Cell Cycle 11:3, 451-459; February 1, 2012; © 2012 Landes Bioscience

Mouse model for probing tumor suppressor activity of protein phosphatase 2A in diverse signaling pathways

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Evidence that protein phosphatase 2A (PP2A) is a tumor suppressor in humans came from the discovery of mutations in the genes encoding the Aα and Aβ subunits of the PP2A trimeric holoenzymes, Aα-B-C and Aβ-B-C. One point mutation, Aα-E64D, was found in a human lung carcinoma. It renders Aα specifically defective in binding regulatory B' subunits. Recently, we reported a knock-in mouse expressing Aα-E64D and an Aα knockout mouse. The mutant mice showed a 50–60% increase in the incidence of lung cancer induced by benzopyrene. Importantly, PP2A's tumor suppressor activity depended on p53. These data provide the first direct evidence that PP2A is a tumor suppressor in mice. In addition, they suggest that PP2A is a tumor suppressor in humans. Here, we report that PP2A functions as a tumor suppressor in mice that develop lung cancer triggered by oncogenic K-ras. We discuss whether PP2A may function as a tumor suppressor in diverse tissues, with emphasis on endometrial and ovarian carcinomas, in which Aα mutations were detected at a high frequency. We propose suitable mouse models for examining whether PP2A functions as tumor suppressor in major growth-stimulatory signaling pathways, and we discuss the prospect of using the PP2A activator FTY720 as a drug against malignancies that are driven by these pathways.

Key words: lung cancer, oncogenic K-ras, p53, Aα mutations in endometrial cancer

Submitted: 12/09/11

Accepted: 12/14/11

http://dx.doi.org/10.4161/cc.11.3.19057

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Understanding how protein phosphatase 2A (PP2A) functions as a tumor suppressor requires knowledge of its complex structure and the roles its numerous regulatory subunits play. The trimeric holoenzyme is composed of a catalytic C subunit, a scaffolding A subunit and one of many regulatory B subunits. The catalytic C subunit exists as two isoforms, Cα and Cβ, that are 96% identical. The scaffolding A subunit also exists as two isoforms, Aα and Aβ, and they are 87% identical. The B subunits fall into four families designated B, B', B" and B'". The B or PR55 family has four members; the B' family (also designated B56 or PR61) consists of five isoforms and additional splice variants, and the B'' or PR72 family has four members including splice variants. B, B' and B" are largely unrelated by sequence. The combination of all subunits could give rise to over 70 distinct holoenzymes. In addition, the ability of PP2A to associate with approximately 150 other proteins further increases its regulatory potential.[1-5] **Figure 1B** shows a schematic diagram of the holoenzyme whose subunit interactions and structure have been revealed initially by biochemical studies[17,18] and subsequently in great detail by crystal structure analyses.[19-23] Through this work and numerous other investigations, it has become increasingly clear over the past 25 years that PP2A is not just a nonspecific phosphatase, as it was thought to be initially, but a highly sophisticated enzyme involved in most, if not all, fundamental cellular processes. One of the most challenging properties of PP2A is its role as a tumor suppressor, which has been covered by excellent reviews in references 24–28. The present report highlights recently developed mouse models for investigating PP2A's tumor suppressor activity.

Aα Subunit Mutations in Human Cancer

The discovery that Aα and Aβ are mutated in a variety of human malignancies, including carcinomas of the lung, breast, colon, skin, ovary and endometrium,[6-10,29,30] provided the first indication that PP2A plays a role as tumor suppressor in humans. A key finding was that E64D and E64G, two Aα substitution mutants that were discovered in a lung and a breast carcinoma, respectively,[10] are specifically defective in binding B'γ subunits, whereas binding of Bα and B" is normal (**Fig. 1F**).[13,31-33] These results raised the question of whether the sole loss of B'γ binding to Aα causes loss of tumor suppressor activity and whether B'γ itself or the B'γ-containing holoenzyme is a tumor suppressor.[32,33] Initially, it appeared that PP2A mutations occur infrequently in human cancer, in particular when compared with the high frequency of mutations in genes encoding the tumor suppressors p53 and PTEN, or in proto-oncogenes encoding K-ras and PI3K.[9] This raised some doubts about the clinical relevance of PP2A as a tumor suppressor. However, due to recent sequencing of a large number of human cancer genomes, it became apparent that Aα mutations occur in 18% of endometrial and in 6% of ovarian cancers.[6-9] Importantly, the incidence of Aα mutations in endometrial carcinomas is comparable to that of K-ras (15%), p53 (20%) and PI3K (24%) and over three times as high as the incidence of Arf mutations (5%) (**Fig. 1E**). Importantly, if one looks specifically at cases with high-grade serous endometrial cancer, 41% reveal Aα mutations.[8] These findings strongly suggest that Aα mutations play an essential role in the development of a significant fraction of endometrial carcinomas. It should be noted that the number of new cases of endometrial cancer in the US is 46,470 per year, i.e., 6% of all new cases, and that 8,120 women die each year from endometrial cancer.[34]

A critical question is whether the newly discovered Aα mutations render Aα defective in B' binding, as did the E64D and E64G mutations. We suggest that this is likely the case for all endometrial and ovarian cancer-associated point mutations listed in **Figure 1A** for two reasons: (1) According to the X-ray structure of Aα,[22] P179, R182 and R183 are located at or near the intra-repeat loop of repeat 5, and R249, S256, W257, R258, R260 are located at or near the intra-repeat loop of repeat 7 (**Fig. 1A and B**). As we reported previously, the intra-repeat loop regions of Aα are important for B and C subunit binding.[17,18] (2) In vitro binding assays with artificial Aα mutations demonstrated that P179A is specifically defective in B'γ binding, and that R183A, R183E, as well as W257A are defective in binding all forms of B subunits (**Fig. 1F**).[14] Therefore, it seems highly likely that all endometrial and ovarian cancer-associated mutations located at or close to P179, R183 and W257 interfere with B' binding only or with binding of all forms of B subunits and, consequently, render PP2A devoid of tumor suppressor activity. It is important to note that the true incidence of Aα mutations in endometrial and ovarian cancers could be considerably higher than reported, since only exons 5 and 6 (encoding repeats 5, 6 and 7) were sequenced in search of mutations.[8] Thus, E64D and E64G, which are located in repeat 2 (**Fig. 1B**), and mutations of other binding sites would have been missed if they occurred. Furthermore, down-regulation of Aα expression at the transcriptional or translational level could be equally or more important in tumor development than A subunit mutations. For example, a 10-fold or more reduced expression of Aα was observed in 43% of primary human gliomas in the absence of Aα mutations.[35]

Which B' Holoenzymes are Tumor Suppressors?

This question has been partially answered in tissue culture. One study demonstrated that B'γ holoenzyme is a tumor suppressor,[36] while others identified tumor suppressor activity of the B'α holoenzyme.[27] If either one of these forms played a significant role on its own, one might expect to find it mutated at a substantial frequency in tumors, which appears not to be the case. An explanation could be that B' subunits functionally overlap. Based on sequencing a large number of tumor samples, cancer-associated mutations of PP2A were found in significant numbers only in the genes encoding Aα and Aβ[29] subunits but not in those encoding regulatory B, B' and B".[9] The COSMIC database reports 65 Aα mutations in 1655 tumor samples (4%), whereas the mutation rate for the various B' subunits is less than 0.2%.[9] This is also the case for all forms of B and B". Since all Aα mutations reported so far interfere, or are expected to interfere, with the binding of most, if not all, B' isoforms,[33,37] it is far more advantageous for tumor development to abolish PP2A tumor suppressor activity by mutating Aα than by independently mutating two or more B' subunits. It is unclear why numerous Aβ mutations described by Wang et al.[29] are not listed in COSMIC.[9] Thus, the conclusions from cancer genome sequencing could still change as more data become available.

Another aspect of B' holoenzymes that needs to be considered is the large difference in their tissue distribution. For example, B'δ is highly expressed in cerebellum and cortex but low in kidney and liver, whereas B'α is low in cerebellum and cortex and high in lung.[37] Which form of B' subunit is used for tumor suppression may depend on their abundance. Furthermore, the affinities of various B' subunits for Aα differ greatly,[33,37] which could play a role in B' holoenzyme formation.

Mouse Model Reveals PP2A Tumor Suppressor Activity

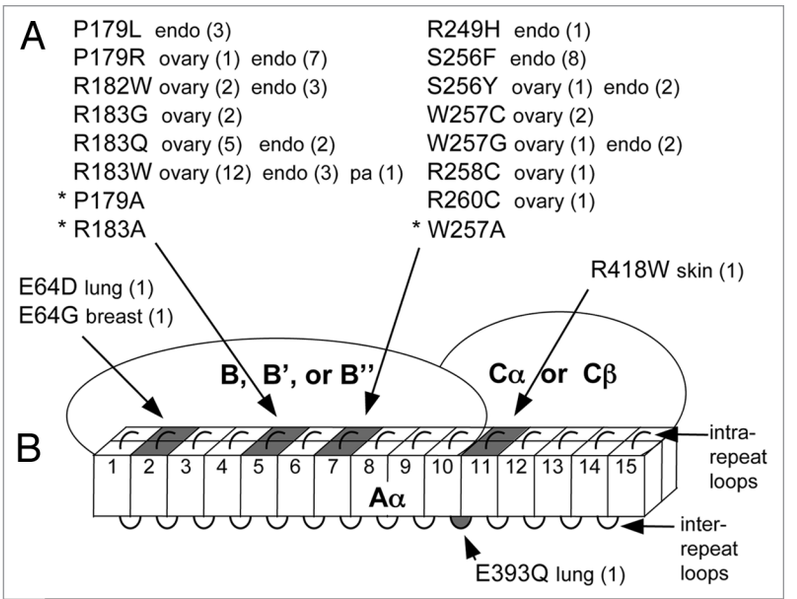
To obtain evidence for the hypothesis that PP2A is a tumor suppressor in mice, and that the human lung cancer-associated E64D mutation abolishes PP2A tumor suppressor activity by preventing formation of B' holoenzymes, we generated the following mouse strains: (1) E64D/+ knock-in; (2) F5–6/+ with exons 5 and 6 floxed for conditional Aα knockout; (3) Δ5–6/+ with exons 5 and 6 deleted. The Δ5–6 allele encodes a truncated Aα subunit (Δ168–589) missing repeats 6–15, very similar to the Δ171–589 mutant in breast cancer (**Fig. 1C**). Both truncation mutants are equivalent to an Aα knockout, since they bind none of the B and C subunits.[37] We discovered a 50–60% increase in lung cancer incidence in mice of strains

E64D/+, Δ5–6/+ and Δ5–6/E64D in comparison with FVB control mice (+/+). All mice were treated at 5 weeks of age with one dose of benzopyrene, a powerful carcinogen in cigarette smoke. We also showed that the formation of B' holoenzymes is strongly reduced in lungs due to the E64D mutation. From these results, we concluded that PP2A is a tumor suppressor in mice, and that the point mutation E64D abolishes this activity. A very similar increase in cancer incidence was obtained in mice with a heterozygous Aα knockout. Since the only defect caused by the E64D mutation is reduced formation of B' holoenzymes, we conclude that one or several forms of B' holoenzymes are tumor suppressors.[37] The data are consistent with previous results in tissue culture.[32,36] Importantly, our results strongly suggest that the E64D mutation enhanced the development of the human lung carcinoma in which it was discovered.

As reported previously, homozygous E64D and E64G mice as well as Δ5–6/E64D and Δ5–6/E64G mice are viable, implying that they survive with little or no B' holoenzyme. We proposed that large amounts of B' holoenzymes are only required as protection against oncogenic stress, such as exposure to benzopyrene or expression of K-ras^{G12D}.[37] In fact, the degree of protection depends on the level of B' holoenzyme expression (gene dosage effect) (see below).

PP2A Tumor Suppressor Activity Depends on p53

We asked whether loss of PP2A tumor suppressor activity is enhanced by p53 inactivation. The expectation was that simultaneous loss of two independent tumor suppressors would have an additive effect on tumor incidence. Surprisingly, this was not the case. Instead we found that the PP2A tumor suppressor function is abolished if p53 is inactivated, suggesting that PP2A either activates the tumor suppressor function of p53 or causes a rise in its level by preventing its degradation. As noted previously, the lung cancer incidence is reduced 35% by PP2A,[37] while p53 causes a 50% reduction,[38] suggesting that PP2A is required for 2/3 of p53's effect.



E

	Endometrium	Ovary
Aα	18	6
K-ras	15	14
Arf	5	7
p53	20	49
PI3K	24	9

F

	Bα	B'γ3	B''
E64D	61	5	88
E64G	62	0	102
P179A	52	7	108
R183A	6	4	22
R183E	7	4	0
W257A	7	5	7

Figure 1. Model of PP2A holoenzyme; location of human cancer-associated Aα mutations; high frequency of Aα mutations in endometrial cancer. (B) Trimeric PP2A holoenzyme consists of one catalytic subunit (Cα or Cβ), one scaffolding subunit (Aα or Aβ) and one of several regulatory subunits (B, B′ or B′′). Aα and Aβ consist of 15 repeats connected by inter-repeat loops. Each repeat consists of two antiparallel α-helices connected by intra-repeat loops. (A) Aα mutations in endometrial (endo) or ovarian (ovary) cancer are clustered at or near intra-repeat loop 5 of repeat 5 (from P179 to R183) and at or near intra-repeat loop 7 of repeat 7 (from R249 to R258). Numbers in parentheses represent number of tumors with a mutation at a particular site.[6-9] E64D, E64G and R418W were found in lung, breast and skin cancer, respectively.[10] Shown in (C and D) are C-terminal truncations, Δ171–589 from breast cancer missing repeats 6 to 15[10] and Δ375–589 from kidney cancer missing repeats 11 to 15.[11] (E) Frequency of Aα mutations in endometrial (18%, 31/171) and ovarian (6%, 27/470) cancers in comparison to K-ras, Arf, p53 and PI3K.[6-9] (F) Loss of Bα, B′γ3 (formerly known as B′α1),[12] and B′′/PR72 binding to mutant Aα. Note: All Aα mutants are defective in B′γ3 binding.[13,14] For E393Q, see reference 15; for R183W in pancreatic (pa) cancer, see reference 16; *indicates synthetic mutant.

PP2A Delays Death from Lung Cancer Triggered by Oncogenic K-ras^{G12D}

Having demonstrated that PP2A suppresses the development of lung tumors in benzopyrene-treated mice, we were interested in identifying the relevant PP2A substrates and the growth stimulatory signaling pathways in which these substrates operate. Because of the dependence of PP2A tumor suppressor activity

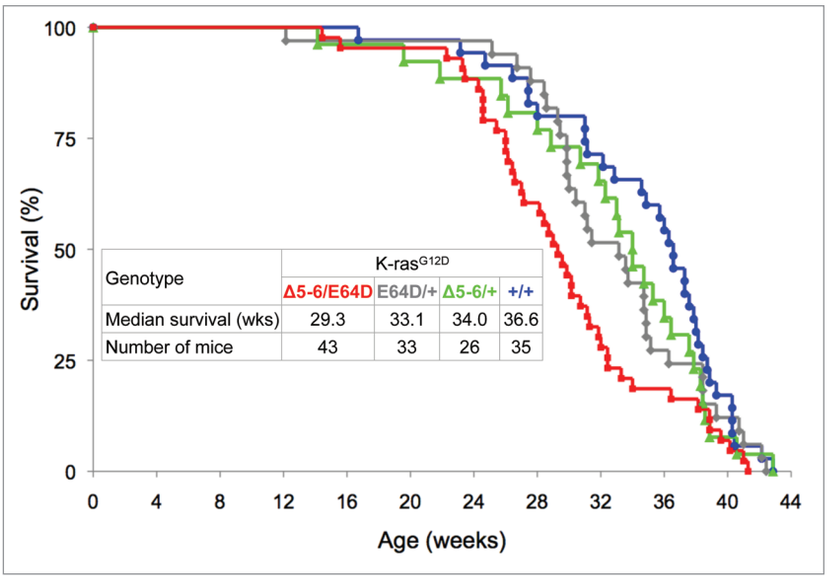


Figure 2. Wild-type PP2A prolongs the survival of mice expressing oncogenic K-ras^{G12D}. Mice expressing oncogenic K-ras^{G12D} (LA2) [42] were crossed with PP2A mutant mice to obtain the four genotypes listed. The mice were observed daily for signs of ill health, and moribund animals were sacrificed. The Δ5–6/E64D;K-ras^{G12D} mice lived statistically significantly shorter than E64D/+;K-ras^{G12D} ($p = 0.01$), Δ5–6/+;K-ras^{G12D} ($p = 0.03$) and +/+;K-ras^{G12D} ($p = 0.0003$) mice. When considering only mice in the 75–25% survival range, p-values are 0.0001 for E64D/+;K-ras^{G12D} vs. +/+;K-ras^{G12D} mice and 0.02 for Δ5–6/+;K-ras^{G12D} vs. +/+;K-ras^{G12D} mice (one-tailed t-test for equal variances; Excel).

on functional p53, we chose to investigate the Ras-MAPK pathway, which involves both PP2A and p53 and is activated by benzopyrene in over 90% of mouse lung tumors due to generation of oncogenic K-ras^{G12D}.[39] Thus, it is likely that K-ras^{G12D} was also generated in our benzopyrene-treated mice, in which case PP2A might have dephosphorylated and inactivated MEK and ERK as well as increased the level of p53 through the Arf-MDM2-p53 pathway that is connected to the Ras-MAPK pathway (**Fig. 3**). To obtain direct evidence that PP2A suppresses tumor formation initiated by K-ras^{G12D}, we made use of a mouse model of K-ras^{G12D}-induced lung carcinogenesis in which a latent allele (LA) of K-ras^{G12D} is turned on by spontaneous recombination throughout the animal, but only in a fraction of cells. In this K-ras^{LA2} mouse,[42] K-ras^{G12D} expression is controlled by the endogenous K-ras promoter, thus mimicking the occurrence of mutations in somatic cells, as is the case during spontaneous tumor development. Despite activation of K-ras^{G12D} in all tissues, the mice develop primarily lung tumors, first detectable at 1 week of age as hyperplastic pleural nodules, which develop into adenocarcinomas. The majority of mice die between 4–8 mo of age.[42] K-ras^{G12D} mice were crossed with Aα-E64D and Aα-Δ5–6 mice, and four genotypes were obtained: (1) E64D/+;K-ras^{G12D}, (2) Δ5–6/+;K-ras^{G12D}, (3) Δ5–6/E64D;K-ras^{G12D} and (4) +/+;K-ras^{G12D} (control). The mice were monitored for survival. If PP2A suppresses lung tumor formation by inhibiting MAP kinase signaling, then E64D mutation, heterozygous Aα knockout or a combination of both are expected to accelerate tumor formation and death.

As shown in **Figure 2**, E64D/+;K-ras^{G12D} mice (gray) died 3.5 weeks earlier than +/+;K-ras^{G12D} mice (blue), while Δ5–6/E64D;K-ras^{G12D} mice (red) died 7 weeks earlier (**Fig. 2**, insert). These results demonstrate that homozygous wild type PP2A extends the median survival time of K-ras^{G12D} mice by 25%. Importantly, the survival times of E64D/+ and Δ5–6/+ mice are very similar, indicating that the heterozygous Aα-E64D mutation has approximately the same negative effect on survival as the heterozygous Aα knockout. These results confirm those obtained with benzopyrene-treated mice, i.e., that one or several B' holoenzyme(s) are PP2A tumor suppressors. The data are consistent with results from tissue culture.[33,36] The observation that the survival times of E64D/+ and Δ5–6/+ mice are approximately in the middle between those of +/+ and Δ5–6/E64D mice demonstrates that the level of tumor suppressor activity correlates with gene dosage. All mice developed multiple adenocarcinomas, as described by Johnson et al. To determine the tumor burden, we weighed the lungs. Compared with normal mouse lungs of approximately 0.14 g, the median weights of lungs from the tumor mice at death were 1.29 g (Δ5–6/E64D;K-ras^{G12D}), 1.23 g (E64D/+;K-ras^{G12D}), 1.16 g (Δ5–6/+;K-ras^{G12D}) and 1.17 g (+/+;K-ras^{G12D}). Since there is no substantial difference in tumor burden, we conclude that the PP2A-mutant mice died sooner, because they reached the lethal tumor burden earlier than the PP2A wild type mice. That all survival curves converge at around 40 weeks of age could be caused by multiple side effects from the high tumor burden, which overshadows the specific effects due to genetic differences. To fully understand how PP2A acts as a tumor suppressor in the Ras-MAPK and Arf-MDM2-p53 pathways, it is necessary to investigate how the different levels of B' holoenzymes in E64D/+;K-ras^{G12D}, Δ5–6/+;K-ras^{G12D} and Δ5–6/E64D;K-ras^{G12D} mice, in comparison to +/+;K-ras^{G12D} mice, affect the phosphorylation state and/or abundance of potential PP2A targets, including Raf, MEK, ERK, Arf, MDM2 and p53.

Is PP2A a Universal Tumor Suppressor?

PP2A is involved in a network of major signaling pathways that control growth and survival as well as growth arrest and apoptosis. Some of these pathways and the positions where PP2A might operate as tumor suppressor are shown in **Figure 3**. It appears that PP2A is involved in all these pathways, raising the question of whether it is a universal tumor suppressor. However, much of the evidence for the presumed role of PP2A as tumor suppressor is derived from tissue culture data, which do not always reflect the situation

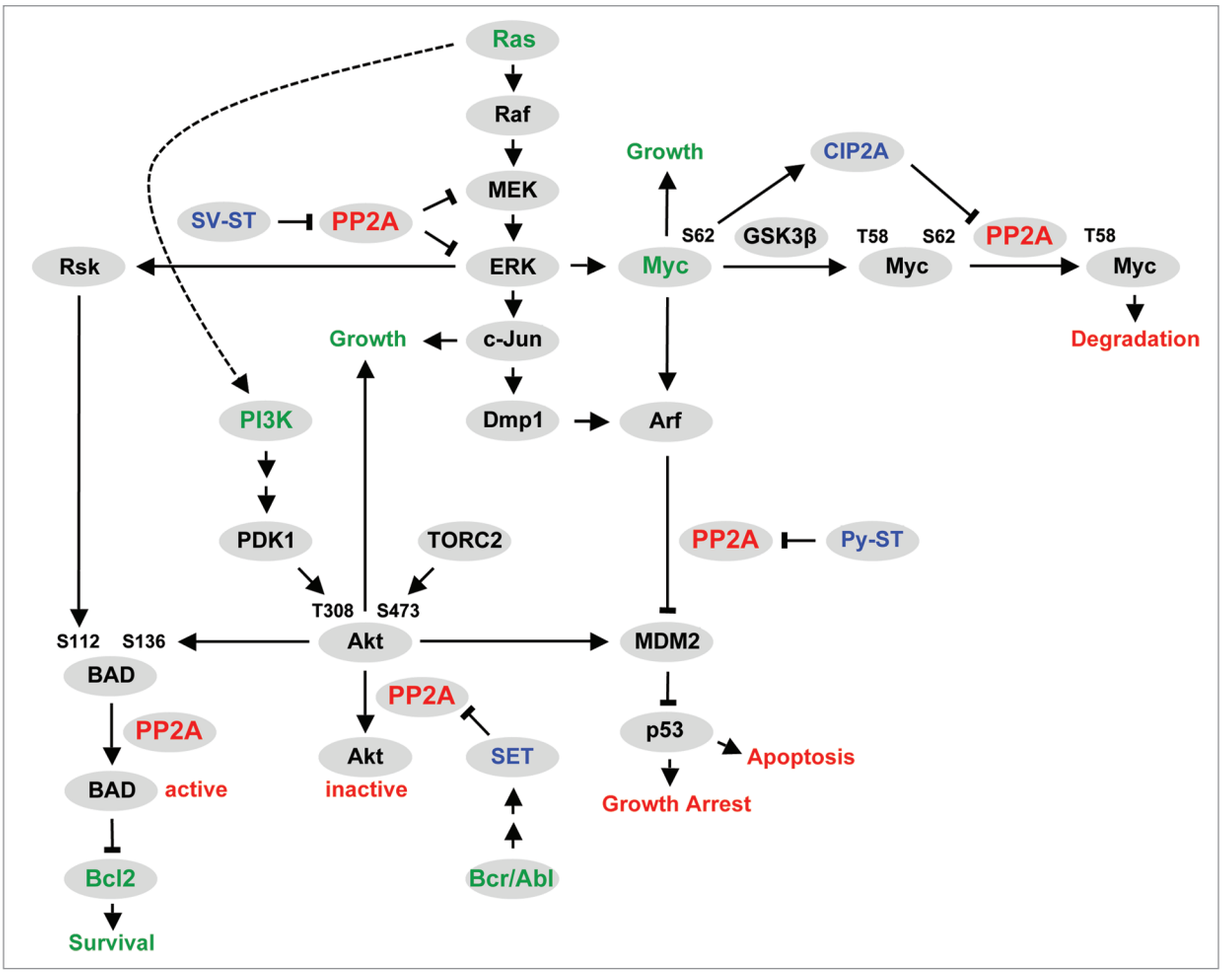


Figure 3. PP2A inhibits oncogene signaling and promotes p53 activation. A large fraction of tumors arise from aberrant Ras, Myc or PI3K signaling. PP2A has been implicated in the suppression of all three pathways. It suppresses MAPK signaling by dephosphorylating MEK and ERK and it reduces the levels of active Myc and active Akt by dephosphorylating S62 as well as T308 and S473, respectively. PP2A is also implicated in promoting the function of Arf, which is crucial for oncogene-induced p53 activation.[40,41]

in vivo. Therefore, our mouse model for testing PP2A's role as a tumor suppressor might represent a suitable tool to answer the above question. If PP2A, indeed, plays a universal role, it could become a drug target for most if not all types of cancer. Here, we briefly summarize how PP2A might function as a tumor suppressor in the various pathways, keeping in mind that, like in benzopyrene-treated mice, it might only do so if p53 is active, which is the case in approximately 50% of all cancers.

Ras-Raf-MEK-ERK. Oncogenic mutation of the K-ras gene was found in 22% of all human cancers and thus may represent one of the most important events in cancer development overall.[9] Oncogenic K-ras constitutively activates the Raf-MEK-ERK kinase cascade,[43] thereby generating a continuous growth-stimulatory signal. PP2A has been shown to inhibit this cascade and cell growth by dephosphorylating MEK and ERK and thereby inactivating their kinase activities.[44,45] While our data clearly demonstrate that PP2A functions as a tumor suppressor of K-ras^{G12D}-induced lung cancer, we have not yet shown that this process depends on p53. This could be determined by crossing PP2A mutant mice with p53$^{-/-}$ mice (**Table 1**).

Ras-Arf-p53. Continuously elevated signaling by oncogenic K-ras is known to induce p53 via transcription of Dmp1, which, in turn, triggers expression of Arf, a potent tumor suppressor.[58] Arf acts as tumor suppressor by inhibiting MDM2-mediated p53 degradation.[59,60] In a large fraction of tumors (e.g., in 41% of non-small cell lung carcinomas and 50% of colon carcinomas),[61] Arf is downregulated to low or undetectable levels by promoter methylation,[62,63] resulting in increased p53 degradation mediated by MDM2. That expression of oncogenic K-ras induces coordinated upregulation of two potent tumor suppressors, Arf and p53, might seem "paradoxical." A creative suggestion has been offered, according to which Arf and p53 upregulation in response to strong oncogenic signals represent a desperate but ultimately unsuccessful attempt to block tumor growth, which eventually will be overcome by Arf downregulation through promoter methylation, p53 loss-of-function mutation, increased oncogene expression or expression of additional oncogenic proteins.[64-67] Importantly, it was discovered

Table 1. Mouse models for testing PP2A's effects on various signaling pathways

Mouse models tested	Organ	Effect of PP2A mutation on carcinogenesis	Conclusion	References
$A\alpha^{E64D}$ + benzopyrene	Lung	Accelerated	PP2A inhibits tumors driven by benzopyrene	37
$A\alpha^{E64D}$;dnp53 + benzopyrene	Lung	None	PP2A requires p53 to inhibit tumors driven by benzopyrene	37
$A\alpha^{E64D}$;K-ras^{G12D}	Lung	Accelerated	PP2A inhibits tumors driven by K-ras^{G12D}	37, 42, this report
Mouse models proposed		**Anticipated effect of PP2A mutation on carcinogenesis**		
$A\alpha^{E64D}$;K-ras^{G12D};$p53^{-/-}$	Lung	None	PP2A requires p53 to inhibit tumors driven by K-ras^{G12D}	42
$A\alpha^{E64D}$;K-ras^{G12D};$Arf^{-/-}$	Lung	None	PP2A requires Arf to inhibit tumors driven by K-ras^{G12D}	42, 46, 47
$A\alpha^{E64G}$;WAP-Myc	Mammary gland	Accelerated	PP2A inhibits tumors driven by Myc	48, 49
$A\alpha^{E64D}$;$PI3K^{H1047R}$	Lung	Accelerated	PP2A inhibits tumors driven by mutant PI3K	50
$A\alpha^{P179A}$;$PTEN^{F/F}$	Endometrium	Accelerated	PP2A inhibits tumors driven by PTEN loss	51
$A\alpha^{P179A}$;$PTEN^{F/F}$ + FTY720	Endometrium	Activation of PP2A by FTY720 inhibits progression of endometrial cancer	Activation of PP2A beneficial for 18% of women with endometrial cancer with Aα mutations	51–57

The Aα-floxed and E64G mice will soon be available from the Jackson Laboratory (order numbers 017441 and 017475).

that the Arf-mediated activation of p53 is inhibited by polyomavirus small T antigen (Py-ST), which binds to the Aα subunit of PP2A replacing B subunits. Mutants of Py-ST that do not bind to PP2A have no effect on Arf. These findings suggest that PP2A is required for the Arf-mediated inhibition of MDM2 and the ensuing accumulation of p53.[40] This implies that PP2A cannot fulfill a role as tumor suppressor in the Arf-p53 pathway, if functional p53 is not present. Similar to the continuous expression of oncogenic K-ras, high levels of Myc also induce p53 through the Arf-p53 pathway.[68,69]

To investigate whether PP2A's tumor suppressor function depends on Arf, $Arf^{-/-}$ mice[46,47] expressing mutant PP2A could be used. If PP2A tumor suppressor activity acts through Arf, and only through Arf, and if this activity is abolished by PP2A mutation, then there should be no difference in tumor incidence between wild type and mutant PP2A mice in the absence of Arf. In this case, PP2A would be at the center of a major tumor suppressor system that preserves p53 activity.

Myc degradation. The Myc gene is amplified in 25% of breast carcinomas.[70] Evidence that PP2A acts as a tumor suppressor on Myc comes from the discovery that the B'α holoenzyme plays a key role in Myc degradation[27] and from the identification of an inhibitor, CIP2A, which binds to PP2A and Myc, thereby preventing B'α holoenzyme from dephosphorylating Myc at serine 62, a prerequisite for Myc degradation (**Fig. 3**).[71] Most importantly, CIP2A has clinical relevance, since patients with gastric cancer have a 10-y survival rate of 37% when the tumor is CIP2A-negative and PP2A is not inhibited and only an 8% survival rate when the tumor is CIP2A-positve and PP2A is inhibited.[72] Whether E64G, which was found in a breast carcinoma, enhances the carcinogenic effect of Myc amplification can be tested in a mouse model that combines PP2A mutant mice with mice expressing Myc in the mammary gland.[48,49] We would expect that tumor development is accelerated in a PP2A-mutant background, because the mutant PP2A fails to dephosphorylate Myc at S62 to prepare it for degradation.

PI3K-Akt-p53. The phosphoinositide-3-kinase (PI3K) pathway is one of the most frequently activated pathways in cancer.[73,74] Upstream receptor tyrosine kinases, PI3K itself and its main effector Akt have all been found amplified or mutated in human tumors.[75] Loss of the PI3K antagonist PTEN also leads to enhanced Akt signaling. Akt is activated by phosphorylation on T308 and S473. One of its many targets is MDM2, which is stabilized through phosphorylation by Akt, resulting in degradation of p53.[76] Importantly, PP2A has been shown to inactivate Akt by dephosphorylating both T308 and S473, leading to reduced phosphorylation and increased degradation of MDM2 while p53 accumulates.[76-79] Whether PP2A mutation cooperates with oncogenic PI3K signaling could be tested by combining Aα-mutant mice with a recently generated transgenic mouse that expresses an activating PI3K mutation.[50]

Ras-PI3K interaction. There is considerable crosstalk between pathways. For instance, it has been demonstrated that Ras is required to physically interact with PI3K in order to promote lung carcinogensis,[80] possibly acting as an adaptor molecule tethering PI3K to a receptor tyrosine kinase.[73] The Ras and PI3K pathways also converge on BAD, each leading to addition of an inhibitory phosphate.[81,82] PP2A has been shown to dephosphorylate BAD, thereby activating its pro-apoptotic function as an inhibitor of Bcl2 [83] (**Fig. 3**).

Activation of PP2A by FTY720 Inhibits Tumor Growth and Metastasis

FTY720, also known as fingolimod or Gilenya (Novartis), is an activator of PP2A core and holoenzymes[26,52] that was used as an immunosuppressant after organ transplantation. It traps lymphocytes in lymphnodes, withholding them from attacking the transplanted organ. In addition, FTY720 induces lymphocyte apoptosis. Subsequently, it was found to induce apoptosis of cultured cells from various malignancies, including melanoma, breast cancer, leukemias and liver cancer.[53-57] Injection of such cells into mice led to tumor growth that was inhibited by FTY720. Most importantly, FTY720 strongly reduces the generation of metastases. It had virtually no adverse effects on the mice, other than a low lymphocyte count. FTY720 has been successfully tested as an anticancer drug in mice injected with human tumor cells, i.e., in xenograft models. To our knowledge, it has not yet been examined on genetically engineered mice that develop tumors in situ. Such a model could be a tool for testing drug efficacy in tumors with reduced PP2A activity. Since endometrial cancers with mutated Aα were found at high frequency (18%), and since mouse models for endometrial cancer exist,[51] mice from such models could be combined with Aα-mutant mice to find out whether Aα mutations accelerate endometrial carcinogenesis and whether activation of PP2A by FTY720 suppresses tumor formation. What makes FTY720 particularly attractive as an anti-cancer drug is the fact that it is already FDA-approved for use in humans, most recently for the treatment of multiple sclerosis.[84] Furthermore, the potentially widespread role of PP2A as a tumor suppressor might qualify FTY720 for the treatment of a variety of cancers. In the long run, one might also consider FTY720 as a prophylaxis for individuals with a genetic predisposition to certain cancers. While activating PP2A by FTY720 cannot prevent initiation of the oncogenic process, it could considerably slow down its development.

Acknowledgements

We thank Jennifer Ruiz for excellent technical assistance. We thank Steven Gonias for critical reading of the manuscript. Minya Pu and Karen Messer are appreciated for their help with statistical analyses. This work was supported by US Public Health Service grant CA118015 and Tobacco-Related Disease Research grant 14RT-0065 to G.W.

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