Published in final edited form as:

Cancer Metastasis Rev. 2008 June ; 27(2): 147–158. doi:10.1007/s10555-008-9128-9.

A tumor suppressor role for PP2A-B56 α through negative regulation of c-Myc and other key oncoproteins

Hugh K. Arnold and Rosalie C. Sears

Department of Molecular and Medical Genetics, Oregon Health & Sciences University, 3181 SW Sam Jackson Park Rd., L103A, Portland, OR 97239, USA

Rosalie C. Sears: searsr@ohsu.edu

Abstract

Loss or inhibition of the serine/threonine protein phosphatase 2A (PP2A) has revealed a critical tumor suppressor function for PP2A. However, PP2A has also been shown to have important roles in cell cycle progression and survival. Therefore, PP2A is not a typical tumor suppressor. This is most likely due to the fact that PP2A represents a large number of different holoenzymes. Further understanding of PP2A function(s), and especially its tumor suppressor activity, will depend largely on our ability to determine specific targets for these different PP2A holoenzymes and to gain an understanding of how these targets confer tumor suppressor activity or contribute to cell cycle progression and cell survival. Recent work has identified c-Myc as a target of the PP2A holoenzyme, PP2A-B56 α . This holoenzyme also negatively regulates β -catenin expression and modulates the anti-apoptotic activity of Bcl2, thus characterizing PP2A-B56 α as a tumor suppressor PP2A holoenzyme. This review will focus on the role of PP2A-B56 α in regulating c-Myc and will place this tumor suppressor activity of PP2A within the context of its other tumor suppressor functions. Finally, the mechanism(s) through which PP2A-B56 α tumor suppressor activity may be lost in cancer will be discussed.

Keywords

PP2A; c-Myc; B56 α ; Tumor suppressor

1 Introduction

A multitude of signaling pathways are utilized to govern virtually all aspects of cellular function thus enabling cells to respond to both their external and internal environment and to maintain “normal” cellular homeostasis. These signaling pathways are exquisitely regulated through a variety of mechanisms including the post-translational modification of proteins by reversible phosphorylation. Two key groups of proteins are involved in the reversible phosphorylation process, kinases that add phosphates to proteins and phosphatases that remove these phosphates from such targets. Interestingly, the number of published reports identifying and/or characterizing kinases exceeds those dealing with the activities of phosphatases by 15:1. Therefore, our understanding of phosphatases significantly lags behind our understanding of kinases.

Over the past 15 years the tumor suppressor function of certain phosphatases have begun to be appreciated. For example, mutation or loss of the tumor suppressor lipid phosphatase, PTEN occurs very frequently in a wide array of human cancers and is only surpassed by the frequency with which the function(s) of the classical p53 tumour suppressor are altered [1]. Additionally, the protein phosphatase, PP2A has received considerable attention as a result of the demonstration that it also possesses tumor suppressor activity [2–4]. However, a somewhat confusing aspect of PP2A function in cancer arises because PP2A also plays important roles in promoting cell cycle progression and cell survival and these are functions which are usually associated with tumor initiation and progression rather than tumor suppression [5–9]. These apparently contradictory functions of PP2A were initially demonstrated following the global inhibition of PP2A catalytic activity using okadaic acid. This can result in either increased or decreased levels of cellular transformation depending in large part upon the biological system being utilized [10–12]. These divergent functions of PP2A are most likely due to the large number of PP2A holoenzymes, all of which are sensitive to inhibition by this marine toxin. Consequently, to further understand the role of PP2A in cancer, the characterization of specific PP2A holoenzymes and their targets is critical. In this review we will discuss work focused on characterizing a subset of PP2A holoenzymes containing the regulatory B subunit B'/PR61/B56/PPP2R5 family members that generally have tumor suppressor activity. In particular, we will focus on the important role of the PP2A-B56 α holoenzyme in negatively regulating several key oncoproteins, specifically Cellular Myc (c-Myc), as well as β -catenin and Bcl2. We will conclude with an exploration of the mechanisms by which this PP2A holoenzyme may become disrupted in human cancers.

2 PP2A is a heterotrimeric substrate specific serine/threonine protein phosphatase

Protein phosphatases are grouped into two general families based on their ability to either dephosphorylate Tyrosine or Serine/Threonine (S/T) residues. PP2A is a member of the S/T family of protein phosphatases, which also includes PP1, PP2B (also called calcineurin), PP2C, PP3, PP4, PP5, PP6, PP7, PP8, and possibly others, which are yet to be identified. Of the S/T family of protein phosphatases, PP2A is considered to be one of the most prevalent members constituting 30–50% of cellular S/T protein phosphatase activity depending upon cell and tissue type. PP2A is a heterotrimeric protein comprised of a structural A subunit, catalytic C subunit, and highly variable, regulatory B subunit (Fig. 1, top). The structural A subunit is found in two isoforms, α or β and serves as a scaffold protein comprised of 15 non-identical huntingtin-elongation-A subunits-TOR-like (HEAT) repeats that facilitate the binding of the B and C subunits [13,14]. Similar to the A subunit, the C subunit also exists in two isoforms, α or β and is the catalytically active phosphatase subunit of PP2A holoenzymes. The regulatory B subunits fall into four unrelated families called B/B55/PPP2R2, B'/PR61/B56/PPP2R5 (referred to as B56 throughout the remainder of this review), B'', and B''' (Fig. 1) that bind the A subunit in a mutually exclusive manner to form distinct PP2A holoenzymes. Importantly, the regulatory B subunits direct PP2A holoenzyme activity to specific substrates as well as acting to target PP2A holoenzymes to various subcellular locations. Altogether, there are 25 reported regulatory B subunits, which allows for ~100 different PP2A holoenzymes to be formed through the various combinations of A and C isoforms along with the different B subunits. However, it is unlikely that all PP2A holoenzymes are formed in all cell types as the regulatory B subunits display differential expression depending upon cell type, tissue type and developmental stage.

3 Summary of the B56 subunit family in tumorigenesis

Considerable research activity has been focused on the tumor suppressor activity of PP2A following the demonstration that global inhibition of PP2A can contribute to the transformation of primary human cells [15–19]. However, there is now growing evidence that PP2A also possesses potentially oncogenic roles [7,20–22]. As mentioned above, much of the ambiguity regarding PP2A tumor suppressor versus oncogenic activity stems from the fact that PP2A represents a large number of distinct holoenzymes. Consequently, significant contributions to our understanding of PP2A function in tumorigenesis have resulted from the identification of specific PP2A holoenzyme/target protein interactions. Of particular interest for the purposes of this review are the B56 regulatory B subunits: α , β , γ , δ , and ϵ , which have been shown to have specific targets that are important in tumorigenesis (Fig. 1). Since their identification in 1995, most of the B56 subunits have been shown to have tumor suppressor activity, detailed in Fig. 1.

Of the B56 regulatory B subunits studied to date, B56 α and B56 γ are perhaps the best characterized in terms of both their cellular targets and tumor suppressor functions. We will discuss B56 α targets later in more detail and in particular its role in negatively regulating c-Myc. There are four splice variants of B56 γ (Fig. 1). Studies using anti-sense knockdown of multiple B56 γ variants have shown that loss of B56 γ expression contributes to the transformation of human kidney epithelial cells that also express Simian virus 40 (SV40)-Large-T-antigen, H-Ras, and hTERT [16]. It has also been shown in cultured human cells that both B56 γ 1 (variant 3) and B56 γ 3 (variant 1) can associate with and dephosphorylate p53 at T55, an event which stabilizes p53 in response to DNA damage [23]. Moreover, RNAi knockdown of B56 γ led to a decrease in p53 stability, reduced expression of Bax, and reduced apoptosis. B56 γ has also been shown to promote the degradation of the transcriptional co-activator, p300 in response to valproic-acid treatment of human cultured cells [24]. The biological importance of B56 γ regulation of p300 is yet to be fully appreciated. However, p300 has been shown to promote cellular proliferation and conversely induce apoptosis depending upon cellular context through its roles as a transcriptional co-activator and its ability to acetylate p53, respectively [25]. In a somewhat contradictory role, B56 γ could help to resolve the DNA damage response as it was shown to negatively regulate Chk2 activity in response to genotoxic stress in cultured human cells [26,27]. In addition, a truncated form of B56 γ 1, called Δ B56 γ 1, was identified in a mouse melanoma cell line that demonstrated increased metastatic potential [28]. It was further demonstrated that paxillin is a direct target of full-length B56 γ and expression of Δ B56 γ 1 could disrupt this regulation leading to increased paxillin phosphorylation and cell motility, suggesting that Δ B56 γ 1 functions as a dominant negative form of B56 γ . Following up on this initial study, the same group demonstrated that Δ B56 γ 1 expression led to increased polyploidy in gamma-irradiated BL6 tumor cells [29]. More recent work has shown that Δ B56 γ 1 expression can disrupt the phosphorylation of Mdm2 in cultured cells in response to irradiation [30]. The phosphorylation of Mdm2 is an important event in activating p53 as phosphorylation of Mdm2 inhibits its ability to target p53 for degradation. Altogether, these studies highlight important roles of B56 γ in activating p53 signaling and apoptosis, inhibiting proliferation and cell motility, and regulating genomic stability.

Two other B56 family members that have been characterized with specific targets are B56 β and B56 δ , with a single transcript reported for B56 β and three variants reported for B56 δ (Fig. 1). Recent work has demonstrated that B56 β specifically interacts with and negatively regulates protein levels of the Pim-1 kinase in 293 cells [31]. This finding suggests a tumor suppressor role for B56 β , since elevated Pim-1 kinase levels have been shown to cooperate with other oncoproteins such as c-Myc in cellular proliferation and lymphomagenesis [32]. A role for B56 δ in dephosphorylating Cdc25 at T138 and thus preventing the release of

14-3-3 from Cdc25 and inhibiting cell cycle progression through mitosis was shown in *Xenopus* egg extract studies [33]. Other B56 δ targets include the Heart and Neural Crest Derivatives Expressed 1 (HAND1) transcription factor, which is important in development, and the Dopamine- and cAMP-Regulated Phosphoprotein, (DARPP-32), which is critical for dopaminergic neurotransmission in striatal neurons [34,35].

Finally, B56 ϵ has been shown to be required for Wnt signaling in *Xenopus* embryogenesis [22]. Although no specific targets were identified for B56 ϵ in this study, it was demonstrated that B56 ϵ is required upstream of Dishevelled (DVL), but downstream of Wnt ligand. Having a positive role in Wnt signaling allows for a potentially oncogenic role for B56 ϵ as Wnt signaling results in increased proliferation. In summary, for all B56 family members, it will be essential to understand what cellular contexts are important for their respective tumor suppressor or oncogenic activities. In particular, understanding the relative importance of specific PP2A holoenzymes for the biology of different cancers, the mechanisms that govern the regulation of different PP2A holoenzymes, and how these mechanisms are perturbed in cancer will give us critical insight into PP2A's role in tumorigenesis.

4 PP2A-B56 α negatively regulates the potent oncoprotein c-Myc

As previously mentioned, B56 α is one of the more extensively characterized regulatory B subunits within the B56 family. Here we will examine in more detail recent work that has identified c-Myc as a B56 α target, followed by a discussion of other B56 α targets and its tumor suppressor functions. C-Myc is a transcription factor that regulates the expression of numerous genes involved in cellular proliferation, cellular growth, apoptosis, angiogenesis, and differentiation [36–40]. Over the past 20 years c-Myc has been intensely studied due to both the requirement of *c-myc* for normal development in mice as well as the high prevalence of elevated c-Myc expression in a wide array of human cancers [41,42]. Moreover, expression of c-Myc in mice using inducible *c-myc* transgenes results in the development of neoplastic pre-malignant and malignant phenotypes that often spontaneously regress when *c-myc* expression is shut off [43,44]. In addition, c-Myc has been shown to be one of four critical factors for the self-renewal of stem cells and could be important in cancer stem cell biology [45,46]. It is therefore critical to maintain tight control of c-Myc expression in order to maintain normal cellular function. Regulation of c-Myc expression occurs at several levels through transcriptional, translational, and post-translational mechanisms [47–52]. The regulation of c-Myc at the post-transcriptional level appears to be particularly critical since elevated expression of c-Myc protein is observed in ~70% of human tumors, but only ~20% of these show amplification of the *c-myc* gene or translocation of the gene to areas of high transcriptional activity [42].

Work from a number of labs including ours has focused on the post-translational regulation of c-Myc through a series of sequential, reversible phosphorylation events on two highly conserved residues, Threonine 58 (T58) and Serine 62 (S62) (Fig. 2(a)). Phosphorylation of T58 and S62 has opposing effects on c-Myc protein stability with S62 phosphorylation stabilizing and T58 phosphorylation destabilizing the c-Myc protein [52]. The PP2A holoenzyme, PP2A-B56 α , plays an important role in the regulation of these reversible phosphorylation events [47, 53]. The regulated phosphorylation of c-Myc on T58 and S62 is summarized in Fig. 2(a). As shown, in response to mitogenic stimulation, c-Myc can be phosphorylated on S62 by ERK through the Ras/Raf/MEK/ERK kinase cascade [54, 55]. It has also been shown that other MAPK and cyclin dependent kinases can phosphorylate c-Myc on S62 [56, 57]. Simultaneous activation of the PI3K/Akt pathway by Ras can result in the phosphorylation and inactivation of GSK3 β [58]. These two Ras regulated pathways keep c-Myc phosphorylated on S62, which stabilizes c-Myc protein. When Ras activity falls, inhibition of GSK3 β activity is relieved, allowing GSK3 β to phosphorylate c-Myc on T58 in

a processive manner, requiring previous phosphorylation on S62 [55, 57]. Following T58 phosphorylation by GSK3 β , the phosphorylation-directed prolyl isomerase, Pin1 associates with c-Myc and mediates isomerization to facilitate dephosphorylation of S62 by PP2A, which is conformation sensitive, dephosphorylating Serine or Threonine residues followed by Proline when the peptidyl prolyl bond is in *trans* [47]. In the case of c-Myc, PP2A-B56 α is the specific PP2A holoenzyme that dephosphorylates S62 leaving c-Myc singly phosphorylated at T58 within this motif [53]. Knockdown of B56 α was shown to increase c-Myc S62 phosphorylation and protein stability, while ectopic expression of B56 α in combination with additional PP2A-C subunit expression substantially reduced c-Myc protein levels [53]. C-Myc phosphorylated at T58 and lacking the stabilizing S62 phosphate is unstable, as it is recognized by the SCF^{Fbw7} ubiquitin machinery, which multi-ubiquitinates c-Myc marking it for degradation by the 26S proteasome [59, 60]. Very recent experiments in our lab have found that multiple components of the degradation pathway for c-Myc are recruited into a complex by the scaffold protein, Axin1 (Fig. 2(a)), which will be discussed below. Altogether, c-Myc protein stability is tightly regulated by these hierarchical, reversible phosphorylation events in which PP2A-B56 α plays an essential role.

Since post-translational regulation of c-Myc expression is likely a key level of regulation to prevent tumorigenic levels of c-Myc expression, our lab has been analyzing cancer cell-lines and patient samples for possible deregulation of c-Myc expression at the post-translational level. Thus far, observations in several breast cancer and leukemia cell-lines and patient samples have shown c-Myc protein stability to be significantly increased when compared to respective control cell-lines or normal samples with unstable c-Myc ([61] and Sears lab unpublished). Moreover, the c-Myc in these cancer cell-lines shows significantly increased levels of S62 phosphorylation when compared with control cell-lines, indicating that PP2A-B56 α function with respect to c-Myc may be impaired in these cancer cell-lines. In support of this hypothesis, a more oncogenic point mutant of c-Myc, c-Myc^{T58A}, is resistant to PP2A-mediated S62 dephosphorylation and is more stable with higher levels of S62 phosphorylation than wild-type c-Myc [47,52]. Moreover, this c-Myc^{T58A} mutant can also transform cultured primary human fibroblasts without any requirement for the presence of SV40 small-T antigen, which has been shown to disrupt PP2A-B56 α as well as other PP2A holoenzymes [16,47]. In contrast, SV40 small-T expression was required for wild-type c-Myc to transform these same cells. Additionally, preliminary analyses demonstrate that specific RNAi-mediated knockdown of B56 α can also facilitate transformation of these cells without the need for global inhibition of PP2A activity by SV40 small-T antigen (Sears lab, unpublished). Altogether, these findings strongly suggest that the maintenance of S62 in the unphosphorylated state by PP2A-B56 α is critical for the negative regulation of c-Myc expression and inhibition of its oncogenic function. Consequently, understanding the mechanism(s) through which B56 α activity may be lost in tumorigenesis is critical and some possible mechanisms are discussed below.

5 PP2A-B56 α associates with the scaffold protein Axin1

The general view of PP2A localization and regulation has been largely reported to be intrinsic, with the regulatory B subunits acting to both localize and regulate PP2A holoenzyme activity. Moreover, post-translational modification of the C subunit by phosphorylation and methylation regulate C subunit activity as well as the binding of particular regulatory B subunits [62–68]. Another level of PP2A regulation now being uncovered is the association of PP2A holoenzymes with adaptor and scaffold proteins (Fig. 2(b)). These proteins may act to both coordinate PP2A with its targets and help to localize PP2A holoenzymes. This mode of regulation was initially revealed by the recruitment of PP2A holoenzyme containing B56 family members to dephosphorylate Mdm2 through association with Cyclin G in cell culture experiments [69]. It was also shown that B56 α can

bind the adaptor protein, ankyrin-B and through this interaction B56 α was correctly localized in primary cardiomyocytes (Fig. 2(b)) [70]. Of particular interest in the context of this review, PP2A-B56 α holoenzymes have also been shown to associate with the scaffold protein, Axin1. This was initially demonstrated in *Xenopus* egg extract experiments [71]. A well characterized function of Axin1 is the coordination of APC, GSK3 β and now PP2A-B56 α into a degradation complex for β -catenin (Fig. 2(b)) [71–74]. However, the exact role of PP2A-B56 α within this Axin1-mediated degradation complex is not clear. Recent work in our lab has now shown that Axin1 also associates with c-Myc and regulates its stability by coordinating a degradation complex containing GSK3 β , PP2A-B56 α , and Pin1 (Sears lab, unpublished). Within the Axin1-mediated degradation complex for c-Myc, one role for PP2A-B56 α would be the direct dephosphorylation of c-Myc at S62. However, it is likely that PP2A-B56 α targets other components as well. Uncovering the regulation of PP2A by adaptor and scaffold proteins will greatly expand our understanding of PP2A biology.

6 PP2A-B56 α negatively regulates β -catenin expression and the anti-apoptotic function of Bcl2

In addition to c-Myc, β -catenin and Bcl2 have been shown to be targets of PP2A-B56 α activity (Fig. 1). β -catenin was the first identified target of B56 α activity, as it was demonstrated that overexpression of B56 α decreased β -catenin expression in mammalian cells and *Xenopus* embryo explants [75]. Additionally, it was also demonstrated that oncogenic point mutations in β -catenin could prevent its down-regulation by B56 α . This has important parallels with the finding that the oncogenic c-Myc^{T58A}-mutant is resistant to PP2A activity [47,52]. Intriguingly, although direct dephosphorylation of β -catenin by PP2A-B56 α has not been demonstrated, PP2A-B56 α was shown to be one component of a degradation complex for β -catenin mediated by the scaffold protein Axin1, as described above. PP2A-B56 α is now thought to have targets within this Axin1-mediated degradation complex for β -catenin. These may include APC, since B56 α and APC were found to interact in yeast-two hybrid experiments [75]. As part of the Axin1-mediated degradation complex for β -catenin, PP2A-B56 α was found to inhibit Wnt signaling and to be critical for normal dorsal/ventral axis formation in *Xenopus* development [71]. Importantly, β -catenin and Wnt signaling are critical for stem-cell maintenance and cellular proliferation, and deregulation of β -catenin and Wnt signaling are often observed in human malignancies [76]. Therefore, as a negative regulator of β -catenin and Wnt signaling, B56 α has been identified as a putative tumor suppressor [71,75].

Following the identification of β -catenin as a B56 α target, Bcl2 was shown to associate with B56 α in cell culture experiments [77]. Furthermore, in this study it was demonstrated that in response to ceramide treatment, PP2A-B56 α dephosphorylated Bcl2 at the mitochondrial membrane. These dephosphorylation events are thought to occur within the flexible loop domain (FLD) of Bcl2, including the key S70 residue, known to be important for Bcl2's anti-apoptotic function [78]. More specifically, phosphorylation of Bcl2 within the FLD increases its pro-survival activity by increasing its association with Bax and decreasing its association with p53 [79,80]. Consequently, PP2A-B56 α -mediated dephosphorylation of Bcl2 decreases its prosurvival activity, supporting a tumor suppressor role for PP2A-B56 α . It is also interesting to note that unspecified PP2A holoenzyme(s) have been shown to promote apoptosis by increasing the pro-apoptotic activity of Bax and BAD [81,82]. Although the specific B subunit responsible for directing PP2A activity to these targets has not been identified, it is becoming clear that PP2A plays an important role in regulating the induction of apoptosis. In particular, the identification of PP2A-B56 α as a regulator of Bcl2 represents the beginning of our understanding of the roles of specific PP2A holoenzymes in controlling key proteins involved in apoptosis.

7 Interplay between PP2A-B56 α targets

An interesting aspect of the identification of these PP2A-B56 α targets arises from the transcriptional interplay and cooperation in tumorigenesis between c-Myc, β -catenin, and Bcl2, summarized in Fig. 3. The *c-myc* gene has been shown to be a transcriptional target of β -catenin/TCF/Lef and observations from different human tumors show elevated co-expression of c-Myc and β -catenin [83–85]. Interestingly, there are also reports that β -catenin and c-Myc expression do not correlate in human tumors and it is suggested from these observations that multiple levels of regulation are responsible for the coordination of β -catenin and c-Myc expression [86,87]. β -catenin/TCF/Lef transcriptional activity has also been shown to induce luciferase activity from a *Bcl2* promoter-luciferase reporter construct [88]. Of note, the induction of luciferase activity in this assay could be suppressed by RNAi knockdown of c-Myc, indicating that β -catenin transcriptional regulation of *Bcl2* is likely dependent upon its ability to induce the expression of c-Myc. In contrast, elevated c-Myc expression has also been shown to repress expression of *Bcl2* by interfering with *Bcl2* gene transcription activated by the Myc Interacting Zinc-finger 1 (MIZ1) (Fig. 3), resulting in the apoptosis of primary human cultured cells [89–91]. The ability of c-Myc to repress *Bcl2* expression through this mechanism is thought to inhibit the transformation of cells due to elevated c-Myc expression. Importantly, this protective mechanism against elevated c-Myc expression is frequently lost in tumors as c-Myc and Bcl2 often cooperate in tumorigenesis [92,93]. Altogether, the expression of c-Myc, β -catenin, and Bcl2 have been shown to affect one another at the transcriptional level, yet discrepancies in a number of reports strongly suggest that other levels of control are also important in the coordinated regulation of these oncoproteins. Therefore, the regulation of c-Myc, β -catenin, and Bcl2 by the same PP2A holoenzyme, PP2A-B56 α at the post-translational level could offer insight into new mechanisms through which these oncoproteins may influence one another. Furthermore, loss of PP2A-B56 α activity could be disastrous as it may result in the simultaneous deregulation of β -catenin, c-Myc and Bcl2.

8 Disruption of PP2A-B56 α holoenzyme function

Since B56 α plays a critical role in regulating multiple oncoproteins, an important question that arises is whether loss of B56 α function occurs in cancer. Furthermore, identifying mechanisms through which loss of B56 α function can occur is a critical aspect to understanding the importance of B56 α function in tumorigenesis. To date, several mechanisms have been reported that could disrupt PP2A-B56 α function and these are summarized in Fig. 4.

One of the first reported mechanisms shown to disrupt PP2A holoenzyme function is the ability of viral proteins such as Polyoma small and middle T antigens and SV40-small T antigen to bind the structural A subunit and prevent the association of regulatory B subunits with the A subunit (Fig. 4) [94–96]. Specifically, expression of SV40 small T antigen has been shown to disrupt PP2A-B56 α and other B56 holoenzymes in cultured human primary cells [16]. Moreover, expression of these viral proteins has been shown to facilitate the transformation of cultured primary human cells [97–99]. Unfortunately, it is not possible to conclude from these experiments which PP2A holoenzymes are critical in preventing transformation since multiple holoenzymes are simultaneously inhibited by the viral T antigens.

There are a growing number of reports that have identified mutations and mis-expression of PP2A in various human cancers. In particular, mutations in the A α and A β subunits have been reported in human melanoma, breast, lung, colon and colorectal cancers, as well as B-cell chronic lymphocytic leukaemia [86,100–102]. Moreover, some of the mutations

identified in the A subunit from breast tumors have been shown to specifically disrupt its interaction with B56 family members [103]. It is important to note that screening by DNA sequence analysis of PP2A-A α / β in some other human cancers such as gliomas, cervical, and Wilms tumor revealed no mutations [104–106]. However, in a study of human gliomas by Colella et al., expression of the PP2A-A α subunit was found to be reduced by 10 fold in 43% of the tumors screened. This suggests that even without mutations in PP2A-A α / β , down-regulation of the PP2A-A α subunit may still play a role in tumorigenesis. Analogous to the mechanisms of viral disruption of PP2A holoenzyme function, mutations or mis-expression of the A subunit, as described above, will most likely affect multiple regulatory B subunits. Consequently, it is again difficult to identify specific PP2A holoenzymes that have tumor suppressor activity from current reports.

An alternative mechanism that could lead to disruption of PP2A-B56 α holoenzyme activity is a “substrate trap” mechanism by which B56 α and/or PP2A-B56 α holoenzymes can be abnormally sequestered. This mechanism was first suggested by the observation that Δ Np63, an oncogenic form of p63, interacted with B56 α resulting in increased β -catenin protein levels and TCF/Lef-mediated transcription in cultured cells [107]. More recently, we reported that c-Myc^{T58A}, a highly oncogenic form of c-Myc, has a significantly increased association with B56 α when compared to c-Myc^{WT} [53]. These results suggest that oncogenes such as Δ Np63 and c-Myc^{T58A} can potentially act as “substrate traps” for B56 α . Consistent with this, data also suggests that the amount of B56 α is limiting [53,108]. Consequently, these oncoproteins could interfere with the ability of B56 α to regulate its other targets, thus resulting in the deregulation of multiple oncoproteins, such c-Myc, β -catenin, and/or Bcl2 at the post-translational level.

Lastly, the association of PP2A-B56 α with adaptor and scaffold proteins opens the door to new mechanisms by which B56 α function could be disrupted. It was recently demonstrated in primary cardiomyocytes that B56 α associated with the adaptor protein, ankyrin-B [70]. Furthermore, the reduced levels of ankyrin-B in primary cardiomyocytes derived from heterozygous ankyrin-B knockout mice resulted in disorganized localization of B56 α and this could be rescued by exogenous expression of ankyrin-B. PP2A-B56 α has also been shown to associate with the scaffold protein Axin1 [71] and several studies have identified mutations in Axin1 from a wide array of human tumors that potentially affect the association of PP2A-B56 α with this scaffold [109]. Clearly, the molecular and biological consequences of these mutations in Axin1 will need to be characterized in order to determine the importance of Axin1/PP2A-B56 α association and its regulation of PP2A-B56 α function in tumorigenesis. Altogether, there are several potential mechanisms that could disrupt normal PP2A-B56 α function resulting in loss of its tumor suppressor activity in cancer (Fig. 4).

9 Conclusions

The results of numerous studies now support the idea that PP2A has important tumor suppressor activities. However, it should also be noted that PP2A has been reported to possess oncogenic potential. An increased understanding of PP2A tumor suppressor function will depend largely on the identification of specific target/PP2A holoenzyme interactions. Recent work discussed in this review has now identified the PP2A holoenzyme containing the regulatory B subunit B56 α as possessing critical tumor suppressor activity via the regulation of several potent oncoproteins. These include, β -catenin, Bcl2, and c-Myc. Furthermore, potential loss of PP2A-B56 α function(s) can occur via several mechanisms resulting in the simultaneous deregulation of these oncoproteins, and may be a critical event in the progression of certain human tumors. Clearly, our current knowledge needs to be greatly expanded and important questions such as the underlying mechanisms and frequency of loss or disruption of B56 α function need to be answered before we can fully understand

the importance of PP2A-B56 α tumor suppressor activity. However, the mechanism of PP2A's tumor suppressor activity is now beginning to be revealed one B subunit at a time.

Abbreviations

APC	adenomatous polyposis coli
Bcl2	B-cell lymphoma 2
Cdc25	cell division cycle 25
c-Myc	cellular Myelocytomatosis
DARPP-32	dopamine- and cAMP-regulated phosphoprotein 32 kD
DVL	dishevelled
ERK	extracellular receptor kinase
FLD	flexible loop domain
GSK3β	glycogen synthase kinase 3 β
HAND1	heart and neural crest derivatives expressed 1
HEAT	huntingtin-elongation-A subunits-TOR-like
hTERT	human telomerase reverse transcriptase
MAPK	mitogen activated protein kinase
Mdm2	mouse double minute 2
MIZ1	Myc interacting zinc-finger 1
PI3K	phosphoinositide-3-kinase
Pin1	protein (peptidylprolyl cis/trans isomerase) NIMA-interacting 1
PP2A	protein phosphatase 2A
PTEN	phosphatase and tensin homolog
S	serine
SCF	Skp/Cullin/F-box
SV40	Simian virus 40
T	threonine
TCF	T cell specific factor
Wnt	wingless/Int

References

1. Sansal I, Sellers WR. The biology and clinical relevance of the PTEN tumor suppressor pathway. *Journal of Clinical Oncology* 2004;22:2954–2963. [PubMed: 15254063]
2. Parsons R. Phosphatases and tumorigenesis. *Current Opinion in Oncology* 1998;10:88–91. [PubMed: 9466490]
3. Van Hoof C, Goris J. PP2A fulfills its promises as tumor suppressor: Which subunits are important. *Cancer Cell* 2004;5:105–106. [PubMed: 14998482]
4. Schonthal AH. Role of serine/threonine protein phosphatase 2A in cancer. *Cancer Letters* 2001;170:1–13. [PubMed: 11448528]
5. Galaktionov K, Lee AK, Eckstein J, Draetta G, Meckler J, Loda M, et al. CDC25 phosphatases as potential human oncogenes. *Science* 1995;269:1575–1577. [PubMed: 7667636]

6. Yan Z, Fedorov SA, Mumby MC, Williams RS. PR48, a novel regulatory subunit of protein phosphatase 2A, interacts with Cdc6 and modulates DNA replication in human cells. *Molecular and Cellular Biology* 2000;20:1021–1029. [PubMed: 10629059]
7. Li X, Scuderi A, Letsou A, Virshup DM. B56-associated protein phosphatase 2A is required for survival and protects from apoptosis in *Drosophila melanogaster*. *Molecular and Cellular Biology* 2002;22:3674–3684. [PubMed: 11997504]
8. Lin XH, Walter J, Scheidtmann K, Ohst K, Newport J, Walter G. Protein phosphatase 2A is required for the initiation of chromosomal DNA replication. *Proceedings of the National Academy of Sciences of the United States of America* 1998;95:14693–14698. [PubMed: 9843951]
9. Mayer-Jaekel RE, Ohkura H, Gomes R, Sunkel CE, Baumgartner S, Hemmings BA, et al. The 55 kd regulatory subunit of *Drosophila* protein phosphatase 2A is required for anaphase. *Cell* 1993;72:621–633. [PubMed: 8382567]
10. Sakai A, Fujiki H. Promotion of BALB/3T3 cell transformation by the okadaic acid class of tumor promoters, okadaic acid and dinophysistoxin-1. *Japanese Journal of Cancer Research* 1991;82:518–523. [PubMed: 1905699]
11. Nagao M, Sakai R, Kitagawa Y, Ikeda I, Sasaki K, Shima H, et al. Role of protein phosphatases in malignant transformation. *Princess Takamatsu Symposia* 1989;20:177–184. [PubMed: 2562181]
12. Zheng B, Woo CF, Kuo JF. Mitotic arrest and enhanced nuclear protein phosphorylation in human leukemia K562 cells by okadaic acid, a potent protein phosphatase inhibitor and tumor promoter. *The Journal of Biological Chemistry* 1991;266:10031–10034. [PubMed: 1645333]
13. Kremmer E, Ohst K, Kiefer J, Brewis N, Walter G. Separation of PP2A core enzyme and holoenzyme with monoclonal antibodies against the regulatory A subunit: Abundant expression of both forms in cells. *Molecular and Cellular Biology* 1997;17:1692–1701. [PubMed: 9032296]
14. Xu Y, Xing Y, Chen Y, Chao Y, Lin Z, Fan E, et al. Structure of the protein phosphatase 2A holoenzyme. *Cell* 2006;127:1239–1251. [PubMed: 17174897]
15. Shenolikar S. Protein serine/threonine phosphatases—new avenues for cell regulation. *Annual Review of Cell Biology* 1994;10:55–86.
16. Chen W, Possemato R, Campbell KT, Plattner CA, Pallas DC, Hahn WC. Identification of specific PP2A complexes involved in human cell transformation. *Cancer Cell* 2004;5:127–136. [PubMed: 14998489]
17. Millward TA, Zolnierowicz S, Hemmings BA. Regulation of protein kinase cascades by protein phosphatase 2A. *Trends in Biochemical Sciences* 1999;24:186–191. [PubMed: 10322434]
18. Virshup DM. Protein phosphatase 2A: A panoply of enzymes. *Current Opinion in Cell Biology* 2000;12:180–185. [PubMed: 10712915]
19. Schonthal AH. Role of PP2A in intracellular signal transduction pathways. *Frontiers in Bioscience* 1998;3:D1262–D1273. [PubMed: 9851914]
20. Jaumot M, Hancock JF. Protein phosphatases 1 and 2A promote Raf-1 activation by regulating 14-3-3 interactions. *Oncogene* 2001;20:3949–3958. [PubMed: 11494123]
21. Abraham D, Podar K, Pacher M, Kubicek M, Welzel N, Hemmings BA, et al. Raf-1-associated protein phosphatase 2A as a positive regulator of kinase activation. *The Journal of Biological Chemistry* 2000;275:22300–22304. [PubMed: 10801873]
22. Yang J, Wu J, Tan C, Klein PS. PP2A: B56epsilon is required for Wnt/beta-catenin signaling during embryonic development. *Development* 2003;130:5569–5578. [PubMed: 14522869]
23. Li HH, Cai X, Shouse GP, Piluso LG, Liu X. A specific PP2A regulatory subunit, B56gamma, mediates DNA damage-induced dephosphorylation of p53 at Thr55. *The EMBO Journal* 2007;26:402–411. [PubMed: 17245430]
24. Chen J, St-Germain JR, Li Q. B56 regulatory subunit of protein phosphatase 2A mediates valproic acid-induced p300 degradation. *Molecular and Cellular Biology* 2005;25:525–532. [PubMed: 15632055]
25. Goodman RH, Smolik S. CBP/p300 in cell growth, transformation, and development. *Genes and Development* 2000;14:1553–1577. [PubMed: 10887150]
26. Dozier C, Bonyadi M, Baricault L, Tonasso L, Darbon JM. Regulation of Chk2 phosphorylation by interaction with protein phosphatase 2A via its B' regulatory subunit. *Biology of the Cell* 2004;96:509–517. [PubMed: 15380617]

27. Liang X, Reed E, Yu JJ. Protein phosphatase 2A interacts with Chk2 and regulates phosphorylation at Thr-68 after cisplatin treatment of human ovarian cancer cells. *International Journal of Molecular Medicine* 2006;17:703–708. [PubMed: 16596250]
28. Ito A, Kataoka TR, Watanabe M, Nishiyama K, Mazaki Y, Sabe H, et al. A truncated isoform of the PP2A B56 subunit promotes cell motility through paxillin phosphorylation. *The EMBO Journal* 2000;19:562–571. [PubMed: 10675325]
29. Ito A, Koma Y, Sohda M, Watabe K, Nagano T, Misumi Y, et al. Localization of the PP2A B56gamma regulatory subunit at the Golgi complex: Possible role in vesicle transport and migration. *American Journal of Pathology* 2003;162:479–489. [PubMed: 12547706]
30. Koma YI, Ito A, Watabe K, Kimura SH, Kitamura Y. A truncated isoform of the PP2A B56gamma regulatory subunit reduces irradiation-induced Mdm2 phosphorylation and could contribute to metastatic melanoma cell radioresistance. *Histology and Histopathology* 2004;19:391–400. [PubMed: 15024700]
31. Ma J, Arnold HK, Lilly MB, Sears RC, Kraft AS. Negative regulation of Pim-1 protein kinase levels by the B56beta subunit of PP2A. *Oncogene* 2007;26:5145–5153. [PubMed: 17297438]
32. Allen JD, Berns A. Complementation tagging of cooperating oncogenes in knockout mice. *Seminars in Cancer Biology* 1996;7:299–306. [PubMed: 9110407]
33. Margolis SS, Perry JA, Forester CM, Nutt LK, Guo Y, Jardim MJ, et al. Role for the PP2A/B56delta phosphatase in regulating 14-3-3 release from Cdc25 to control mitosis. *Cell* 2006;127:759–773. [PubMed: 17110335]
34. Ahn JH, McAvoy T, Rakhilin SV, Nishi A, Greengard P, Nairn AC. Protein kinase A activates protein phosphatase 2A by phosphorylation of the B56delta subunit. *Proceedings of the National Academy of Sciences of the United States of America* 2007;104:2979–2984. [PubMed: 17301223]
35. Firulli BA, Howard MJ, McDaid JR, McIlreavey L, Dionne KM, Centonze VE, et al. PKA, PKC, and the protein phosphatase 2A influence HAND factor function: A mechanism for tissue-specific transcriptional regulation. *Molecular Cell* 2003;12:1225–1237. [PubMed: 14636580]
36. White RJ. RNA polymerases I and III, growth control and cancer. *Nature Reviews. Molecular Cell Biology* 2005;6:69–78.
37. Cole MD. The myc oncogene: Its role in transformation and differentiation. *Annual Review of Genetics* 1986;20:361–384.
38. Luscher B, Eisenman RN. New light on Myc and Myb. Part I. Myc. *Genes and Development* 1990;4:2025–2035. [PubMed: 2269425]
39. Prendergast GC. Mechanisms of apoptosis by c-Myc. *Oncogene* 1999;18:2967–2987. [PubMed: 10378693]
40. Baudino TA, McKay C, Pendeville-Samain H, Nilsson JA, Maclean KH, White EL, et al. c-Myc is essential for vasculogenesis and angiogenesis during development and tumor progression. *Genes and Development* 2002;16:2530–2543. [PubMed: 12368264]
41. Davis AC, Wims M, Spotts GD, Hann SR, Bradley A. A null c-myc mutation causes lethality before 10.5 days of gestation in homozygotes and reduced fertility in heterozygous female mice. *Genes and Development* 1993;7:671–682. [PubMed: 8458579]
42. Nesbit CE, Tersak JM, Prochownik EV. MYC oncogenes and human neoplastic disease. *Oncogene* 1999;18:3004–3016. [PubMed: 10378696]
43. Felsher DW, Bishop JM. Reversible tumorigenesis by MYC in hematopoietic lineages. *Molecular Cell* 1999;4:199–207. [PubMed: 10488335]
44. Pelengaris S, Littlewood T, Khan M, Elia G, Evan G. Reversible activation of c-Myc in skin: Induction of a complex neoplastic phenotype by a single oncogenic lesion. *Molecular Cell* 1999;3:565–577. [PubMed: 10360173]
45. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;126:663–676. [PubMed: 16904174]
46. Coppola JA, Cole MD. Constitutive c-myc oncogene expression blocks mouse erythroleukemia cell differentiation but not commitment. *Nature* 1986;320:760–763. [PubMed: 3458027]
47. Yeh E, Cunningham M, Arnold H, Chasse D, Monteith T, Ivaldi G, et al. A signalling pathway controlling c-Myc degradation that impacts oncogenic transformation of human cells. *Nature Cell Biology* 2004;6:308–318.

48. Flinn EM, Busch CM, Wright AP. myc boxes, which are conserved in myc family proteins, are signals for protein degradation via the proteasome. *Molecular and Cellular Biology* 1998;18:5961–5969. [PubMed: 9742113]
49. Jones TR, Cole MD. Rapid cytoplasmic turnover of c-myc mRNA: Requirement of the 3' untranslated sequences. *Molecular and Cellular Biology* 1987;7:4513–4521. [PubMed: 3325826]
50. Kelly K, Cochran BH, Stiles CD, Leder P. Cell-specific regulation of the c-myc gene by lymphocyte mitogens and platelet-derived growth factor. *Cell* 1983;35:603–610. [PubMed: 6606489]
51. Sears R, Leone G, DeGregori J, Nevins JR. Ras enhances Myc protein stability. *Molecular Cell* 1999;3:169–179. [PubMed: 10078200]
52. Sears R, Nuckolls F, Haura E, Taya Y, Tamai K, Nevins JR. Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. *Genes and Development* 2000;14:2501–2514. [PubMed: 11018017]
53. Arnold HK, Sears RC. Protein phosphatase 2A regulatory subunit B56alpha associates with c-myc and negatively regulates c-myc accumulation. *Molecular and Cellular Biology* 2006;26:2832–2844. [PubMed: 16537924]
54. Seth A, Gonzalez FA, Gupta S, Raden DL, Davis RJ. Signal transduction within the nucleus by mitogen-activated protein kinase. *The Journal of Biological Chemistry* 1992;267:24796–24804. [PubMed: 1332967]
55. Pulverer BJ, Fisher C, Vousden K, Littlewood T, Evan G, Woodgett JR. Site-specific modulation of c-Myc cotransformation by residues phosphorylated *in vivo*. *Oncogene* 1994;9:59–70. [PubMed: 8302604]
56. Noguchi K, Kitanaka C, Yamana H, Kokubu A, Mochizuki T, Kuchino Y. Regulation of c-Myc through phosphorylation at Ser-62 and Ser-71 by c-Jun N-terminal kinase. *The Journal of Biological Chemistry* 1999;274:32580–32587. [PubMed: 10551811]
57. Lutterbach B, Hann SR. Hierarchical phosphorylation at N-terminal transformation-sensitive sites in c-Myc protein is regulated by mitogens and in mitosis. *Molecular and Cellular Biology* 1994;14:5510–5522. [PubMed: 8035827]
58. Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* 1995;378:785–789. [PubMed: 8524413]
59. Welcker M, Orian A, Jin J, Grim JA, Harper JW, Eisenman RN, et al. The Fbw7 tumor suppressor regulates glycogen synthase kinase 3 phosphorylation-dependent c-Myc protein degradation. *Proceedings of the National Academy of Sciences of the United States of America* 2004;101:9085–9090. [PubMed: 15150404]
60. Yada M, Hatakeyama S, Kamura T, Nishiyama M, Tsunematsu R, Imaki H, et al. Phosphorylation-dependent degradation of c-Myc is mediated by the F-box protein Fbw7. *The EMBO Journal* 2004;23:2116–2125. [PubMed: 15103331]
61. Malempati S, Tibbitts D, Cunningham M, Akkari Y, Olson S, Fan G, et al. Aberrant stabilization of c-Myc protein in some lymphoblastic leukemias. *Leukemia* 2006;20:1572–1581. [PubMed: 16855632]
62. Chen J, Martin BL, Brautigan DL. Regulation of protein serine-threonine phosphatase type-2A by tyrosine phosphorylation. *Science* 1992;257:1261–1264. [PubMed: 1325671]
63. Xie H, Clarke S. Methyl esterification of C-terminal leucine residues in cytosolic 36-kDa polypeptides of bovine brain. A novel eucaryotic protein carboxyl methylation reaction. *The Journal of Biological Chemistry* 1993;268:13364–13371. [PubMed: 8514774]
64. Lee J, Stock J. Protein phosphatase 2A catalytic subunit is methyl-esterified at its carboxyl terminus by a novel methyltransferase. *The Journal of Biological Chemistry* 1993;268:19192–19195. [PubMed: 8396127]
65. Favre B, Zolnierowicz S, Turowski P, Hemmings BA. The catalytic subunit of protein phosphatase 2A is carboxyl-methylated *in vivo*. *The Journal of Biological Chemistry* 1994;269:16311–16317. [PubMed: 8206937]
66. Bryant JC, Westphal RS, Wadzinski BE. Methylated C-terminal leucine residue of PP2A catalytic subunit is important for binding of regulatory Balpha subunit. *Biochemical Journal* 1999;339(Pt 2):241–246. [PubMed: 10191253]

67. Tolstykh T, Lee J, Vafai S, Stock JB. Carboxyl methylation regulates phosphoprotein phosphatase 2A by controlling the association of regulatory B subunits. *The EMBO Journal* 2000;19:5682–5691. [PubMed: 11060019]
68. Yu XX, Du X, Moreno CS, Green RE, Ogris E, Feng Q, et al. Methylation of the protein phosphatase 2A catalytic subunit is essential for association of B α regulatory subunit but not SG2NA, striatin, or polyomavirus middle tumor antigen. *Molecular and Cellular Biology* 2001;21:185–199. [PubMed: 11113193]
69. Okamoto K, Li H, Jensen MR, Zhang T, Taya Y, Thorgeirsson SS, et al. Cyclin G recruits PP2A to dephosphorylate Mdm2. *Molecular Cell* 2002;9:761–771. [PubMed: 11983168]
70. Bhasin N, Cunha SR, Mudannayake M, Gigena MS, Rogers TB, Mohler PJ. Molecular basis for PP2A regulatory subunit B56 α targeting in cardiomyocytes. *American Journal of Physiology. Heart and Circulatory Physiology* 2007;293:H109–H119. [PubMed: 17416611]
71. Li X, Yost HJ, Virshup DM, Seeling JM. Protein phosphatase 2A and its B56 regulatory subunit inhibit Wnt signaling in *Xenopus*. *The EMBO Journal* 2001;20:4122–4131. [PubMed: 11483515]
72. Hart MJ, de los Santos R, Albert IN, Rubinfeld B, Polakis P. Downregulation of beta-catenin by human Axin and its association with the APC tumor suppressor, beta-catenin and GSK3 β . *Current Biology* 1998;8:573–581. [PubMed: 9601641]
73. Ikeda S, Kishida S, Yamamoto H, Murai H, Koyama S, Kikuchi A. Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3 β and beta-catenin and promotes GSK-3 β -dependent phosphorylation of beta-catenin. *The EMBO Journal* 1998;17:1371–1384. [PubMed: 9482734]
74. Sakanaka C, Weiss JB, Williams LT. Bridging of beta-catenin and glycogen synthase kinase-3 β by axin and inhibition of beta-catenin-mediated transcription. *Proceedings of the National Academy of Sciences of the United States of America* 1998;95:3020–3023. [PubMed: 9501208]
75. Seeling JM, Miller JR, Gil R, Moon RT, White R, Virshup DM. Regulation of beta-catenin signaling by the B56 subunit of protein phosphatase 2A. *Science* 1999;283:2089–2091. [PubMed: 10092233]
76. Reya T, Clevers H. Wnt signalling in stem cells and cancer. *Nature* 2005;434:843–850. [PubMed: 15829953]
77. Ruvolo PP, Clark W, Mumby M, Gao F, May WS. A functional role for the B56 α -subunit of protein phosphatase 2A in ceramide-mediated regulation of Bcl2 phosphorylation status and function. *The Journal of Biological Chemistry* 2002;277:22847–22852. [PubMed: 11929874]
78. Ruvolo PP, Deng X, May WS. Phosphorylation of Bcl2 and regulation of apoptosis. *Leukemia* 2001;15:515–522. [PubMed: 11368354]
79. Deng X, Gao F, Flagg T, Anderson J, May WS. Bcl2's flexible loop domain regulates p53 binding and survival. *Molecular and Cellular Biology* 2006;26:4421–4434. [PubMed: 16738310]
80. Deng X, Gao F, Flagg T, May WS Jr. Mono- and multisite phosphorylation enhances Bcl2's antiapoptotic function and inhibition of cell cycle entry functions. *Proceedings of the National Academy of Sciences of the United States of America* 2004;101:153–158. [PubMed: 14660795]
81. Xin M, Deng X. Protein phosphatase 2A enhances the proapoptotic function of Bax through dephosphorylation. *The Journal of Biological Chemistry* 2006;281:18859–18867. [PubMed: 16679323]
82. Chiang CW, Kanies C, Kim KW, Fang WB, Parkhurst C, Xie M, et al. Protein phosphatase 2A dephosphorylation of phosphoserine 112 plays the gatekeeper role for BAD-mediated apoptosis. *Molecular and Cellular Biology* 2003;23:6350–6362. [PubMed: 12944463]
83. He TC, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT, et al. Identification of c-MYC as a target of the APC pathway. *Science* 1998;281:1509–1512. [PubMed: 9727977]
84. Ozaki S, Ikeda S, Ishizaki Y, Kurihara T, Tokumoto N, Iseki M, et al. Alterations and correlations of the components in the Wnt signaling pathway and its target genes in breast cancer. *Oncology Reports* 2005;14:1437–1443. [PubMed: 16273236]
85. Shiina H, Igawa M, Shigeno K, Terashima M, Deguchi M, Yamanaka M, et al. Beta-catenin mutations correlate with over expression of C-myc and cyclin D1 genes in bladder cancer. *Journal of Urology* 2002;168:2220–2226. [PubMed: 12394763]

86. Wang SS, Esplin ED, Li JL, Huang L, Gazdar A, Minna J, et al. Alterations of the PPP2R1B gene in human lung and colon cancer. *Science* 1998;282:284–287. [PubMed: 9765152]
87. Takayasu H, Horie H, Hiyama E, Matsunaga T, Hayashi Y, Watanabe Y, et al. Frequent deletions and mutations of the beta-catenin gene are associated with overexpression of cyclin D1 and fibronectin and poorly differentiated histology in childhood hepatoblastoma. *Clinical Cancer Research* 2001;7:901–908. [PubMed: 11309340]
88. Li Q, Dashwood WM, Zhong X, Nakagama H, Dashwood RH. Bcl-2 overexpression in PhIP-induced colon tumors: Cloning of the rat Bcl-2 promoter and characterization of a pathway involving beta-catenin, c-Myc and E2F1. *Oncogene* 2007;26:6194–6202. [PubMed: 17404573]
89. Eischen CM, Packham G, Nip J, Fee BE, Hiebert SW, Zambetti GP, et al. Bcl-2 is an apoptotic target suppressed by both c-Myc and E2F-1. *Oncogene* 2001;20:6983–6993. [PubMed: 11704823]
90. Eischen CM, Woo D, Roussel MF, Cleveland JL. Apoptosis triggered by Myc-induced suppression of Bcl-X(L) or Bcl-2 is bypassed during lymphomagenesis. *Molecular and Cellular Biology* 2001;21:5063–5070. [PubMed: 11438662]
91. Patel JH, McMahon SB. BCL2 is a downstream effector of MIZ-1 essential for blocking c-MYC-induced apoptosis. *The Journal of Biological Chemistry* 2007;282:5–13. [PubMed: 17082179]
92. Strasser A, Harris AW, Bath ML, Cory S. Novel primitive lymphoid tumours induced in transgenic mice by cooperation between myc and bcl-2. *Nature* 1990;348:331–333. [PubMed: 2250704]
93. Letai A, Sorcinelli MD, Beard C, Korsmeyer SJ. Antiapoptotic BCL-2 is required for maintenance of a model leukemia. *Cancer Cell* 2004;6:241–249. [PubMed: 15380515]
94. Pallas DC, Shahrik LK, Martin BL, Jaspers S, Miller TB, Brautigan DL, et al. Polyoma small and middle T antigens and SV40 small t antigen form stable complexes with protein phosphatase 2A. *Cell* 1990;60:167–176. [PubMed: 2153055]
95. Mumby M. Regulation by tumour antigens defines a role for PP2A in signal transduction. *Seminars in Cancer Biology* 1995;6:229–237. [PubMed: 8541518]
96. Sontag E, Fedorov S, Kamibayashi C, Robbins D, Cobb M, Mumby M. The interaction of SV40 small tumor antigen with protein phosphatase 2A stimulates the map kinase pathway and induces cell proliferation. *Cell* 1993;75:887–897. [PubMed: 8252625]
97. Hahn WC, Dessain SK, Brooks MW, King JE, Elenbaas B, Sabatini DM, et al. Enumeration of the simian virus 40 early region elements necessary for human cell transformation. *Molecular and Cellular Biology* 2002;22:2111–2123. [PubMed: 11884599]
98. Rundell K, Parakati R. The role of the SV40 ST antigen in cell growth promotion and transformation. *Seminars in Cancer Biology* 2001;11:5–13. [PubMed: 11243894]
99. Yu J, Boyapati A, Rundell K. Critical role for SV40 small-t antigen in human cell transformation. *Virology* 2001;290:192–198. [PubMed: 11883184]
100. Tamaki M, Goi T, Hirono Y, Katayama K, Yamaguchi A. PPP2R1B gene alterations inhibit interaction of PP2A-Abeta and PP2A-C proteins in colorectal cancers. *Oncology Reports* 2004;11:655–659. [PubMed: 14767517]
101. Kalla C, Scheuermann MO, Kube I, Schlotter M, Mertens D, Dohner 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. *European Journal of Cancer* 2007;43:1328–1335. [PubMed: 17449237]
102. Calin GA, di Iasio MG, Caprini E, Vorechovsky I, Natali PG, Sozzi G, et al. Low frequency of alterations of the alpha (PPP2R1A) and beta (PPP2R1B) isoforms of the subunit A of the serine-threonine phosphatase 2A in human neoplasms. *Oncogene* 2000;19:1191–1195. [PubMed: 10713707]
103. Ruediger R, Pham HT, Walter G. Disruption of protein phosphatase 2A subunit interaction in human cancers with mutations in the A alpha subunit gene. *Oncogene* 2001;20:10–15. [PubMed: 11244497]
104. Yeh LS, Hsieh YY, Chang JG, Chang WW, Chang CC, Tsai FJ. Mutation analysis of the tumor suppressor gene PPP2R1B in human cervical cancer. *International Journal of Gynecological Cancer* 2007;17:868–871. [PubMed: 17343570]
105. Ruteshouser EC, Ashworth LK, Huff V. Absence of PPP2R1A mutations in Wilms tumor. *Oncogene* 2001;20:2050–2054. [PubMed: 11360189]

106. Colella S, Ohgaki H, Ruediger R, Yang F, Nakamura M, Fujisawa H, et al. Reduced expression of the Aalpha subunit of protein phosphatase 2A in human gliomas in the absence of mutations in the Aalpha and Abeta subunit genes. *International Journal of Cancer* 2001;93:798–804.
107. Patturajan M, Nomoto S, Sommer M, Fomenkov A, Hibi K, Zangen R, et al. DeltaNp63 induces beta-catenin nuclear accumulation and signaling. *Cancer Cell* 2002;1:369–379. [PubMed: 12086851]
108. Martens E, Stevens I, Janssens V, Vermeesch J, Gotz J, Goris J, et al. Genomic organisation, chromosomal localisation tissue distribution and developmental regulation of the PR61/B' regulatory subunits of protein phosphatase 2A in mice. *Journal of Molecular Biology* 2004;336:971–986. [PubMed: 15095873]
109. Salahshor S, Woodgett JR. The links between axin and carcinogenesis. *Journal of Clinical Pathology* 2005;58:225–236. [PubMed: 15735151]

Fig. 1. Schematic of heterotrimeric PP2A holoenzyme, which is comprised of a structural A subunit, catalytic C subunit and a variable regulatory B subunit from one of four families of regulatory B subunits. The B56/PPP2R5 family of regulatory B subunits are encoded by five genes and the γ and δ genes encode several splice variants. A Subunit Binding Domain 1 and 2 (ASBD1 and 2) are regions of higher amino acid sequence homology that facilitate the association of the B subunits with the A subunit. Known targets of specific B56 family members are shown with indication of suppression or activation by a particular PP2A-B56 holoenzyme

Fig. 2.
(a) Schematic showing the sequential reversible phosphorylation pathway that regulates c-Myc protein stability and turnover via the 26S proteasome. Several of the proteins involved in this pathway are coordinated into a degradation complex for c-Myc by the scaffold protein Axin1. (b) Schematic of complexes that PP2A-B56α has been shown to be a part of

Fig. 3. Schematic of known B56α targets and target sequences if available. Also shown is the transcriptional cross-talk connection between known B56α targets and the biological function of these targets

Fig. 4.
Schematic representations of mechanism through which B56α function could be lost