diterpene derived from the roots of *Coleus forskohlii*, is known for its ability to induce adenylate cyclase and, recently, as a PP2A activator(14). Importantly forskolin

inhibits growth and/or induces apoptosis of leukemic cells(14). 1,9-dideoxy forskolin lacks the adenylate cyclase-activating function while retaining the ability to activate PP2A(14). In CML, restoration of PP2A activity via treatment with these drugs induces marked apoptosis, reduces proliferation, impairs colony formation, inhibits tumorigenesis and restores differentiation of patient-derived myeloid CML-BC^{CD34+} progenitors and/or BCR/ABL-transformed cell lines regardless of their sensitivity to imatinib(14). Accordingly, *in vivo* administration of forskolin and/or 1,9-dideoxy forskolin severely impacted the development of wild-type and T315I BCR/ABL-induced acute leukemia-like disease in immunocompromised mice without signs of toxicity(14). Remarkably, these drugs did not induce apoptosis of CD34⁺ normal marrow cells(14).

5.2 FTY720 (Fingolimod; Gilenya) and non-immunosuppressive FTY720 derivatives

FTY720 is an oral sphingosine analog currently used in relapsing multiple sclerosis patients as an immunosuppressant that, upon phosphorylation by sphingosine kinase 2 (Sphk2), is internalized by the sphingosine-1-phosphate receptor (S1PR1)(67). Phosphorylated FTY720 does not impair T- and B-lymphocyte activation but it interferes with immune cell trafficking between lymphoid organs and peripheral blood(67). FTY720 has high oral bioavailability and is not toxic nor tumorigenic in animals(68). Interestingly, FTY720 seems to be also a potent inhibitor of tumor growth and angiogenesis(69). Several *in vitro* studies suggest the possible inclusion of FTY720 in the therapy of multiple myeloma, hepatocellular, bladder, breast and prostate carcinomas because of its ability to induce apoptosis by interfering with Bcl-2 and suppress mitogenic/survival signals by inhibiting the ERK and PI-3K/Akt(70–77). FTY720's anti-cancer activity does not require Sphk2 phosphorylation or S1PR1 interaction but depends on its ability, at least in leukemias, to act as a potent PP2A activator (48, 53, 75). In CML and Ph⁺ BALL progenitors, FTY720-induced PP2A activity promotes BCR-ABL1 inactivation/degradation and inhibition of survival factors (e.g. JAK2, Akt and ERK1/2)(14, 53). This results in apoptosis of CD34⁺ progenitors from TKI-sensitive and –resistant CML patients and translates into long-term survival with normal myelopoiesis and absence of toxicity in BCR-ABL1⁺ leukemic mice(14, 53). In fact, long-term FTY720 treatment (27 weeks) of leukemic animals extensively prolongs survival and restores normal myelopoiesis without exerting any toxic effects in hematopoietic and non-hematopoietic organs(53). Restoration of PP2A activity by FTY720 or its non-immunosuppressive derivatives (*S*)-FTY720-OMe, (*S*)-FTY720-regioisomer and OSU-2S selectively suppresses the survival and self-renewal of CML but not normal quiescent HSCs and progenitors both *in vitro* and in animal models (Neviani and Perrotti, manuscript submitted 2012)(66, 78–80). These FTY720 derivatives neither induce lymphopenia nor undergo phosphorylation or interact with the S1PR1 receptor but still activate PP2A(66, 80). Mechanistically, FTY720 disrupts the SET-PP2A interaction, allowing PP2A activation, which inhibits JAK2 and impairs β -catenin-dependent survival through GSK3 β activation (Neviani P. and Perrotti D., manuscript submitted)(66, 80). These findings hold the promise to bring CML therapy further into a setting where patients are brought into remission by TKIs and possibly cured by FTY720 or its derivatives.

Furthermore, *in vitro* and animal studies with hematopoietic cells lines and/or *ex vivo* PV progenitors indicated that FTY720 also has a therapeutic potential toward Jak2^{V617F} MPDs (Oaks J.J and Perrotti D., manuscript in preparation)(60). Likewise, three different groups highlighted the efficacy of FTY720 in AML, in which FTY720 restored PP2A activity, decreased clonogenicity and/or significantly suppressed AML in animal models(48, 81). FTY720 also seems to have anti-leukemic activity toward Ph-negative lymphoid malignancies; in fact, FTY720 diminishes the neoplastic cell burden and potentiates the effects of conventional anti-cancer drugs in studies with cell lines, *ex vivo* patient cells, and in animal models of CLL, T-cell large granular lymphocyte leukemia, NK cell leukemia,

Mantle cell lymphoma, and Ph-negative ALL. However, it is unclear whether the FTY720 anti-cancer activity in some lymphoid malignancies should be attributed to activation of PP2A or other mechanisms (e.g. autophagy)(82–86).

5.3 SET Binding Peptides

SET Binding Peptides COG112, COG/OP449 and apoE-mimetics reactivate PP2A upon interaction with SET, preventing SET-PP2Ac interaction and inhibition of PP2A activity(87–89). In CLL and NHL cells, these compounds inhibit Mcl-1 expression and are cytotoxic both *in vitro* and in tumor xenografts. Likewise, apoE-mimetic peptides suppress phosphorylated kinase signaling and inflammatory response. In cancer cells, the COG112-SET interaction releases the SET-mediated inhibition of PP2A and augments the activity of the metastasis suppressor nm23-H1. As a result of PP2A activation, COG112 also decreases Akt signaling and cellular proliferation. Recently, it was reported that COG/OP449 induces apoptosis of CML progenitors through a mechanism that depends on PP2A re-activation(87).

5.4 Other PP2A Activating Drugs

Other PP2A activity-inducing compounds are being evaluated as anticancer agents. Noteworthy are the following drugs: promethylating agents like the *Chloroethyl Nitrosourea* (*CENU*) that inhibits growth of melanoma cells upon induction of PP2A methylation (activation)(90); the *α -tocopheryl succinate* (*α -TOS*) that induces cancer cell apoptosis through the induction of PP2A(91); *Vorinostat* and *sorafenib* that induce gastrointestinal tumor cells death through induction of PP2A activity in a C16 dihydro-ceramide-dependent manner(92); *Carnosic acid* induces apoptosis of human prostate carcinoma cells through the PP2A-dependent modulation of Akt/IKK/NF- κ B signaling (93); *methylprednisolone* induces complete differentiation of leukemic cells by enhancing expression of PP2A regulatory subunits(94); *ceramide* produces apoptosis through induction of p27(kip1) by PP2A-dependent Akt dephosphorylation in prostate cancer cells(95); and, *exogenous TGF β* uses Smad4-independent, PP2A-dependent signaling pathways for apoptosis induction in lymphoma cells(96).

6. Conclusion

Over the past two decades PP2A has emerged as a major player in cancer transformation, and its central role as a regulator of cell growth and survival has unraveled its function as a tumor suppressor. It is generally accepted that partial loss of PP2A phosphatase activity may be sufficient to unleash oncogenic signals induced by unrestrained oncogenic kinase activities. In CML, PP2A loss-of-function likely contributes to disease maintenance, transformation and, likely, to survival of the leukemia-initiating cells. Importantly, the observation that PP2A activity can be pharmacologically induced in different neoplastic malignancies with PADs (e.g. FTY720 and its derivatives) that show strong anti-cancer activity and safety profile, makes PP2A an attractive therapeutic target for the development of alternative and feasible protocols for the treatment and, perhaps, eradication at stem cell level of different types of cancer characterized by functional inactivation of this tumor suppressor.

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Figure 1. Physiological functions of PP2A

Schematic representation of some of the pathways controlled by PP2A. The figure shows the many levels at which PP2A affects the normal physiology of cells. PP2A, by virtue of its phosphatase activity counteracts most of the signal triggered by protein kinases.

Figure 2. Opposing roles of PP2A and BCR-ABL1 in CML

Schematic representation of the major targets of BCR-ABL1, whose activity is counteracted by the phosphatase activity of PP2A. In CML, PP2A is inhibited in a SET/JAK2/CIP2A dependent manner. PP2A, when active, targets most of the downstream effectors of BCR-ABL1 and can also induce BCR-ABL1 inactivation/degradation through the Shp-1 tumor suppressor phosphatase.

Table 1

PP2A subunits and binding proteins.

Subunits	Gene Name	Aliases (isoforms)
PP2A_C	PPP2CA	PP2A α (C α)
	PPP2CB	PP2A β (C β)
PP2A_A	PPP2R1A	PR65 α (A α)
	PPP2R1B	PR65 β (A β)
PP2A_B	PPP2R2A	B55 α , PR55 α (B α)
	PPP2R2B	B55 β , PR55 β (B β)
	PPP2R2C	B55 γ , PR55 γ 1(B γ)
	PPP2R2D	B55 δ , PR55 δ (B δ)
PP2A_{B'}		B56 α , PR61 α (B' α)
		B56 β , PR61 β (B' β)
	PPP2R5A	B56 γ , PR61 γ (B' γ 1)
	PPP2R5B	
	PPP2R5	(B' γ 2)
	CPPP2R5D	(B' γ 3)
PP2A_{B''}	PPP2R5E	B56 δ , PR61 δ (B' δ)
		B56 ϵ , PR61 ϵ (B' ϵ)
	PPP2R3A	PR130 (B'' α 1), PR72 (B'' α 2)
	PPP2R3B	PR48 (B'' β)
	PPP2R3C	G5PR (B'' γ)
Binding partners		
I1PP2A	ANP32A	PHAP1, PP32
I2PP2A	SET	PHAPII, TAF-IBETA
CIP2A	KIAA1524	p90
TIP	TIPRL	TIP41
PTPA	PPP2R4	PR53

Table 2

Alterations of PP2A subunits and binding proteins responsible for disease development.

Subunits	Alteration	Disease	Reference
PP2A_C	Decreased expression, increased phosphorylation, decreased methylation.	Alzheimer's	(32)
PR65/A	Decreased expression, loss of heterozygosity, deletion, point mutations.	Alzheimer's, lung cancer, colorectal, hepatocellular, breast, ovarian, cervical, endometrial, stomach, bladder carcinoma, glioma, melanoma, B-CLL, AML	(18, 32, 42–45, 47)
B55α	Decreased expression.	Alzheimer's, AML, breast cancer	(32, 38, 41, 48)
B56α	Decreased expression.	Melanoma, AML	(35, 40, 48)
B56γ	Decreased expression.	Melanoma, AML	(40, 48)
B56ε	Decreased expression.	AML	(48)
Binding partners			
I1PP2A	Increased expression	Alzheimer's	(32)
SET (I2PP2A)	Increased expression, increased activity.	Alzheimer's, CML, AML, Ph+ ALL, T-cell ALL, B-cell CLL, B-cell NHL, PV	(14, 32, 37, 51–54)
CIP2A	Increased expression.	Hepatocellular, breast, colorectal, ovarian, cervical, prostate, lung, head and neck cancer, CML, AML	(20–23, 33, 34, 49, 50, 62)
SETBP1	Increased expression	T-ALL, AML	(36, 61)

Abbreviations. CML: Chronic Myeloid Leukemia; AML: Acute Myeloid Leukemia, Ph: Philadelphia-chromosome; ALL: Acute Lymphoblastic Leukemia; NHL: Non-Hodgkin Lymphoma.