Cancerous Inhibitor of Protein Phosphatase 2A, an Emerging Human Oncoprotein and a Potential Cancer Therapy Target

Anchit Khanna¹,², John E. Pimanda¹,², and Jukka Westermarck³,⁴

Abstract

Protein phosphatase 2A (PP2A) complexes function as tumor suppressors by inhibiting the activity of several critical oncogenic signaling pathways. Consequently, inhibition of the PP2A phosphatase activity is one of many prerequisites for the transformation of normal human cells into cancerous cells. However, mechanisms for PP2A inactivation in human cancers are poorly understood. The aberrant expression of cancerous inhibitor of protein phosphatase 2A (CIP2A), a recently identified endogenous PP2A inhibitor in malignant cells, is one such mechanism. Various independent studies have validated CIP2A’s role in promoting tumor growth and resistance to apoptosis and senescence-inducing therapies. Notably, high CIP2A expression predicts poor patient prognosis in several human cancer types. Among the oncogenic proteins dephosphorylated by PP2A, the MYC oncoprotein, which is phosphorylated at serine 62, has surfaced as a marker for the oncogenic activity of CIP2A. The positive-feedback loop between CIP2A and MYC augments the activity of MYC in cancer cells. In addition, CIP2A promotes the phosphorylation and activity of additional oncoproteins, including E2F1 and AKT. However, CIP2A is not essential for normal mouse growth and development. These findings indicate that CIP2A is a novel anticancer target based on PP2A reactivation and inhibition of the oncogenic activity of its downstream effectors. The potential approaches and feasibility of targeting CIP2A are discussed here. Cancer Res; 73(22); 6548–53. ©2013 AACR.

Introduction

Reversible protein phosphorylation is one of the most common mechanisms for regulating protein function and transmitting signals throughout the cell. Phosphoregulation is tightly controlled by protein kinases and phosphatases, and these two classes of enzymes thus act as complementary molecular switches to determine the phosphorylation status of a large fraction of the human proteome. It has become evident that the deregulation of this balance contributes to the pathogenesis of several human diseases, including cancer. Constitutively activated protein kinase driver mutations are found in many cancers, and they constitute an important class of cancer therapy target proteins (1). However, despite the relative effectiveness of kinase inhibitors as cancer drugs, most cancers eventually become resistant to these inhibitors (1). This limitation, in addition to the limited number of drug targetable kinases, creates an urgent need to develop and implement new strategies to prolong the survival of cancer patients. As phosphatases are equally important for determining the phosphorylation status of oncogenic phosphoproteins, increasing the activity of tumor suppressor phosphatases is an alternative or complementary strategy to target increased phosphorylation-dependent signaling in cancer cells (2, 3). However, the feasibility and potential of this cancer therapy strategy remain largely unexplored.

A critical role for inhibition of phosphatase activity in cancer is strongly supported by functional experiments using immortalized human cells. Findings from several independent studies demonstrate that activation of kinase signaling via the expression of the activated RAS oncoprotein is not sufficient to transform immortalized cells if the major human serine/threonine phosphatase complex, protein phosphatase 2A (PP2A), is not simultaneously inhibited (3). These results strongly support the rationale for PP2A reactivation in combination with kinase signaling inhibition as an effective approach for cancer therapy (2, 3).

Of the various phosphatases, PP1 and PP2A account for most of the serine/threonine phosphatase activity in the cell (2, 3). The first indication that PP1 and PP2A complexes have tumor suppressor activities was obtained from studies using the serine/threonine phosphatase inhibitor okadaic acid, which is a potent inhibitor of PP2A but also inhibits PP1 at higher concentrations (2, 3). The potential relevance of PP1 inhibition and specific PP1 inhibitors, such as NIPPP1 and DARPP-32, for cancer is still emerging, but the subsequent work has established the role of PP2A as an important human tumor suppressor (2–4). PP2A is a trimeric complex consisting of the scaffolding A-subunit, the catalytic C-subunit, and various

Authors' Affiliations: ¹Adult Cancer Program, Lowy Cancer Research Centre and Prince of Wales Hospital, University of New South Wales (UNSW) Medicine; ²Translational Cancer Research Network, University of New South Wales (UNSW), Sydney, Australia; ³Turku Centre for Biotechnology, University of Turku and Åbo Akademi University; and ⁴Department of Pathology, University of Turku, Turku, Finland

Corresponding Authors: A. Khanna, University of New South Wales (UNSW), Level2, Kensington Campus, Building C25, Sydney, NSW 2052, Australia. Phone: 61-2-9385-2527; Fax: 61-2-9385-1510; E-mail: a.khanna@unsw.edu.au; and J. Westermarck, jukka.westermarck@utu.fi
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substrate-determining B-subunits (Fig. 1; refs. 2 and 3). PP2A tumor suppressor activity is inhibited by several oncogenic DNA/RNA viruses (3). The best understood viral protein that inhibits PP2A tumor suppressor activity is simian virus 40 (SV40) small-t antigen, and using small-t, inhibition of PP2A was shown to be required for malignant transformation of several types of normal human cells (3). Mechanistically, dephosphorylation of several well-known oncogenes, such as MYC, β-catenin, AKT, and BCL2, by PP2A explains its potent tumor-suppressive role (2, 3).

The essential role of PP2A inhibition in human cell transformation and cancer progression makes the identification of PP2A-inhibitory mechanisms vital for understanding phosphorylation-based signaling in human cancers. Several endogenous inhibitors of PP2A, such as I1PP2A, I2PP2A/SET, and PME-1, have been identified previously (2, 3). Both SET and PME-1 are overexpressed in several tumor types, and they have been shown to promote the malignant growth of human cancer cells (3). Using the tandem affinity purification method, we identified a novel endogenous interacting partner of PP2A, which was named cancerous inhibitor of PP2A (CIP2A; alias p90, KIAA1524; refs. 4 and 5). CIP2A binds to the PP2A complex, and inhibition of CIP2A has been shown to increase the catalytic phosphatase activity of the PP2A complex in several different human cancer cell types (4, 6, 7). Moreover, a recent study demonstrated that codepletion of the PP2A regulatory B-subunits B55a or B56b rescued the inhibitory effects of CIP2A depletion, as determined by both colony growth and MYC expression (8). However, the exact molecular mechanism by which CIP2A inhibits PP2Ac catalytic activity is unclear.

Oncogenic functions of CIP2A

CIP2A was demonstrated to be an oncogenic PP2A inhibitor protein in human cells due to its ability to replace the role of small-t antigen in the transformation of immortalized human HEK-TERV cells (4). Moreover, CIP2A expression induces the transformation of JNK2−/− mouse embryo fibroblasts (MEF), which are otherwise defective for RAS-driven transformation (9). In addition to evidence indicating that CIP2A directly promotes malignant transformation, several recent studies have shown that CIP2A inhibition in fully malignant cancer cells results in decreased cell viability and anchorage-independent growth (4, 6–8, 10–15). In addition, CIP2A promotes progenitor cell self-renewal and protects cancer cells from therapy-induced apoptosis or senescence induction (6, 16–19).

Importantly, several independent studies have demonstrated that CIP2A depletion via small interfering RNAs (siRNA) inhibits the growth of xenografted tumors of various cancer cell types (4, 11, 15). Independent of RNAi, hypomorphic CIP2A mutant mice display decreased HER2-driven mammary tumorigenesis, and the mammary tumors in CIP2A-deficient mice show a senescence phenotype in vivo (16). Nevertheless,
these CIP2A mutant mice are healthy and viable, except for a defect in spermatogenesis (16, 19). It is possible that the viability of CIP2A mutant mice is related to the relative selectivity of CIP2A for regulating the phosphorylated (Ser 62) oncogenic form of MYC (21), allowing sufficient expression of unphosphorylated MYC to support viability and normal tissue function. In contrast to testicular progenitors and various tumor cells, CIP2A expression is relatively low in most normal human and mouse tissues (4, 19), which may also explain the viability of CIP2A mutant mice. Altogether, these findings establish CIP2A as an emerging human oncoprotein and as a potential anticancer target.

**CIP2A expression in human malignancies**

CIP2A overexpression is observed in several types of tumors (4, 9–13, 15, 22). Strikingly, CIP2A is overexpressed in approximately 39% to 90% of the patient samples, establishing it as one of the most frequent molecular alterations in human cancers. The clinical relevance of CIP2A overexpression as a prognostic marker has been established in various solid and hematological cancers, including gastric, bladder, ovarian, tongue, hepatocellular, colon, non–small cell lung carcinoma (NSCLC), and chronic myelogenous leukemia (10, 12–15, 23, 24). CIP2A expression has been correlated with tumor grade in many cancers (11–13, 15, 16). Importantly, two recent studies have provided evidence that cancer patients with CIP2A negative tumors respond significantly better to cancer therapies (10, 16). CIP2A expression in cancer cells is driven by several oncogenic signaling mechanisms, including MYC (9, 13), activation of the EGFR–MEK–ETS1 pathway (22), inactivation of p53 (16), or the overexpression of E2F1 (16) and ATF-2 (9). Although ETS1, ATF2, and E2F1 have been shown to directly bind to the CIP2A promoter, the MYC-responsive region within the CIP2A promoter has not yet been identified. More recently, we have identified DNA-damage kinase, CHK1, as a stimulator of CIP2A transcription in cancer cells in which CHK1 is constitutively phosphorylated on serine 345 by another DNA-damage kinase, DNA-PK (25). As DNA damage is present in most, if not all, cancer cells, these results may explain the widespread overexpression of CIP2A across multiple human cancer types.

**CIP2A and MYC**

MYC is one of the most-studied oncoproteins, and it is involved in several malignant cellular processes and is regulated in multiple ways. Notably, MYC mRNA expression and gene amplification fail to explain the high frequency of MYC protein overexpression in various human cancers. This may be indicative of MYC posttranslational regulation, leading to high MYC levels in human tumors. At the posttranslational level, MYC is regulated by phosphorylation at several phosphorylation sites, and several kinases, such as ERK, JNK2, CK2, and Cdk1, have been demonstrated to phosphorylate MYC on serine 62. The PP2A target residue (21, 26). PP2A inhibition increases MYC serine 62 phosphorylation, leading to increased MYC stability and enhanced transcriptional and oncogenic activities (3, 21, 26). In accordance with its role as a PP2A inhibitor protein, CIP2A expression promotes the serine 62 phosphorylation of MYC and MYC protein stability in several human cancer types (4, 8, 11–13, 15, 18, 24).

Gastric cancer is one of the diseases in which the oncogenic activity of MYC is implicated in its pathogenesis. However, less than 20% of gastric cancers show amplification of the c-MYC gene. Interestingly, CIP2A and MYC proteins are coexpressed in human gastric cancer samples, and CIP2A depletion in gastric cancer cells results in decreased expression of serine 62 phosphorylated MYC and destabilization of MYC (13). Furthermore, CIP2A and MYC are coexpressed in hepatocellular, colon, and NSCLC tissue samples (9, 12, 13). In addition to CIP2A promoting MYC expression, MYC is also able to stimulate CIP2A mRNA and protein expression (13), establishing a positive feedback loop between these 2 human oncoproteins. The existence of the positive feedback loop between MYC and CIP2A has been verified by 2 additional studies (9, 18). This bistable relationship between CIP2A and MYC may in part account for the prevalent MYC protein expression and activity in different human cancers. Convincing evidence for a functional relationship between these proteins was provided by experiments in which ectopic expression of CIP2A rescued both MYC- and RAS-signaling defects in JNK2-null MEFs, which were similarly rescued by ectopic MYC expression (9). Another study demonstrated that both MYC and CIP2A induce the expression of the other protein and promote the self-renewal and proliferation capacity of neural progenitor cells (18). Recently, pathway analysis of the transcriptional signature regulated by CIP2A revealed a significant association with MYC-associated signaling, and CIP2A was verified to regulate expression of MYC target genes (8). However, this study also identified MYC-independent functions for CIP2A in promoting cellular migration.

In breast cancer, which is another MYC-driven human cancer, CIP2A overexpression was observed in approximately 40% of unselected patient populations (8, 11, 16). However, 79% of the breast cancers with characteristics of advanced metastatic disease showed CIP2A overexpression (16), confirming the link between increased malignancy and elevated CIP2A expression in human tumors (11–13, 15). As in gastric cancer, prevalent overexpression of CIP2A may explain the disparity between the frequency of MYC amplification (approximately 15%) and protein overexpression (approximately 45%) in breast cancers. Furthermore, MYC is closely linked to basal-type and HER2-positive breast cancers, and these are also the subtypes of breast cancer in which CIP2A is most prevalently expressed (8, 16). At the level of gene expression signatures, the CIP2A-regulated signature clustered together with signatures for these breast cancer subtypes (8). Triple-negative breast cancer (TNBC) shares common features of basal-type breast cancers, and CIP2A is overexpressed in 64% of tumor samples from this therapeutically challenging breast cancer subtype (17).

**CIP2A and E2F1**

Recently, we uncovered a positive feedback loop between oncogenic transcription factor E2F1 and CIP2A (16). E2F1 binds to the CIP2A promoter 375 base pairs upstream of the transcription start site, and CIP2A inhibited the ability of PP2A
to phosphorylate E2F1 on serine 364. Interestingly, impaired p53 activity, which is observed in most cancers, was demonstrated to increase CIP2A expression via E2F1, whereas forced expression of CIP2A fully prevented p53-induced inhibition of E2F1 and senescence induction (16). Together, these results indicate a critical role for the CIP2A–E2F1 feedback loop in modulating the p53 response in cancer cells and as a potential novel target mechanism for pro-senescence therapies, particularly in cancer cells containing mutated p53. We also demonstrated that human breast cancer patients expressing high levels of CIP2A have significantly poorer prognosis upon treatment with senescence-inducing vinca-alkaloid drugs (16). Therefore, the reduced expressions of both E2F1 and CIP2A proteins may serve as biomarkers to stratify cancer patients for pro-senescence therapies.

CIP2A and AKT

The AKT signaling pathway is activated in a wide range of tumor types and drives cancer cell proliferation and survival. PP2A complexes control AKT activity by dephosphorylating both threonine 308 and serine 473 of AKT in a context-dependent manner (27). CIP2A overexpression in liver cancer cells increased AKT phosphorylation on both of these sites, and CIP2A inhibition caused dephosphorylation of serine 473 of AKT in liver cancer and TNBC cells (6, 17). Moreover, resistance to drugs that act via the AKT pathway seems to occur at least partly because of their effects on CIP2A expression (17). As such, CIP2A ablation might be useful for overcoming resistance to these drugs. However, CIP2A depletion did not affect AKT serine 473 phosphorylation in various colon cancer cell lines in which it potently inhibited MYC expression (24).

CIP2A–PP2A–UNC5H2–DAPk complex

UNC5H2 is a member of the dependence group of receptors that induces apoptosis in the absence of ligand (netrin-1) binding. This mechanism of apoptosis induction leads to the elimination of tumor cells that would otherwise invade or metastasize to different parts of the body. This UNC5H2-induced apoptosis is mediated by the recruitment of a protein complex that includes the death-associated protein kinase (DAPk); PP2A catalytic subunit; and PPP2R1B (PR65α), a scaffolding subunit of the PP2A holoenzyme (28). UNC5H2-mediated PP2A activation has been shown to inhibit the auto-phosphorylation of DAPk at serine 308 (28), and phosphorylation at this site renders it active, which leads to cellular apoptosis. In cancer, UNC5H2 interacts with CIP2A, which increases the survival of cancer cells by inhibiting PP2A-mediated dephosphorylation of DAPk serine 308 (28).

CIP2A in cancer therapeutics

Based on the genetic evidence that the generation of malignant human cancer cells requires both the activation of oncogenic kinase pathways (RAS) and the inhibition of their antagonistic phosphatases (PP2A; ref. 3), it can be proposed that an effective therapeutic response in cancer requires simultaneous inhibition of kinase signaling and reactivation of PP2A. It is important to note that compared with the p53 tumor suppressor, which is mostly inhibited in human cancers because of inactivating genetic mutations, PP2A complex proteins are infrequently mutated in various cancers (http://www.cbioportal.org/). Therefore, therapeutic PP2A reactivation via targeting of PP2A inhibitor proteins is a feasible option.

Based on the CIP2A-mediated regulation of multiple pathways that drive the development of various cancer types (MYC, AKT, E2F1, DapK, BCR/ABL, JAK2, and PLK1; refs. 3, 4, 6, 14, 16, 20, and 28), therapeutic targeting of CIP2A could facilitate a multitarget cancer therapy approach. Moreover, the fact that the CIP2A mutant mouse model shows no growth or development defects (16, 19) suggests that CIP2A-targeting therapies could be tolerated. CIP2A does not belong to any gene family known to encode proteins with enzymatic activity, and the structure of CIP2A remains unknown. Thus, it is not clear whether CIP2A is a "druggable" protein.

A possible method to overcome the limitations related to protein druggability is RNA interference (RNAi). A number of RNAi-based preclinical and clinical trials are ongoing, and a recent study showed several durable responses and an example of a cured patient following an RNAi trial therapy in patients with liver metastases (29). Natural accumulation of RNAi molecules in liver cells in addition to the role of CIP2A in liver cancer (6, 23) suggest that primary liver cancers and liver metastases of other cancer types could be tested with CIP2A RNAi therapy. CIP2A may be a particularly good target for RNAi because single transfection of CIP2A siRNA results in long-lasting inhibition of xenograft tumor growth (up to 59 days; refs. 4, 11, and 12). The bi-stable relationship between CIP2A and oncogenic proteins, such as MYC (13) and E2F1 (16), is a possible explanation for this sustained CIP2A RNAi effect. An initial study using intratumoral injection of CIP2A siRNA showed almost complete inhibition of tumor growth more than 40 days using repeated injections with a 5-day interval (15).

Based on currently available data, CIP2A binding to PP2A complexes involved in tumor suppression is likely critical for CIP2A's oncogenic activity. Although protein–protein interactions are generally considered to be challenging targets for therapeutics, recent advances in the field encourage the consideration of direct targeting of CIP2A–PP2A interactions using small molecules or peptidomimetics (30). Another potential approach could be allosteric or interfacial small molecule modulators of CIP2A that would affect its PP2A and/or MYC binding properties (30). Significantly, determining CIP2A's protein structure, alone and when interacting with PP2A and other targets, will be instrumental in validating these currently hypothetical assumptions.

Another alternative for inhibiting CIP2A is to target signaling mechanisms driving high CIP2A expression in cancer. Several small molecules have been shown to inhibit CIP2A expression, including 10058-F4 (MYC inhibitor; ref. 13), AG1478 (EGFR inhibitor; ref. 22), U0126 (MEK inhibitor; ref. 22), bortezomib (proteosome inhibitor; refs. 6 and 17), SP600125 (JNK inhibitor; ref. 31), and imatinib (BCR/ABL–ABL inhibitor; ref. 14). In addition, small molecule inhibitors of the DNA-PK and CHK1 DNA damage signaling kinases inhibit CIP2A expression, and they induce PP2A activity in human cancer cells (25). Importantly, CIP2A downregulation was shown to be crucial for induction of the CHK1 inhibitor response, affecting

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cell survival in different cancer cell lines (25). These new results that link Chk1 activity to oncogenic phosphoprotein signaling via the CIP2A–PP2A axis (25) may promote the development of single-agent anticancer Chk1 inhibitors that can block MYC signaling and inhibit the survival of cancer cells.

Future Perspectives

To therapeutically benefit from CIP2A targeting in human cancers, barriers must still be breached. First, numerous functions of distinct trimeric PP2A complexes are observed in normal physiology; thus, it is essential to identify the specific PP2A complexes that are reactivated upon CIP2A inhibition. Currently, 2 specific B-subunits have been demonstrated to be involved in response to CIP2A inhibition (8). Second, for drug discovery purposes, CIP2A structural information and the mechanism by which CIP2A inhibits PP2A activity must be solved. Furthermore, despite the convincing effects of CIP2A inhibition on xenograft tumor growth (4, 9, 11, 12, 15), we lack genetic in vivo models to test the therapeutic potential of acute CIP2A inhibition for cancer therapy. This type of data should motivate researchers to target CIP2A in human cancers using newer generations of protein–protein interaction inhibitors or RNAi strategies. Even if CIP2A turns out to be an undruggable protein, studies on CIP2A function will broaden our understanding of the importance of PP2A inhibition in cancer and may facilitate alternative strategies for PP2A reactivation as a cancer therapy approach. Finally, the identification of the underlying mechanisms behind CIP2A’s role in chemoresistance, in conjunction with using CIP2A expression as a biomarker for patient stratification, may lead to more successful clinical trials with a higher probability of attaining better patient outcomes.

Disclosure of Potential Conflicts of Interest

A. Khanna has ownership interest (including patents) in CIP2A expression as marker for patient stratification for anticancer therapy. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: A. Khanna, J.E. Pimanda, J. Westermarck
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Khanna
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Khanna
Writing, review, and/or revision of the manuscript: A. Khanna, J.E. Pimanda, J. Westermarck

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