Cell. Mol. Life Sci. 63 (2006) 2979–2991
1420-682X/06/242979-13
DOI 10.1007/s00018-006-6300-7

Cellular and Molecular Life Sciences

Review

Regulation of cell adhesion by PP2A and SV40 small tumor antigen: An important link to cell transformation

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Received 29 June 2006; received after revision 3 August 2006; accepted 20 September 2006
Online First 28 October 2006

Abstract. The serine/threonine protein phosphatase 2A (PP2A) represents a large family of highly conserved heterotrimeric enzymes. Their critical importance in cell homeostasis is underlined by the fact that they are targets of natural toxins like the tumor promoter okadaic acid, and of simian virus 40 small tumor antigen (SV40 small t), a viral protein known to promote cell transformation. Furthermore, mutated or lower expression levels of PP2A subunits have been found in certain cancers. One major known event in PP2A-dependent cell transformation is the alteration of key signaling pathways that control cell growth and survival. In this review, we focus on how PP2A enzymes also affect cell adhesion and cytoskeletal dynamics, the disruption of which is linked to loss of cell polarity, increased cell motility and invasiveness. We also examine how those various pathways participate in the transforming activity of SV40 small t.

Keywords. Phosphatase 2A, SV40 small t, transformation, junction, adhesion, cytoskeleton.

Introduction

The Ser/Thr protein phosphatase 2A (PP2A) is a large family of heterotrimeric enzymes [1, 2]. The core enzyme is a dimer formed by association of a catalytic C (PP2A-C) to a scaffolding/regulatory A (PP2A-A) subunit. PP2A substrate specificity is achieved in part through interaction of the core enzyme with different types of regulatory B subunits (PP2A-B). B subunits are catalogued into four families (B, B', B", B"'), each containing multiple isoforms and/or splice variants [1, 2]. A unique feature of the B-type subunits is their ability to compete *in vitro* for (AC) binding and differentially exchange and replace each other in the PP2A complex [3]. In all organisms examined, PP2A activity is essential for cell survival, cell cycle regulation, DNA damage response and embryonic development [1, 2]. The general importance of PP2A in cell homeostasis is illustrated by the vast array of naturally occurring PP2A inhibitors [1, 2, 4], including okadaic acid (OA), calyculin A, microcystin-LR and fostriecin, with OA being the most widely utilized. OA, calyculin A and microcytin-LR are potent tumor promoters [5–7]. However, in some cell types, OA inhibits transformation and promotes growth [2]. Moreover, fostriecin is an antibiotic with anti-tumor properties [8]. Additional evidence for a role of PP2A in tumorigenesis comes from the identification of specific alterations (mutations, deletions, loss of heterozygosity) and decreased expression of selective subunits (Aα, Aβ and B'/B56γ) in lung, colorectal, breast and brain cancer, and various cancer cell lines [9, 10].

Transformation of cells requires notable changes in their cytoskeletal organization and adhesive properties. Not surprisingly, decreased PP2A activity observed in human head and neck squamous carcinoma [11, 12], metastatic Lewis lung carcinoma [13] and melanoma BL6 [14] cells is associated with increased cell motility and invasiveness. OA-mediated PP2A inhibition also enhances cell

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motility in keratinocytes [15], non-metastatic Lewis lung carcinoma [13] and endothelial [16] cells. In these cells, altered PP2A activity is accompanied by decreased cell adhesion and cytoskeletal reorganization. Cell transformation also results from alterations in signal transduction cascades governing cell proliferation [17]. A remarkable example of the clever utilization of key regulatory proteins to subvert cell growth control is provided by studying the DNA tumor virus, simian virus 40 (SV40). This virus expresses two antigens, large T (LT) and small t (ST), which are solely responsible for cell transformation in non-permissive cells. SV40 ST specifically targets endogenous PP2A, resulting in changes in PP2A substrate specificity and targeting, and subsequent deregulation of PP2A-controlled mitogenic signaling pathways [2, 9, 10, 18]. Like OA, ST also affects the cytoskeleton [19, 20] and cell adhesion [20].

In this review, we first examine the role of PP2A in the regulation of cell adhesion and cytoskeletal dynamics. We then describe how ST targets PP2A to affect signal transduction pathways controlling cell adhesion, growth and survival, in order to promote cell transformation.

PP2A and cell adhesion

Multi-protein complexes mediate the adherence of cells to each other and the elaborate mesh that composes the extracellular matrix (ECM). Cell-cell and cell-matrix adhesion are important processes that control biological mechanisms as varied as cell migration, embryogenesis, angiogenesis, tissue repair, inflammation, invasion and metastasis [21–26]. As described below, there is growing evidence that PP2A participates in the assembly/disassembly mechanisms regulating cell-matrix and cell-cell contacts, either directly by controlling structural adhesion proteins, or indirectly by affecting proteins involved in the signaling pathways that initiate from and converge to these adhesion platforms.

PP2A and cell-matrix interactions

Cell interactions with the surrounding ECM provide both selective adhesive information and specific signaling instructions, the outcome of which influences stable cell adhesion, migration, proliferation, or apoptosis. Cell adhesion to the substratum leads to the formation and stabilization of focal adhesions, specialized sites of convergence for the actin cytoskeleton, integrins and an interconnection of protein complexes [23]. The role of PP2A in cell-ECM interactions is highlighted by a study showing that PP2A levels increase immediately after suspension of anchorage-dependent fibroblasts [27]. Recent data suggest that PP2A co-localizes with β1 integrin at focal adhesion sites, and promotes the formation of focal adhesions through dephosphorylation of the β1A integrin subunit at Thr-788/789 and Ser-785 residues [28, 29]. PP2A actively participates in the maintenance of β1 integrin-F-actin anchoring at focal adhesion sites [30] (Fig. 1). In quiescent human mammary endothelial (HME) cells, PP2A is located in a protein complex that includes β1 integrin, the Rho-family GTPase, Rac, and the actin cross-linking protein, IQGAP1 [31, 32]. OA-mediated inhibition of PP2A activity induces the loss of IQGAP1, disassembly of F-actin and decrease of β1 integrin-actin interactions in HME cells. Epidermal growth factor (EGF)-stimulation of HME cells activates calmodulin kinase II (CamKII), resulting in subsequent dissociation of the PP2A-IQGAP1 complex from the β1 integrin-Rac proteins [33]. PP2A-Aα subunit may directly associate with IQGAP1. Interestingly, human breast cancer MCF-7 cells express very little PP2A-Aα relative to normal HME cells [34], and IQGAP1 does not associate with the β1 integrin-Rac protein complex in these cells [30]. PP2A also drives cell adhesion through β2 integrin signaling [35].

Focal adhesions are sites of signal transduction that control diverse cell functions, including membrane extension, cytoskeletal tension, motility, matrix assembly, and tissue invasion. At the center of this multifunctional signaling core is the focal adhesion kinase (FAK). FAK is phosphorylated on Tyr-397 in response to integrin-dependent cell adhesion. Phosphorylated FAK interacts with Src-family kinases (Src), which, in turn, phosphorylate two FAK-interacting proteins, Crk-associated substrate (p130CAS) and paxillin. Both proteins regulate Rho-family GTPase activity and cell motility [36, 37]. PP2A is an important regulator of FAK/Src/paxillin complexes (Fig. 1). PP2A inhibition induces the disorganization of focal adhesion sites and increases cell migratory activity in endothelial cells [16], non-metastatic Lewis Lung carcinoma (LLC-C8) tumor variants [38–40] and keratinocytes [15]. In these cells, there is increased Ser phosphorylation of paxillin and reduced Tyr phosphorylation of paxillin, FAK and Src. PP2A holoenzymes containing the B'/B56γ1 isoform co-localize with, interact with, and dephosphorylate paxillin [14], possibly at Ser-457 and Ser-481 residues [39], the phosphorylation of which is critical for cell migration [39]. In contrast, the regulation of Tyr phosphorylation by PP2A is indirect, and may involve Tyr phosphatases like Shp-2 [40]. PP2A inhibition leads to dissolution of FAK/Src/paxillin complexes, activation of Src and increased Src-dependent cell motility [38, 39]. Interestingly, BL6 mouse melanoma cells, which express an N-terminally truncated form of B'/B56γ1, are metastatic cells [14]. Besides paxillin, PP2A also directly interacts with and dephosphorylates p130CAS, which regulates cell entry into mitosis [41].

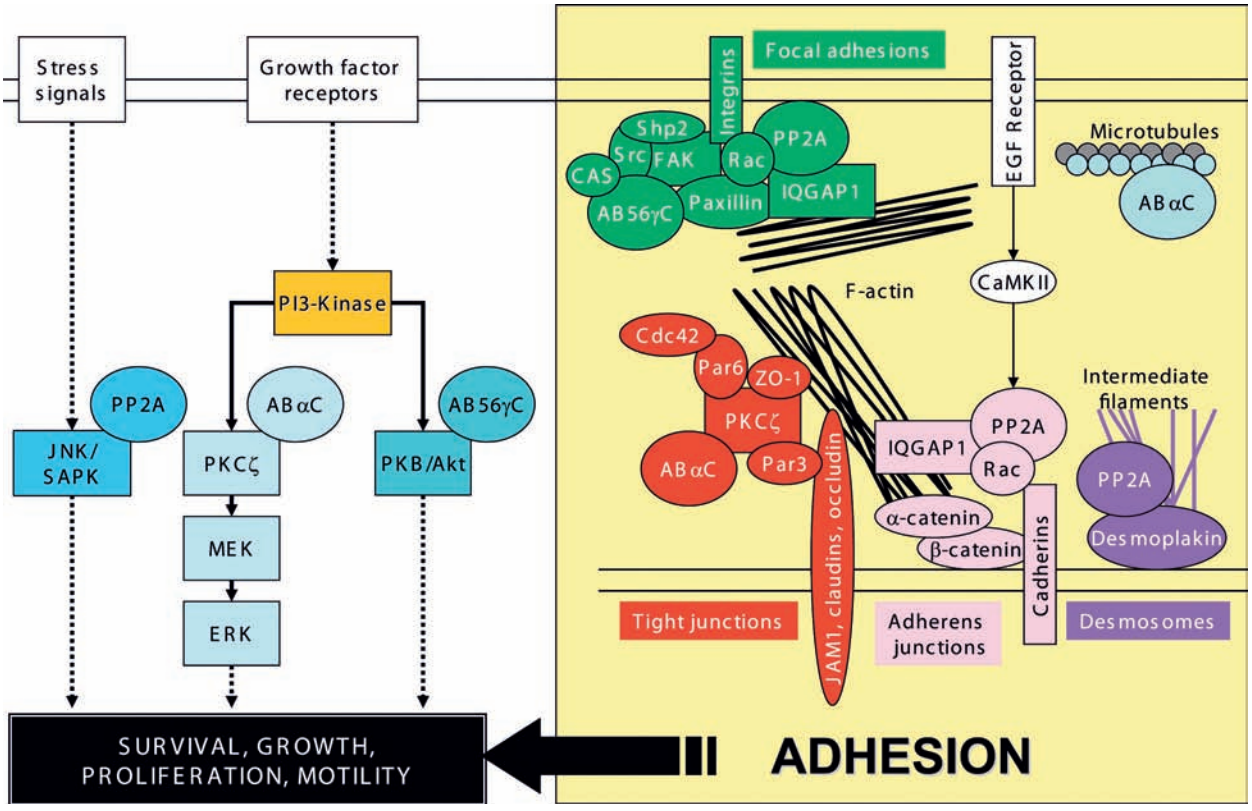

Figure 1. Schematic representation of major PP2A-dependent pathways that are targeted by SV40 ST to promote cell transformation. PP2A enzymes are essential regulators of intertwined signaling cascades and multi-protein adhesion complexes that converge to regulate cell polarity, growth, survival and proliferation. These PP2A-dependent pathways become altered in cancer, resulting in increased cell motility and invasiveness. Whenever possible, the specific PP2A heterotrimer involved in the illustrated mechanisms is indicated. 'PP2A' refers to unidentified phosphatase isoforms.

PP2A and cell-cell adhesion

Cell-cell interactions are mediated by tight junctions (TJs), adherens junctions (AJs), and desmosomes. Gap junctions (GJs) are a fourth-type of junction responsible for intercellular communication. The transmembrane proteins that form these junctions are linked to components of the cytoskeleton and a growing number of cytoplasmic scaffolding molecules. These molecules, in turn, regulate cellular processes as diverse as transcription, proliferation, trafficking, polarization and permeability.

PP2A and tight junctions

TJs are the most apical components of the epithelial junctional complex and are crucial for the formation and functioning of epithelial and endothelial barriers. Within confines of the TJ, the cell membranes of adjacent cells are brought into intimate focal contact sites in which the intercellular space is obliterated [22, 24]. TJs regulate selective diffusion of ions and solutes along the paracellular pathway and restrict apical/basolateral intramembrane diffusion of lipids. They serve as barriers that prevent the mixing of molecules in the apical domain with those in the lateral domain. TJ integrity is compromised almost universally in epithelial and endothelial cancers [42]. Loss of polarity is a critical step in promoting tumorigenesis. It is often a consequence of changes of expression levels and patterns of TJ proteins, encompassing: (1) decreased expression of occludin in endometrical cancer, prostate cancer and adenocarcinoma, claudins-1 and -7 in colon cancer, glioblastoma and breast tumors, and ZO-1 in colon cancer; and (2) increased protein expression of claudin-1 in colorectal cancer, claudins-3 and -4 in ovarian, breast, pancreatic and prostate cancers, and claudin-16 in certain ovarian tumors. The first evidence of a potential role of PP2A in TJ regulation comes from the cellular effects of naturally occurring, PP2A-inhibitory toxins that are responsible for diarrheic shellfish poisoning in humans [43, 44]. High concentrations or prolonged incubation of epithelial cells with OA or calyculin A induce cell rounding and loss of barrier properties [43, 44]. The precise mechanisms underlying OA- and calyculin A-induced TJ leakiness are not fully deciphered, but probably involve disruption of F-actin, and/or hyperphosphorylation and

subsequent activation of protein kinases that stimulate TJ disassembly. In this context, it is especially interesting that activation of protein kinase C by exposure of epithelial cells to another tumor promoter, phorbol ester, results in a sustained increase in paracellular permeability [45]. This enables growth factors to leak from luminal fluid compartments into lateral intercellular and interstitial fluid spaces of epithelia. It is believed that such alterations in TJ permeability play an important role in carcinogenesis, and may be mediated by the deregulation of downstream kinases and/or phosphatases [45]. One such candidate may be PP2A, since it directly interacts with the multi-protein TJ complex [46] (Fig. 1). During junctional biogenesis, the Bα-containing PP2A isoform (ABαC) is recruited from the cytosol to the apical plasma membrane where it co-localizes and interacts with TJ proteins. Notably, ABαC associates with and negatively regulates the activity of atypical protein kinase C ζ (PKCζ), and the PKCζ/Par3/Par6 protein complex is critically required for TJ formation and establishment of cell polarity [22, 24]. Overexpression of PP2A-Cα subunit in MDCK cells slows down TJ assembly, while inhibition of PP2A activity promotes the phosphorylation and recruitment of PKCζ, ZO-1, occludin and claudin-1 to the TJ. The effects of OA are blocked following incubation of cells with PKCζ inhibitory peptides. Thus, ABαC negatively regulates TJ assembly, probably at the level of the PKCζ/Par3/Par6 signaling complex. In further support for an important role of ABαC in TJ regulation, expression of SV40 ST is associated with altered distribution and reduced expression levels of ZO-1, occludin and claudin-1 [20]. These effects also correlate with deregulation of cell growth. Sure enough, TJ proteins like ZO-1, which is homologous to *Drosophila* discs-large tumor suppressor proteins, critically participate in the regulation of epithelial cell proliferation and differentiation [47]. Altogether, these observations suggest that altered ABαC expression or activity can lead to TJ disruption and initiate the first steps of neoplasia.

PP2A and adherens junctions

AJs are located at the lateral border of epithelial cells. In AJs, E-cadherins connect to actin filaments by way of their undercoat proteins called catenins [21]. Cell-cell adhesion is often reduced in cancer. Tumorigenesis can be the result of the inactivation of E-cadherin cell adhesion system by various mechanisms, including: (1) silencing of the E-cadherin gene; (2) mutations of the genes encoding E-cadherin and α-/β-catenins; (3) inactivation of E-cadherin-dependent cell adhesion system by Tyr phosphorylation of β-catenin; and (4) decrease of E-cadherin function following internalization or endocytosis [21]. Several observations indicate that PP2A is critical for membrane targeting of E-cadherin and catenins, and maintenance of E-cadherin-mediated cell-cell contact. Treatment of keratinocytes with OA or calyculin A induces β-catenin hyperphosphorylation, decreased E-cadherin phosphorylation, and AJ disruption [48, 49]. In mouse embryos, PP2A-Cα knockout induces the redistribution of E-cadherin and β-catenin to the cytosol [50]. In non-malignant HME cells, PP2A interacts with a protein complex involving E-cadherin, β-catenin, Rac and IQGAP1 [51] (Fig. 1). PP2A targets IQGAP1 to the cadherin-catenin-Rac complex and allows linkage of the AJ complex to F-actin [51]. Incubation of HME cells with OA or silencing of PP2A-Aα provokes AJ dissociation and E-cadherin internalization [51]. Thus, PP2A plays an important role in maintaining AJ structural and functional integrity, but the nature of the PP2A isoform responsible for this function is unknown. In contrast, PP2A may not be required for *de novo* AJ formation, based on the following observations. First, OA has no apparent effect on E-cadherin expression levels or membrane redistribution, or AJ formation during assembly of epithelial intercellular junctions [46, 52]. Second, in polarized MDCK cells and colon, ABαC holoenzymes are localized at apical TJs, but not AJs [46]. However, it has been reported that PP2A-Cα associates with and stabilizes the β-catenin/E-cadherin complex in immature blastocysts from mouse embryos at an early developmental stage [50]. Interestingly, B'/B56-, but not Bα-containing PP2A isoforms are involved in the regulation and degradation of β-catenin during Wnt signaling, a cascade that plays an important role during embryonic patterning and carcinogenesis [53]. Thus, B'/B56 subunits may target PP2A to AJs. Alternatively, specific PP2A isoforms could become segregated from AJs at later stages of development upon completion of epithelial tissue polarization. This assumption is based on the observation that the TJ protein, ZO-1, is initially found with catenins in E-cadherin-enriched lateral membranes during early stages of junction formation, but later redistributes exclusively to apical mature TJs after cell polarization has occurred [54].

PP2A and desmosomes

Desmosomes are highly organized intercellular junctions that provide mechanical integrity to tissues, such as skin and heart, by anchoring intermediate filaments to sites of strong adhesion [25]. Desmosomes may have a tumor suppressor function because expression of desmosomal components is reduced in some human cancers, and desmosomal cadherins have the capacity to suppress the invasiveness of cells in culture [25]. PP2A activity appears to be required for final stages of desmosome plaque assembly and its maintenance [52, 55]. Treatment of hepatocytes with microcystin-LR induces disruption of des-

moplakin organization and dissociation of desmosomes [55]. OA inhibits desmosome assembly in MDCK cells [52]. However, much remains to be learned on the precise mechanisms involved in the regulation of desmosomes by PP2A.

PP2A and gap junctions

GJs are membrane structures made of intercellular channels, which allow the diffusion of small hydrophilic molecules from cytoplasm to cytoplasm. GJs maintain tissue homeostasis and are implicated in cell growth, development and differentiation. Defective GJs contribute to cancer development [56]. Loss of functional GJs occurs in cancer cells, and expression of GJ proteins in these cells can slow down growth or even suppress tumorigenicity. The GJ components, connexins, undergo phosphorylation/dephosphorylation cycles that regulate GJ functions [26]. The utilization of PP2A inhibitors on different cell types suggest that connexin-43 phosphorylation, which decreases GJ intercellular communication, is regulated either directly or indirectly by PP2A [57].

PP2A and the cytoskeleton

Cytoskeletal dynamics rely on the interplay of three filament systems, microtubules (MTs), microfilaments, and intermediate filaments (IFs), which are integrated into a complex network regulated by associated and cytolinker proteins. They play important functions in maintenance of cell architecture, adhesion, migration, differentiation, division, and organelle transport. PP2A enzymes play a key role in regulating these cytoskeletal structures via their direct interaction with cytoskeletal proteins and proteins that partake in the assembly/disassembly mechanisms, and the modulation of signal transduction pathways that control these events. There are clear correlations between certain cancer phenotypes and changes in the properties of cytoskeletal proteins. It is likely that some of these phenotypes result in part from PP2A deregulation.

PP2A and actin

The first evidence of a regulatory role of PP2A in actin assembly comes from PP2A inhibitor studies. Incubation of platelets [58], neutrophils [59], hepatocytes [60], and neuroblastoma [61], endothelial [62, 63] and epithelial [43, 44, 64] cells with OA, calyculin A or microcystin-LR leads to F-actin disorganization, cell rounding and loss of cell polarity. In *Acetabularia* [65], renal epithelial cells [66] and sea urchin eggs [67], treatment with OA or calyculin A induces F-actin phosphorylation, leading to depolymerization and changes in key cellular functions, such as organelle transport, adhesion and cytokinesis. The well-documented effect of OA on the actin network even serves as a diagnostic tool to determine whether patients have been poisoned by shellfish toxins [68]. The spatiotemporal modulation of actin remodeling and branching is required for maintenance of actin-containing structures at the leading cell edge. These processes are tightly controlled by actin-binding proteins involved in actin polymerization/depolymerization, gelation and cross-linking [69]. Among those, actin-depolymerizing factor/cofilin proteins assist filament disassembly and possess actin-severing properties. Significantly, PP2A associates with and dephosphorylates cofilin, possibly at Ser-3 [70], inducing its activation and binding to F-actin [71]. Remodeling of the actin cytoskeleton is also critical for assembly/disassembly of cell adhesion structures. As stated before, PP2A regulates the interaction of microfilaments with AJs and focal adhesion proteins, through interaction with the actin-cross-linking protein IQGAP1, and the FAK/paxillin/Src protein complex. Studies with SV40 ST have shown that ABαC also plays a role in F-actin organization at TJs [20]. Expression of wild-type ST, but not a ST mutant unable to interact with PP2A, induces marked disorganization of F-actin in MDCK cells. The remodeling of actin caused by ST is likely mediated by PP2A-dependent activation of Rac and cdc42, and loss of Rho [20]. Like adhesion, cell migration is dependent on the extensive remodeling of the actin cytoskeleton. Several observations indicate that a decrease or suppression of PP2A activity promotes cell motility and invasiveness in various cell types [14–16, 39]. For instance, the highly motile and invasive Lewis lung carcinoma 7 (LLC-7) cells are deficient in PP2A compared with non-metastatic LLC-8 cells. Conversely, OA-treated LLC-8 cells become migratory and invasive, as a result of redistribution of F-actin towards the periphery of the cells, and eventual loss of the filamentous actin network [13, 72]. In mouse melanoma cells, expression of a truncated isoform of B'/B56γ regulatory subunit alters PP2A substrate specificity, resulting in enhancement of their metastatic potential [73, 74].

PP2A also controls the F-actin network at the beginning of mitosis and during cytokinesis. The interaction of the PP2A-IQGAP1 protein complex with F-actin and integrins is lost in HME cells during the G1 to the G2/M cell cycle transition [75]. Treatment of NIH3T3 cells with OA increases phosphorylation of p130CAS [41], which is necessary for cell entry into mitosis and is maintained until mitosis is complete [76]. These observations suggest that PP2A inhibition is necessary for decreased cell adhesion at focal adhesion sites, and this step is a prerequisite for cell entry into mitosis. The role of PP2A in cytokinesis has been primarily studied in yeast. PP2A deletion mutants (heterotrimers contain homologues of

A, C or Bα subunits) induce delayed and anomalous septation and cytokinesis in *S. cerevisiae* and *S. pombe* [77–82]. In *S. pombe*, disruption of the *par1* and *par2* genes (B'/B56 homologues) show abnormal septum positioning and growth and cytokinesis defects [82, 83]. In *S. cerevisiae*, mutations in Cdc55 (Bα homologue) result in highly elongated and multiple budded cells indicative of delayed cytokinesis [79]. Mutations in PPH21/PPH22 (C homologues) also induce defects in *S. cerevisiae* bud morphogenesis, and actin [81]. In *S. cerevisiae*, activation of the mitotic exit network triggers both septin ring splitting and actomyosin ring contraction [84]. The PP2A B'/Rts1p subunit is involved in the regulation of septin dynamics, through Shs1 dephosphorylation [85].

PP2A and microtubules

In eukaryotic cells, MTs form a well-organized network in which the minus ends are generally anchored at the centrosome or MT organizing center, whereas the free plus ends probe the cytoplasm, in a search and capture process, to reach specific targets. MT dynamics are tightly regulated both spatially and temporally. This regulation involves post-translational modifications [86], true MT-associated proteins (MAPs), MT-binding proteins including kinases and phosphatases [87], as well as coordinated interactions with other cytoskeletal components, e.g. actin [88]. PP2A has emerged as an important regulator of MT dynamics, being capable of dephosphorylating several major MAPs, such as tau [89] and MAP2 [90], that critically control neuronal MT assembly and stabilization. PP2A also affects the activity of enzymes like tubulin carboxypeptidase [91], which regulates tubulin tyrosination/detyrosination cycles, a process essential for the control of MT dynamics. Indeed, deregulation of PP2A has been linked to the destabilization of stable MT populations in numerous cell types [58, 63, 89, 92–95]. Notably, pools of ABαC heterotrimers directly bind to MTs *in vitro* and in mammalian cells [90, 92, 96–99]. Although PP2A-C can directly bind assembled, but not monomeric tubulin, heterotrimeric enzymes containing the regulatory B subunit are required for efficient binding and targeting of PP2A to the MT cytoskeleton [97, 99]. Interestingly, MT binding is associated with pronounced inhibition of PP2A catalytic activity [97, 99]. Cytoskeletal anchoring may serve to sequester selective intracellular pools of enzymes in an inactive state, and promote kinase-mediated phosphorylation of MAPs, such as tau [97, 99]. Conversely, cholesterol depletion leads to a loss of MT-bound PP2A, and concomitant increase in cytosolic PP2A activity and dephosphorylation of MAP2 [90].

The regulation of MTs by PP2A likely plays an important role during morphogenesis and tumorigenesis. PP2A regulates MT stability during podocyte process formation in the newborn kidney [100]. Changes in MT patterns mediated by deregulation of PP2A contribute to increased cell motility and invasiveness in various cell types [13, 98, 101]. OA treatment of human head and neck squamous cell carcinoma cells results in a loss of MT-bound PP2A pools and increased cell invasiveness [11]. Fostriecin induces abnormal centrosome replication and the formation of aberrant mitotic spindles in Chinese hamster ovary cells [102]. Notably, ABαC and possibly other PP2A heterotrimers are the only enzymes capable of efficiently dephosphorylating phosphorylated, assembled β-III tubulin [97, 103], a specific tubulin isoform that plays a critical role in development, differentiation, and tumor formation and progression [104].

Interestingly, the amounts and activity of MT-associated ABαC fluctuate during the cell cycle, which may be critical for proper mitosis [96]. Genetic studies have revealed how PP2A enzymes can regulate MT structure and dynamics during cell division. In *S. cerevisiae*, PP2A-C mutants are hypersensitive to nocodazole-induced MT destabilization [105]. They induce a blockage of cells into G2, because mitotic spindles are unable to form or extend [78]. Bα homologue mutants lack a functional kinetochore/spindle assembly checkpoint [106]. Disruption of the *paa1* gene (A homologue) in *S. pombe* causes anomalies in MT distribution [77]. During *Drosophila* embryogenesis, deficiency of PP2A-C uncouples the nuclear and centrosome cycles, and inhibits the attachment of MTs to the kinetochore, leading to acute disorganization of MT arrays, centrosome multiplication and mitotic defects [107]. In *Xenopus* eggs, PP2A is required to maintain the short steady length of MTs during mitosis, in part by regulating Op18/stathmin, a molecule involved in MT dynamics [108]. Lastly, expression of SV40 ST in fibroblasts abolishes normal centrosome functions in a PP2A-dependent manner, resulting in a mitotic block [109]. PP2A holoenzymes containing the Bα [96] or B'/PR130 [110] subunit have been identified at the centrosome in mammalian cells, and are likely players in the regulation of MTs during mitosis.

PP2A and intermediate filaments

IFs are the major component of the cytoskeleton and nuclear envelope. Originally, IFs were classified according to their tissue-specific expression: keratins in epithelia, desmin in muscle, vimentin in cells of mesenchymal origin, glial fibrillary acidic protein (GFAP) in glial cells, and the neurofilament-triplet proteins (NF-L, NF-M, and NF-H) in neuronal cells. These proteins maintain cell shape and structural integrity of the cell content, and provide protection against various types of stresses

(mechanical and others) [111]. IF proteins are among the most prominent cellular phosphoproteins, and reversible phosphorylation plays a key role in regulating dynamic aspects of IF organization, structure and signaling [111]. PP2A enzymes control the phosphorylation levels of many IF proteins, thereby greatly influencing IF regulation. Incubation of BHK-21 cells [112, 113], epidermal cells [48], colonic and kidney epithelial cells [114], oligodendrocyte precursors [115], human fibroblasts [116], 9L rat brain tumor cells [117, 118], hepatocytes [55] and hamster kidney cells [119], with OA, calyculin A, microcystin-LR or fostriecin promotes the hyperphosphorylation of major IF proteins, including nestin, vimentin, cytokeratins-8 and -18, and neurofilaments. It leads to the disassembly of IF networks, solubilization of IF proteins and disruption of desmosomes. Vimentin dephosphorylation occurs through direct binding to PP2A [113, 118, 120], probably via the Bα subunit [120], and becomes impaired in mutant PP2A-C transgenic mice [121]. PP2A also directly interacts with neurofilaments [122]. In the human colonic cells HT29, PP2A dephosphorylates cytokeratin-8 at Ser-431 when cells are under hyposmotic stress [114].

PP2A deregulation is utilized by SV40 ST to promote cell transformation

The interest in the transforming ability of SV40 has been intensified by the detection of SV40 DNA sequences in various human tumors over the last decade [123]. While both ST and LT can induce cell transformation, ST is only necessary for cell transformation when LT is expressed at low levels in cells, or when cells are quiescent [124–126]. It is worthwhile noting that while the transforming effects of ST are well recognized, some studies show that this protein does not always promote proliferation, but rather induces apoptosis in some cell types [127]. The only confirmed cellular target of ST identified so far is PP2A. The formation of a (AC-ST) complex between small t and the (AC) core enzyme of PP2A alters PP2A substrate specificity and activity, and is required for the transforming activity of ST [125, 126, 128, 129]. ST interacts directly with PP2A-A [130, 131]. *In vitro* experiments with purified proteins have shown that ST and regulatory B-type subunits have differential binding affinity for PP2A-A subunit, and therefore compete for binding to PP2A-A [3]. It was initially reported that ST could displace B-, but not the B' family of subunits from corresponding PP2A heterotrimers [3]. However, recent data suggest that ST exerts its transforming activity by exchanging with the B'/B56γ subunit [132]. Similarly to ST expression, knockdown of B'/B56γ, but not of Bα subunit in human epithelial cells co-expressing the telomerase catalytic subunit (hTERT), an oncogenic allele of H-Ras and SV40 LT, induces cell immortalization and tumorigenicity [132]. Conversely, overexpression of B'/B56γ3 in tumorigenic epithelial cells expressing ST partially reverses the transforming effect of ST. Thus, PP2A tumor suppressor activity may be mediated by the B'/B56γ subunits. In support of these observations, several lung tumor cell lines and malignant melanoma have been identified that express truncated or lower levels of B'/B56γ [14, 73, 74]. It is important to emphasize that, although knockdown of B'/B56γ could recapitulate the effects of ST in a transformed human cell model, the potential role of other PP2A isoforms in ST-dependent cell transformation should not be quickly dismissed. First, the artificial cell line utilized in these studies represents a valuable model for looking at certain mechanisms involved in human cancer formation, but is highly transformed and certainly not a natural host cell for SV40. Second, ST can be detected in both the cytoplasm and nucleus of cells [20, 129]. Since B'/B56γ subunits are concentrated in the nucleus [133], ST likely targets other PP2A complexes localized in the cytosol. Third, B'/B56γ knockdown required normal serum levels to mimic some of the serum-independent cellular effects of ST [134], suggesting that ST activates other PP2A isoform-dependent signaling cascades (see below). The relative expression and intracellular distribution of distinct PP2A isoforms vary among cell types and tissue [1], and may influence which pathways become preferentially activated following ST expression. Lastly, many questions remain as to how exactly ST forms a complex with PP2A *in vivo*. The AC-ST interaction may be more dynamically regulated than is initially apparent. It is also conceivable that ST directly associates with newly synthesized AC dimers, therefore bypassing the need to compete with B-type subunits for AC binding. Since intracellular PP2A-C expression levels are autoregulated [133, 135] and individual PP2A monomeric subunits are usually quickly degraded [136], binding of ST to AC dimers may even prevent the *de novo* biogenesis of certain PP2A heterotrimers. Consequently, there is probably extensive reshuffling of the relative subunit composition of PP2A heterotrimers in cells after ST is expressed. Indeed, stable expression of ST in monkey kidney CV-1 and epithelial MDCK cells induces the down-regulation of PP2A Bα subunit, which likely contributes to alterations in PP2A-controlled signaling pathways [20, 129]. It remains to be seen whether similar changes in PP2A subunit composition occur in cells in which silencing of B'/B56γ has been performed.

Regardless of the mechanisms involved, it is clear that many pathways influencing cell survival, proliferation, apoptosis, adhesion and growth, are globally affected by ST, and all converge to facilitate cell transformation. There is increasing evidence that selective PP2A isoforms participate in the regulation of these specific

cascades, so that the widespread effects of ST should logically result from targeting of signaling cascades controlled by more than one PP2A isoform. In support of this hypothesis, several signaling pathways involved in cell proliferation, growth and survival, including the JNK/SAPK, MEK-ERK, phosphatidylinositol 3-kinase (PI3-kinase)/Akt and PI3-kinase/PKCζ pathways, are perturbed by the interaction of ST with PP2A [1, 2, 9, 17, 137] (Fig. 1). Interestingly, Akt activity is regulated by B'/B56-containing PP2A holoenzymes [138], confirming the close relationship between ST-mediated cell transformation and B'/B56 inactivation [132]. However, it is known that activation of Akt alone is insufficient to achieve cell transformation. ABαC heterotrimers specifically regulate PKCζ [46, 139] and the MEK-ERK pathway [129, 138, 140], implicating these isoforms as another target of ST. It is especially significant that PI3-kinase is required for the cellular effects and transforming activity of ST [9, 139, 141], since it is required for activation of both Akt and PKCζ-dependent pathways (Fig. 1). The stimulation of PI3-kinase-dependent pathways activates different transcription factors whose function is also targeted by ST to promote cell growth and survival. Those include CREB, Sp1, AP-1, NF-κB and c-Myc, most of which are directly dephosphorylated and inactivated by PP2A [9, 139, 141–143]. ST also controls the induction of cell-cycle regulators, such as cyclin D1 [144] and cyclin A [145]. It affects the expression of genes involved in apoptosis, development, and the immune response [134, 146]. The expression of several components of the Notch, Hedgehog and Wnt developmental signaling pathways becomes altered in immortalized ST-expressing human cells [146].

Since most human cancers arise from epithelial cells, it is not surprising that ST also targets PP2A-dependent processes governing epithelial cell adhesion. In MDCK cells, ABαC regulates TJ assembly in a PKCζ-dependent manner, and ST induces deregulation of this pathway to promote TJ leakiness [20]. TJ disruption is associated with down-regulation of TJ proteins, a feature of many cancers. PP2A functional disruption by ST also induces F-actin rearrangements, encompassing increased Rac1-induced membrane ruffling and lamellipodia, Cdc42-initiated filopodia and loss of RhoA-dependent stress fibers. These F-actin changes coincide with elevated levels of Rac1 and Cdc42 and decreased amounts of RhoA in ST-expressing cells [20]. Rac1, Cdc42 and RhoA are essential in the control of actin remodeling during junctional assembly and cell migration. Since alterations in the actin cytoskeleton have been linked to loss of cell polarity and tumor invasiveness, ST-induced PP2A-dependent disorganization of F-actin in selective cell types [19, 20] likely contributes to the role of SV40 in cell transformation. Moreover, expression of ST can promote MT destabilization [89]. Analysis of changes in gene expression patterns show that ST may also affect intercellular adhesion by decreasing the levels of β-catenin and protocadherin γ family members at AJs, plakoglobin at desmosomes, and JAM-1 and claudin-11 at TJs [134]. Lastly, ST affects genes involved in integrin signaling [134], further illustrating the incredible variety of ways that ST can utilize to alter cell adhesion and promote tumorigenesis.

Conclusion

Current knowledge indicates that deregulation of PP2A activity and subunit composition can induce cell transformation by affecting cell adhesion properties, cytoskeleton dynamics, and signaling pathways involved in cell proliferation, growth and survival. Based on the current number of identified PP2A subunits, at least 72 distinct PP2A heterotrimers may be generated in mammalian cells. There is increasing evidence from yeast and mammalian studies that PP2A holoenzyme subfamilies have distinct cell and tissue expression and subcellular distribution, and display different affinities for various protein targets, thereby ensuring PP2A functional specificity. Like their Ser/Thr kinase counterparts, 'PP2A' should now be regarded as a family of highly regulated enzymes dedicated to accomplish specialized cellular tasks. So far, most studies assessing the role of PP2A in tumorigenesis have relied on strategies (utilization of inhibitors or silencing of PP2A-C or PP2A-A) that cannot discriminate the contribution of a specific PP2A isoform. Although expression of SV40 ST has proven to be a valuable tool to investigate PP2A-dependent mechanisms responsible for cell transformation, many questions remain as to how exactly ST affects PP2A isoform-specific signaling. The development of B-type subunit-specific antibodies and silencing tools should give novel insights into the role of selective PP2A enzymes in tumor suppression.

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