Trends in Cancer

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Review

CIP2A coordinates phosphosignaling, mitosis, and the DNA damage response

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Human cancers share requirements for phosphorylation-dependent signaling, mitotic hyperactivity, and survival after DNA damage. The oncoprotein CIP2A (cancerous inhibitor of PP2A) can coordinate all these cancer cell characteristics. In addition to controlling cancer cell phosphoproteomes via inhibition of protein phosphatase PP2A, CIP2A directly interacts with the DNA damage protein TopBP1 (topoisomerase II-binding protein 1). Consequently, CIP2A allows DNA-damaged cells to enter mitosis and is essential for mitotic cells that are defective in homologous recombination (HR)-mediated DNA repair (e.g., BRCA mutants). The CIP2A-TopBP1 complex is also important for clustering fragmented chromosomes at mitosis. Clinically, CIP2A is a disease driver for basal-like triple-negative breast cancer (BL-TNBC) and a promising cancer therapy target across many cancer types.

Highlights

CIP2A (cancerous inhibitor of PP2A) coordinately controls the essential characteristics of cancer cells such as protein phosphorylation, mitosis, and the DNA damage response.

CIP2A allows cell-cycle progression under DNA damage by dampening the G2/M checkpoint, and protects the chromosomal integrity of DNA-damaged mitotic cells.

CIP2A binds directly to TopBP1 (topoisomerase II-binding protein 1) and is codependent upon several key DNA damage control proteins (RHNO, POLQ, NBN, and PARP1).

CIP2A is essential for the survival of homologous recombination deficient and BRCA mutant cancer cells.

CIP2A is a potential therapeutic target in cancer owing to its nonessential role in normal growth and development, as well as its broad relevance across different cancer types.

CIP2A paradigm shift

CIP2A was originally discovered as an oncogenic inhibitor of the tumor-suppressor protein phosphatase PP2A (Box 1) [1]. Based on its overexpression in many human cancers, its oncogenic activity, and its nonessential role in normal physiology, it has been considered a potential target for cancer therapy. However, it has been unclear whether the PP2A inhibitory capacity alone can explain the strong oncogenic activity of CIP2A. Recent studies have identified novel roles for CIP2A in the **DNA damage response** (**DDR**; see Glossary), the **G2/M checkpoint**, protection of chromosomal integrity during mitosis, and mitotic clustering of pulverized chromosomes from micronuclei (**chromothripsis**) [2–7]. These CIP2A functions are linked to its direct interaction with the DNA repair scaffold protein TopBP1. In addition to TopBP1, CIP2A was found to mimic several critical DNA damage factors in their essentiality for cancer cells [2,3]. Together these new results represent a paradigm shift in our understanding of CIP2A and indicate that the oncogenic power of CIP2A can be explained by its capacity to coordinately regulate the **phosphoproteome**, mitotic entry, and the DDR. Herein we review recent developments in CIP2A research and provide a balanced view of its role and functions. We focus on the ability of CIP2A to coordinate essential cancer functions as understanding the many functions of this oncoprotein will be key to the development of diagnostics and therapies targeting it.

CIP2A is associated with poor patient survival and therapy resistance across human cancers

CIP2A is overexpressed in ~70% of cancers [8–11] and is associated with poor patient prognosis (this effect is also seen in pan-cancer meta-analyses) [8–12]. In addition, high CIP2A expression in several cancers correlates with poor therapy response [9,13,14]. The general role for CIP2A in cancer therapy resistance was revealed by two drug screening studies in which CIP2A inhibition sensitized cancer cells to dozens of clinically available cancer therapies [15,16]. As a specific recent example, 20% of patients whose ovarian cancer did not express CIP2A had longer relapse-free survival after standard chemotherapy, and CIP2A-deficient tumors are highly sensitive to the

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reactive oxygen species (ROS) inducer APR-246 (Eprenetapopt®) [17]. Therefore, tumor CIP2A status can be used as a biomarker of cancer aggressiveness and therapy sensitivity.

Regulation of CIP2A

CIP2A expression is very low in most normal cells, except sperm cells [18], intestinal stem cells [19], and some immune cells [20], whereas expression is several-fold higher in many cancer cells [8,9]. *CIP2A* is neither genetically amplified nor mutated in most human cancers [8], but CIP2A protein is instead overexpressed by both transcriptional mechanisms as well as through increased protein stability. Several oncogenic transcription factors, for example MYC, E2F1, STAT3, and ETS family proteins, promote CIP2A expression by binding to the *CIP2A* gene promoter [14] (Figure 1). Furthermore, CIP2A post-translationally stabilizes both MYC and E2F1 proteins, and stimulates mitogen-activated protein kinase (MAPK) signaling upstream of ETS factors. It is likely that the involvement of CIP2A in the feed-forward loops in which CIP2A and its effector proteins support each other's expression explains the high expression of CIP2A in most cancer cells. Inactivating mutations in the tumor-suppressor gene *TP53* are found in the majority of cancers, and these mutations correlate positively with CIP2A expression [2,13,14,21]. Mechanistically, *TP53* inactivation drives *CIP2A* mRNA expression via the P21-E2F1 axis [21] (Figure 1). Functionally, high CIP2A expression is an essential senescence-evasion mechanism in TP53 mutant breast cancer cells [21]. Furthermore, DNA damage signaling promotes CIP2A expression via constitutive CHK1 activity (Figure 1) [22]. Outside cancer, CHK1 was recently shown to also drive high CIP2A expression in Alzheimer's disease where constitutive DDR activity is a known hallmark of the disease [22]. Further, a recent study demonstrated that *Cip2a* expression is induced by the DNA-damaging compound dimethylbenz(a)anthracene (DMBA) in premalignant mouse mammary tissue before detectable tumors [2].

Many compounds downregulate CIP2A expression, but none are direct CIP2A inhibitors [9]. EGFR and CHK1 inhibitors inhibit *CIP2A* transcription [22–24], and another group of drugs cause proteasomal degradation of CIP2A (e.g., celastrol) [25]. E3 ubiquitin ligases mediating proteasomal degradation of CIP2A may include CHIP [25] and UBR5 [2]. Consistent with ubiquitination-mediated degradation, mutation of the most prevalently ubiquitinated lysine 647 stabilized CIP2A protein [26].

CIP2A protein functions as an **obligate homodimer**. CIP2A monomers dimerize via a flat dimerization surface located at amino acids 522–546, and mutations that weaken dimerization also decrease CIP2A protein stability [27]. In addition, CIP2A is stabilized by its direct interaction with the B56 family of PP2A subunits (Box 1) [26,27]. Interestingly, metformin, a widely used metabolic drug, inhibits CIP2A expression, and CIP2A inhibition was shown to be required for the antitumor effects of metformin [28]. One of the cellular targets of metformin is the mitochondrial complex I which is the first enzyme of the mitochondrial electron transport chain. Complex I inhibition by either metformin or its specific inhibitor IACS-010759 induces ROS, detaches CIP2A from the PP2A complex, and induces subsequent CIP2A degradation [29] (Figure 1). The role of phosphorylation in CIP2A regulation has not been systematically studied, but a recent study demonstrated that phosphorylation of the Polo-like kinase 1 (PLK1) target phosphosite serine 904 in the CIP2A C-terminal tail recruits 14-3-3 binding [30]. Although the functional relevance of this phosphorylation and increased 14-3-3 interaction remains unclear, CIP2A and PLK1 seem to reciprocally regulate each other as CIP2A prevents PLK1 degradation [31], whereas PLK1 inhibition increases CIP2A expression [32]. Finally, CIP2A function is affected by its subcellular localization and protein interactions. Whereas CIP2A is predominantly cytoplasmic owing to its active nuclear export [4], a ~10% pool of CIP2A protein constitutively colocalizes with MYC to the inner nuclear membrane in **interphase** cells [19] (Figure 2). Nuclear CIP2A in interphase cells was also recently

Glossary

BRCA mutation: an inactivating mutation in either the *BRCA1* or the *BRCA2* gene which encode tumor suppressors involved in homologous recombination (HR)-mediated DNA repair. HR deficiency (HRD) induced by BRCA mutations makes cancers cells dependent on alternative DNA repair mechanisms such as micro-homology mediated end joining (MMEJ), for their survival.
Chromothripsis: a mutational process in which up to thousands of clustered chromosomal rearrangements occur in a single event at localized genomic regions on one or a few chromosomes.
cGAS-STING: cyclic GMP-AMP synthase (cGAS) and stimulator of interferon genes (STING) constitute the major signaling pathway in vertebrates that recognizes foreign DNA and activates an immune response. The cGAS–STING system is also activated if nuclear DNA leaks into cytoplasm, as occurs for example, during chromothripsis.
DNA damage response (DDR): cellular response to either external or endogenous conditions that damage DNA. Involves phosphorylation-dependent signaling cascades including DDR kinases such as ATR, ATM, CHK1, and CHK2.
Dominant negative: a mutant or fragment of a protein which when expressed in the cells inhibits the endogenous form of the protein by competition.
FKBP12 degron: a protein sequence that can be engineered onto a protein of interest to attract ubiquitin ligase to ubiquitinate the target protein and direct it to proteasomal degradation.
G2/M checkpoint: a cell-cycle checkpoint in which normal cells are stalled until their DNA is repaired before entering to mitosis (M). This is often compromised in cancer cells, resulting in passing mutations to the next generation of cells at mitosis.
γ-H2AX: the phosphorylated form of histone H2AX. γ-H2AX recruits DNA repair proteins to chromatin in response to DNA damage and it remains phosphorylated until completion of DNA repair.
Interphase: cell-cycle phases outside mitosis (G1, S, and G2).
Microhomology-mediated end-joining (MMEJ): an error-prone DNA repair mechanism for double-strand DNA breaks (DSBs). Similarly to HR, it is active in the G2 and M phases of the cell cycle.

found to interact with TopBP1 [2,4], and this interaction prevented DNA damage-induced TopBP1 chromatin recruitment [2]. Furthermore, during mitotic nuclear envelope breakdown, CIP2A is found in the nucleus, and in response to DNA damage localizes to chromatin together with TopBP1 [3,4,31] (Figure 2).

CIP2A drives tumorigenesis

Inhibition of CIP2A by siRNA or short hairpin (sh)RNA has been demonstrated to inhibit xenograft tumor growth in several independent studies, including most common human cancer types [1,9,17,23]. Furthermore, when *Cip2a* knockout mice were treated with DMBA, or tumorigenesis was induced by crossing with HER2 oncoprotein-overexpressing mice, significantly fewer mammary tumors were formed compared with *Cip2a* wild-type mice [2,21]. Interestingly, there were no discernible differences in the tumorigenesis patterns of other tissues studied in the DMBA-treated mice [2], and *Cip2a* was not required for skin carcinogenesis by topical phorbol ester/DMBA treatment [2] or for transgene-induced mouse high-grade ovarian cancer initiation [17]. Together these data indicate that, at least in mice, *Cip2a* has tissue- or tumor type-selective roles in tumor initiation.

Mammary tumors induced by DMBA were found to be of basaloid origin, indicating, together with clinical data that, CIP2A plays a selective role as a driver of BL-TNBC [2]; a breast cancer subtype for which there are no previously known drivers [33]. The best-known mechanism by which CIP2A serves as an oncoprotein is by direct interaction with the B56 family of B subunits of PP2A [26,27], whereby inhibition of PP2A-B56 is tumor-suppressive [34,35] (Box 1). Based on emerging evidence from CIP2A structural studies, a single amino acid point mutation (K21A) in the N-terminal head domain of CIP2A diminished PP2A-B56 binding and ultimately prevented tumor growth in a TNBC xenograft model [26]. Interestingly, this mutation did not impact on cell proliferation but affected anchorage-independent growth, demonstrating that it selectively impaired true malignant growth characteristics.

In the context of *Cip2a* knockout mouse studies [2,17], these results raise an interesting question about the role of PP2A-dependent tumor initiation and progression. A rarely considered notion is that, although PP2A inhibition is essential for neoplastic transformation of human cells, it is not required for mouse cell transformation [36,37]. Indeed, this species-specific requirement for PP2A inhibition could explain why most tested mouse tumor types [2,17] were not dependent on *Cip2a*-mediated PP2A inhibition. The reason why mouse BLBCs were *Cip2a*-dependent could be because these tumors are initiated by the DNA-damaging agent DMBA, and CIP2A interacts with the DDR protein TopBP1 in a PP2A-independent manner [2–4]. However, human tumors may still be fully dependent on CIP2A-mediated PP2A inhibition as it is one of the molecular

Obligate homodimer: a situation in which two monomers of the same protein bind to each other as a dimer, that is required for the proper function or for example stability of the protein.
Phosphoproteome: a snapshot of the proteins/amino acids that are phosphorylated at a particular time or in response to a given manipulation, with the limitation that only a fraction of phosphosites are generally detected. Usually refers to mass spectrometry-based proteomic analysis of cells or tissues.
Synthetic lethal: a condition in which simultaneous cotargeting of two genes or functions is lethal, whereas separate targeting of either is not deleterious. An example of this is the targeting of alternative DNA repair mechanism in BRCA mutant cells.

Box 1. Protein phosphatase 2A and its regulation by CIP2A

Phosphorylation is the most prevalent post-translational protein modification, and the phosphorylation balance determined by kinases and phosphatases controls all cellular functions including the DDR and mitosis [49,50,71]. Protein phosphatase 2A (PP2A) is a serine/threonine phosphatase complex which functions as a trimeric complex of the scaffolding A subunit, the catalytic C subunit, and one of the various substrate-determining B subunits (Figure IA) [36]. The B subunits are highly conserved and comprise four families. As there are also two A and two C subunits, there can be ~70 different ABC trimers that each have different properties, regulation, and substrates. Of the B subunit families, B56 proteins are in most cases tumor-suppressive, in other words they inhibit cancer development and progression by dephosphorylating their phosphoprotein (p-protein) substrates (Figure IB) [36,37]. PP2A is a human-relevant tumor suppressor as its inhibition is essential for neoplastic transformation of human cells but not of mouse cells [38,39]. Another peculiarity among the tumor suppressors is that PP2A is seldom lost or inhibited genetically in human cancers but is instead inhibited by nongenetic mechanisms such as interaction with CIP2A that is widely overexpressed by human cancers [1,9,36,37]. CIP2A inhibits PP2A-B56 function by a unique 'hijack and mute' mechanism involving radical changes in PP2A complex composition (Figure IB). Direct interaction between CIP2A and B56 is mediated by the N terminal head domain of CIP2A [27]. When CIP2A binds to B56 it initiates a cascade in which a scaffolding A subunit is released from the PP2A complex, and CIP2A instead functions as a pseudo-A subunit by interacting with the C subunit (Figure IB). In this CIP2A-B56-C complex CIP2A also interacts with the B56 protein region that B56 uses for substrate phosphoprotein recognition [27]. Thereby CIP2A 'hijacks' the PP2A complex and 'mutes' its function towards oncogenic phosphoproteins (Figure IB).

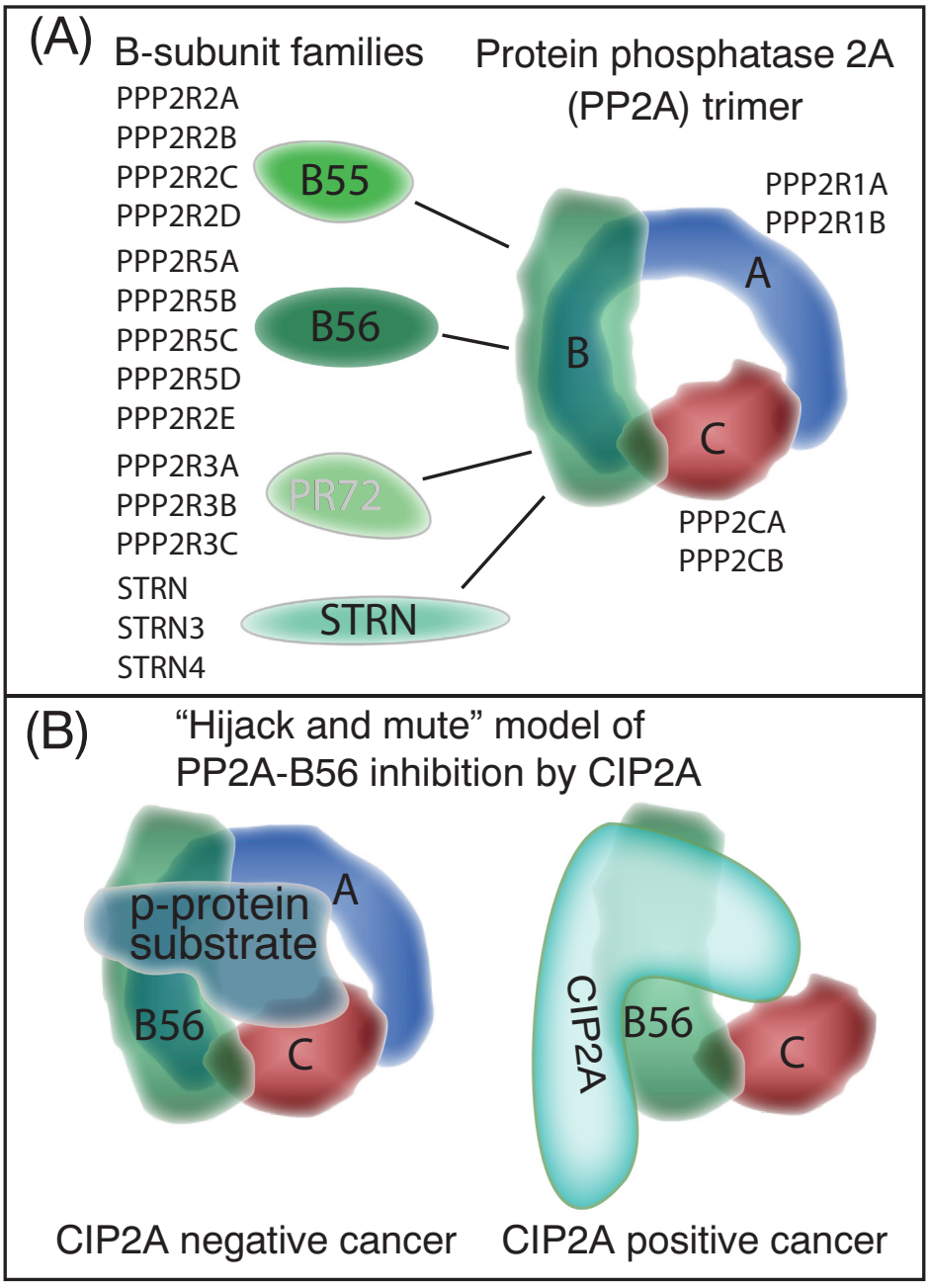

Figure I. Protein phosphatase 2A (PP2A) structure and regulation by cancerous inhibitor of PP2A (CIP2A). (A) PP2A functions as a heterotrimer between the scaffolding A subunit, the catalytic C subunit, and the regulatory B subunit. Both the A and C subunits are encoded by two highly homologous genes whereas the 15 B subunits are structurally divided into four families (B55, B56, PR72, and STRN) whose members are structurally and functionally closely related. The gene names of individual subunits are listed. Collectively, 60 different PP2A trimers can be constituted from the subunits, each potentially having different biochemical properties and roles in cancer. The expression of B subunits varies in a tissue-specific manner, and they can also have distinct subcellular localizations. (B) Schematic presentation of the inhibition of the PP2A complex containing B56 subunit (PP2A-B56) by CIP2A by the hijack and mute mechanism. In normal cells with low CIP2A expression, trimeric PP2A-B56 binds to its phosphorylated substrate protein (p-protein substrate) via a specific LxxIxE groove region, resulting in substrate dephosphorylation. In cancer cells with high CIP2A expression, CIP2A binding to the PP2A-B56 complex results in expulsion of A and the formation of a CIP2A-B56-PP2Ac trimer (hijack). In this alternative trimer CIP2A shields the LxxIxE groove from B56 substrates (mute) and thereby the p-protein substrate remains phosphorylated. Binding of CIP2A to B56 further protects CIP2A protein from degradation.

requirements for human tumorigenesis [36,37], and the only known molecular alteration in the tumor growth-deficient K21A mutant of CIP2A is its inability to bind to PP2A-B56 [26].

Novel experimental approaches to target CIP2A in cancer have recently been reported. Consistent with the finding that CIP2A interacts with TopBP1 and is **synthetic lethal** with **BRCA mutation** [2–4], ectopic expression of a TopBP1 **dominant negative** construct (that inhibits the CIP2A-

TopBP1 interaction) decreased BRCA mutant tumor growth *in vivo* [3,7]. In addition, **FKBP12 degron**-based depletion of CIP2A in mitosis was shown to efficiently kill cancer cells [6,7]. Furthermore, overexpression of the long noncoding RNA (lncRNA) *LINC00665*, which encodes an endogenous CIP2A inhibitor peptide, released CIP2A from the PP2A-B56 complex and resulted in PP2A activation [38]. Functionally, this led to decreased migration, invasion, and lung metastatic nodules in two TNBC metastatic mouse models. Finally, engineered tricyclic neuroleptic compounds that engage PP2A complexes at low micromolar concentrations [39,40] were recently identified as potent transcriptional CIP2A inhibitors [2]. Their growth inhibitory effects were at least partly mediated by CIP2A inhibition, and they inhibited the viability and tumor growth of several TNBC cell lines including patient-derived TNBC cancer stem cells resistant to standard chemotherapies [2].

CIP2A and the DDR

Eukaryotic cells have evolved a complex integrated and interconnected signaling cascade collectively known as the DDR which detects and repairs DNA damage [41]. The DDR also activates cell-cycle checkpoints that arrest cells and provides the time necessary to repair the damage before progressing further in the cell cycle. DDR mechanisms are becoming increasingly important targets for cancer therapies [41,42]

Until 2021 CIP2A was known only as an oncogenic inhibitor of PP2A, but the recent finding that CIP2A is a direct interaction partner for the central DDR scaffold protein TopBP1 changed that

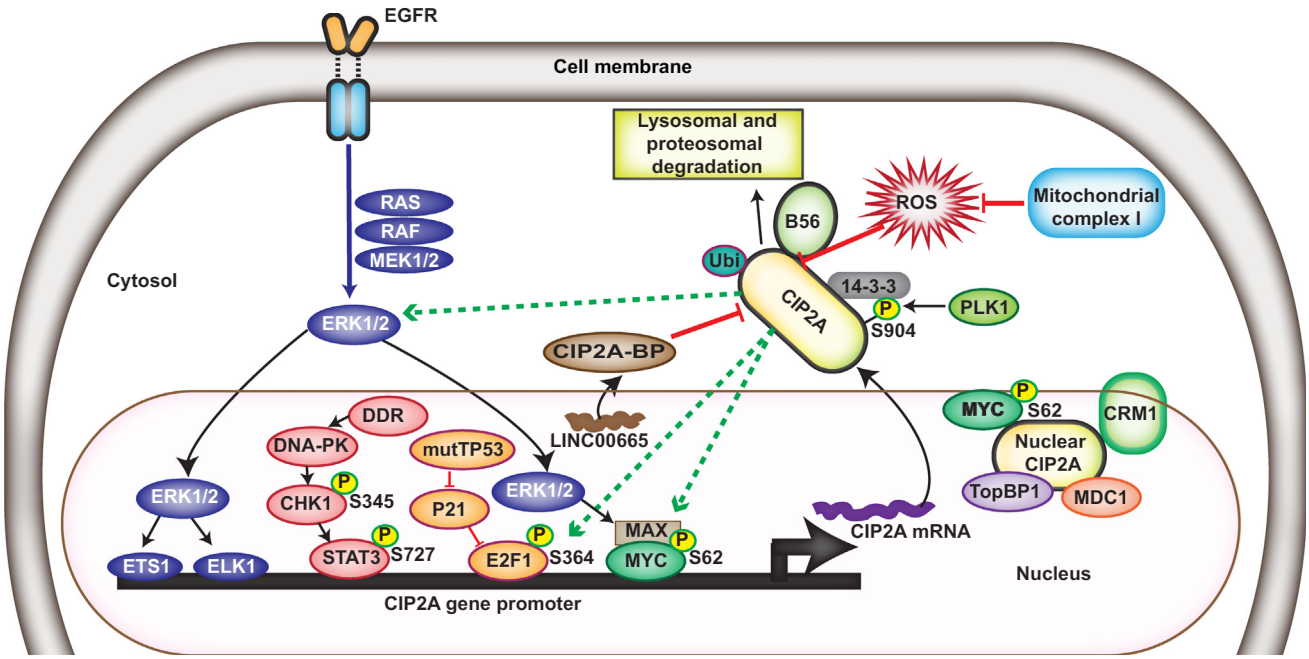

Figure 1. Transcriptional and post-translational regulation of CIP2A. *CIP2A* transcription is driven by three main signaling pathways: (i) the EGFR-activated MAPK pathway leading to MYC-MAX or ETS1/ELK1 activity (blue), (ii) the constitutive DNA damage response (DDR) via the DNA-PK-CHK-STAT3 axis (pink), and (iii) increased E2F1 activity downstream of mutated TP53 (orange). The broken green arrows indicate the positive feedback loops of CIP2A regulation. Post-translationally CIP2A is negatively regulated by the CIP2A-binding peptide (CIP2A-BP) which is encoded by long noncoding RNA LINC00665. Conversely, CIP2A protein stability is increased by an interaction with PP2A B56 subunits, but this interaction is antagonized by excessive ROS resulting from inhibition of mitochondrial complex I. In response to inhibition of the CIP2A-B56 interaction, CIP2A is ubiquitinated and degraded by both lysosomes and proteasomes. CIP2A is phosphorylated by PLK1 kinase and this increases 14-3-3 binding. In the nucleus, CIP2A interacts with TopBP1, MYC, and MDC1. In interphase cells most CIP2A protein is exported to the cytoplasm by CRM1. Abbreviations: CIP2A, cancerous inhibitor of PP2A; CRM1, chromosome region maintenance 1; P, phosphorylation; MAPK, mitogen activated protein kinase; PLK1, Polo-like kinase 1; PP2A, protein phosphatase 2A; ROS, reactive oxygen species; TopBP1, topoisomerase II-binding protein 1; Ubi, ubiquitin.

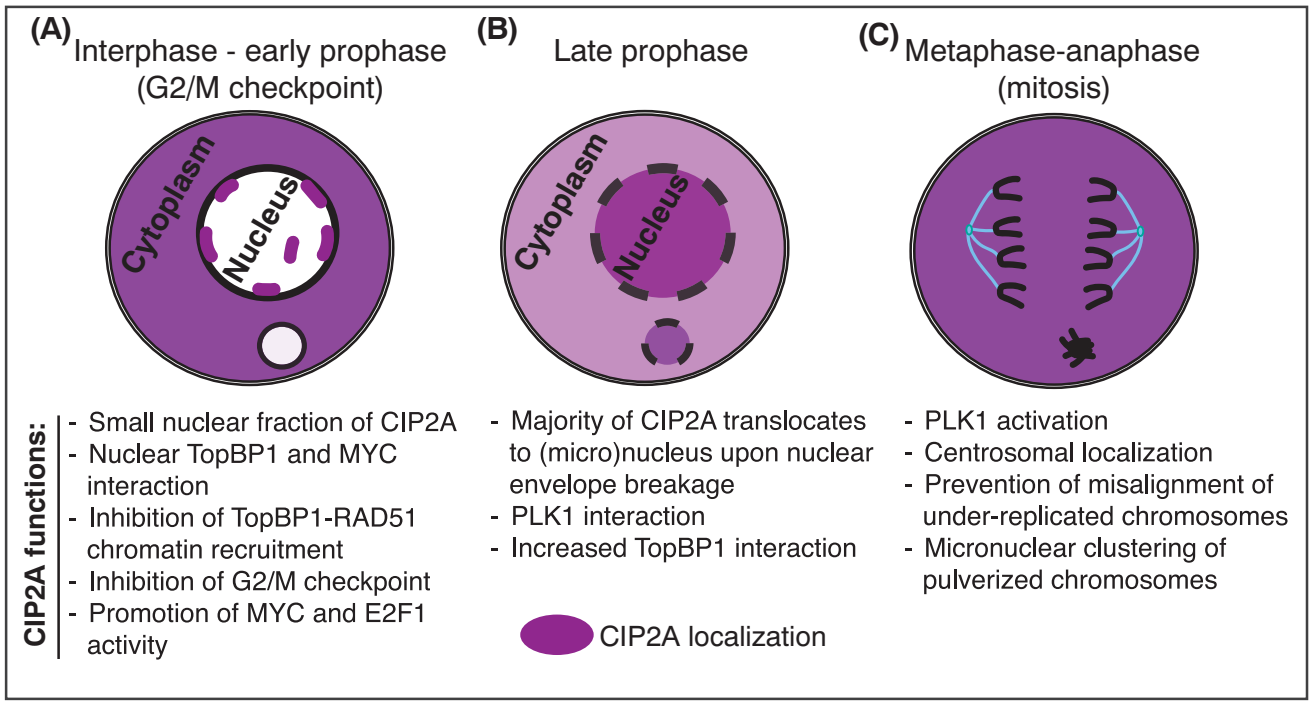

Figure 2. Cytoplasmic–nuclear shuttling of CIP2A during the cell cycle. The intensity of the purple coloration is an estimate of the proportional location of CIP2A between cytoplasm and nucleus during the indicated cell-cycle and mitotic phases. The nuclear functions of CIP2A in each of these phases are listed below each cartoon. In interphase cells (A), the majority of CIP2A resides in the cytoplasm, but there is a small and functionally important nuclear fraction as well. This interphase nuclear CIP2A resides in a complex with serine 62-phosphorylated MYC and is physically associated with the proteinaceous compartment of the nuclear envelope. Beginning from nuclear envelope breakage at late prophase (B), more CIP2A localizes to the nucleus, resulting in increased interaction with ToPBP1 and interaction with PLK1. In mitosis (C) there is no nuclear envelope, and CIP2A is fully associated with nuclear structures that are necessary for chromosome segregation and for DNA repair of mitotic chromosomes. Abbreviations: CIP2A, cancerous inhibitor of PP2A; PLK1, Polo-like kinase 1; TopBP1, topoisomerase II-binding protein 1.

view [2]. Further, in DepMap cancer cell gene essentiality screening data [43], *CIP2A* was identified as the closest functional homolog of *TOPBP1*, and was found to be functionally codependent with many other DDR genes such as *RHNO1*, *H2AX*, *MDC1*, *POLQ*, and *NBN* [2]. In addition, there is significant coexpression correlation between *CIP2A*, *TOPBP1*, and *POLQ*. Functionally, CIP2A was found to be required for DNA damage-induced mouse mammary tumorigenesis, as well as for the survival of BRCA-deficient breast cancer cells [2]. Further, CIP2A was essential for the survival of HAP1 cells under repeated low-dose irradiation. The finding that *CIP2A* is an essential gene in HR-deficient (HRD) (e.g., BRCA mutant) and/or DNA-damaged cells was consistent with the results of CRISPR/Cas9 screens where *CIP2A* was identified among the genes whose loss sensitized cancer cells to inhibition of TopBP1 effector kinase ATR and treatment with genotoxins [44,45].

These results were soon confirmed when Durocher and colleagues also found direct a CIP2A-TopBP1 interaction and confirmed the essential role of CIP2A in HRD cells [3]. Further, MDC1 was shown to be a part of the CIP2A-TopBP1 complex, and CIP2A inhibition impaired the survival of irradiated cells [4]. However, there were significant differences in the design of the studies. Whereas Laine *et al*. focused on the role of the CIP2A-TopBP1 interaction in continuously cycling interphase cells, especially at the G2/M checkpoint [2], the two follow-up studies focused on the role of the CIP2A-TopBP1 interaction only in mitotic cells [3,4]. In DNA-damaged interphase MCF10A cells, CIP2A prevented TopBP1 chromatin recruitment [2], but in mitotic cells CIP2A

and TopBP1 were dependent on each other for recruitment to the DNA damage-induced foci [3,4]. This may also explain the controversy regarding the role of CIP2A in ATR activation which was not observed in mitotic cells. The study by Adam *et al.* also supported the original *in vivo* observation by Laine *et al*. that CIP2A deficiency does not *per se* increase the amount of DNA mutations in replicating cells [2,3]. Notably, although the exact molecular mechanism underlying the essentiality of CIP2A in HRD-deficient cells remains unresolved, these studies make interesting observations. First, the CIP2A-dependent survival of BRCA mutant cells was not dependent on PARP or APEX2, but was linked to the decrease in chromosomal misarrangements and the appearance of micronuclei [3]. The CIP2A-dependency of HRD cells was also linked to replication stress, which together with earlier screening results [45] suggested that mitotic targeting of CIP2A in combination with replication stressors such as ATR inhibitors could be a novel therapy strategy for HRD cancer cells.

Importantly, all three studies concluded that the direct CIP2A interaction domain of TopBP1 is located at amino acids 829–853 that lie between the BRCT (BRCA1 C-terminus) domains 5 and 6 of TopBP1 [2–4]. Furthermore, the CIP2A domain that interacts with TopBP1 of CIP2A was the N-terminal 1–560 region [3]. In interphase cells, the interaction between CIP2A and TopBP1 was enhanced in the presence of the TopBP1 ATR activation domain (AAD) [2], a finding that was subsequently verified [4]. Indeed, CIP2A depletion in the nontransformed basal epithelial MCF10A cell line increased phosphorylated ATR, indicating a direct role of CIP2A binding to TopBP1 in DDR signaling [2]. In addition, levels of **γ-H2AX**, a target of phosphorylated ATR, were increased in CIP2A-depleted cells overexpressing a TopBP1 AAD mutant [2]. In addition, in mitotic cells CIP2A and TopBP1 resided in a stable complex, but DNA damage increased tertiary MDC1-CIP2A-TopBP1 interaction which was important for recruitment to DNA damage foci [4]. Furthermore, inhibition of the CIP2A-TopBP1 interaction was sufficient to induce synthetic lethality in BRCA mutant cells [3], and transcriptional inhibition of *CIP2A* was lethal for human BRCA mutant TNBC cells both *in vitro* and *in vivo* [2].

Although these studies provide the first direct indications that CIP2A is centrally involved in the DDR, previous *in vivo* studies indirectly support these conclusions. First, *Cip2a* is expressed in mouse intestinal stem cells, and *Cip2a*-deficient intestine tissue is severely compromised in regeneration following γ-irradiation- or cisplatin-induced DNA damage [19]. Second, similarly to *Oct4* (encoding a *bona fide* stem cell factor involved in radioresistance), *Cip2a* was also found to be expressed in a radioresistant population of testicular cells based on an *in vivo* irradiation screen [46]. Last, as high CIP2A expression is a prognostic factor for several solid cancer types [8,10,14], and most patients are treated with DNA-damaging irradiation or chemotherapies, these findings provide indirect support for the clinical relevance of CIP2A in influencing the response to DNA-damaging therapies.

CIP2A in mitosis and meiosis

Increased mitotic activity is one of the most common characteristics of cancer cells. In the context of CIP2A as a critical regulator of cancer cell phosphoproteomes via PP2A inhibition [15,26], mitotic activity is tightly controlled by the balance of serine/threonine kinase and phosphatase activities [47,48]. Current data indicate that CIP2A controls the mitotic progression of precancerous and cancer cells at the G2/M checkpoint and at mitosis, and also guards DNA fidelity during mitosis (Figure 2).

Independent lines of evidence demonstrate that CIP2A overexpression dampens the DNA damage-induced G2/M checkpoint, and thereby allows progression of DNA-damaged cells into mitosis [2,31]. Mechanistically, the role of CIP2A in regulating DNA damage-induced G2/M arrest can

be linked to its newly identified direct interaction with TopBP1. CIP2A silencing increased the accumulation of TopBP1 at DNA double-strand breaks (DSBs) [2]. Mouse mammary epithelial cells (MMECs) from *Cip2a* knockout mice also formed more RAD51 foci upon irradiation compared with wild-type cells. CIP2A also dampened radiation-induced γ-H2AX induction [2]. Based on previous data that chromatin recruitment of the TopBP1-RAD51 complex is important for G2/M checkpoint activity [49–51], CIP2A-mediated inhibition of TopBP1 and RAD51 chromatin recruitment provides a mechanism for dampening the G2/M checkpoint in CIP2A-positive cells. These earlier results are supported by a recent study demonstrating that TopBP1 is recruited to DNA DSBs together with RAD51 in G2 cells, and that TopBP1 inhibition resulted in inhibition of HR efficiency [52]. Further, as chromatin recruitment of TopBP1 is necessary for ATR activation [49], CIP2A-mediated inhibition of the TopBP1-RAD51 complex may coordinate both G2/M checkpoint and DDR signaling in interphase cells. In addition, nuclear CIP2A promotes MYC activity in interphase cells [19]; because MYC has a critical role in G2/M progression [53], this could be a further mechanism underlying how high CIP2A expression promotes the transition of G2 phase cells to mitosis.

At the onset of mitosis, CIP2A interacts with PLK1 to protect this critical mitotic kinase from degradation [31]. Upon nuclear membrane breakage at mitosis, CIP2A locates to the nucleus where it is found at kinetochores (Figure 2). PLK1 and PP2A-B56 are localized at kinetochores at the spindle assembly checkpoint [31,54,55]. Although PP2A-B56 (together with protein phosphatase 1, PP1) is responsible for removing PLK1 from the BUB complex, and thus allows the pulling apart of the sister chromatids in anaphase [55], the PLK1-CIP2A interaction was also shown to be decreased at anaphase [31]. Based on these results, the most likely model for how CIP2A is involved in mitosis progression is through coordinated CIP2A-mediated inhibition of PP2A-B56 and stabilization of PLK1, until CIP2A is released from PLK1 (by a so far unknown mechanism) to allow chromosome segregation. An important role for CIP2A in mitosis was also indicated by another study in which CIP2A inhibition led to aberrations in centrosome separation through inhibition of NIMA-related kinase 2 activity [56]. The CIP2A-PLK1 complex also controls meiosis [57]. Further, as meiotic recombination is very dependent on active DNA repair mechanisms in which TopBP1, RAD51, and other CIP2A-codependent DDR proteins are involved, the lessons learned from mitotic CIP2A functions may be well applicable to meiosis (and vice versa). Indeed, it was recently demonstrated that repair of meiotic DNA damage involves the CIP2A-MDC1-TopBP1 complex [5]. The importance of CIP2A in meiosis may also help to explain why it is important for spermatogenesis [18,58].

CIP2A protects genome stability during mitosis

The recent CIP2A-TopBP1 studies focusing on mitotic HRD cells revealed an important role for CIP2A in protecting genome stability and in the proper segregation of chromosomes after DSBs that arise in mitosis [3,4,59]. In response to DNA damage, CIP2A, MDC1, and TOPBP1 colocalized at γ-H2AX-positive foci in mitotic cells, whereas in nondamaged mitotic cells CIP2A was centromeric [3,4]. Similarly to what was shown previously for TopBP1 [60], CIP2A was found to protect the under-replicated chromosomes and prevent misalignment of chromosomes in cells that were DNA-damaged during mitosis [3,4,59]. Furthermore, in some cancers, complex genome rearrangements can result from mitotic pulverization of mis-segregated chromosomes [6]. However, in ~5% of cancers [6], these pulverized and acentric DNA pieces are gathered together and encapsulated into micronuclei with a nuclear envelope [61] (Figure 2). In the absence of gene copy-number changes, this process is called balanced chromothripsis, but it was not understood how the pulverized pieces of chromosomes could be tethered together and organized into micronuclei. Two recent studies discovered that the CIP2A-TopBP1 complex clusters the fragmented chromosomes together during mitosis and determines their localization to micronuclei that are inherited by a single daughter cell [6,7]. The molecular details of these

findings are well summarized in a recent review [61]. From the cancer biology point of view it is important to note that this mechanism is not only relevant to genome integrity but potentially also to tumor immunology as it prevents cytosolic exposure of naked DNA fragments and blunts **cGAS-STING** activation that is crucial for activating immune attack against cancer cells [62]. Finally, the clinical relevance of nuclear CIP2A has not been addressed widely, but it is intriguing that, in ovarian cancers in which cytoplasmic CIP2A is a marker of poor prognosis, nuclear CIP2A predicts better patient overall survival [13].

CIP2A coordinates oncogenic phosphosignaling, mitosis, and DDR

The oncogenic role of CIP2A has been connected to its role in promoting the phosphorylation of oncogenic transcription factors such as MYC or E2F1, or of oncogenic pathways such as ERK, AKT, and NF-κB [2,9,15,17,19]. However, recent data have revealed that MYC regulation cannot comprehensively explain why CIP2A is particularly essential for the survival of HRD cancer cells, or its role in mammary tumorigenesis in mice [2]. Therefore, it is essential to comprehensively understand the CIP2A-regulated target proteins and processes in cancer cells. A recent mass spectrometry proteomic analysis revealed that CIP2A regulates the phosphorylation of >100 proteins involved in the cytoskeleton, nuclear envelope, RNA splicing, epigenetics, and gene expression [15,63]. Interestingly, the phosphorylation site in MYC that is mostly associated with CIP2A activity [9,19], the serine 62, was dephosphorylated by CIP2A depletion in the dataset, although it was far less impacted than many other unstudied CIP2A target phosphosites [15]. For example, CIP2A prevents the dephosphorylation of negative transcription elongation factor NELF-A, and thereby promotes gene expression and cancer progression [64]. CIP2A was also found to regulate the phosphorylation of some DDR proteins [2,15], but the functional relevance of this remains to be determined.

Therefore, we postulate that CIP2A is a powerful human oncoprotein through its role at the crossroads of oncogenic phosphorylation (beyond MYC and the other usual suspects), mitosis, and the DDR (Figure 3, Key figure). This coordination function might be particularly relevant for cancer types such as BL-TNBC that are dependent on both high proliferation and DNA repair activities [33]. Indeed, CIP2A expression is driven by both EGFR activity and TP53 mutations, which are both hallmarks of BL-TNBC. CIP2A is also positively regulated by constitutive DDR and mitochondrial complex I activities in HRD cancer cells [23,29,33,65] (Figure 3). In these HRD BL-TNBC cells, CIP2A promotes MYC and E2F1 activity [2,19,21], blunts DNA damage-induced G2/M checkpoint activation [2,31], and safeguards chromosomal stability during mitosis [3,4]. This unique coordination ability of CIP2A (Figure 3) can therefore explain why its high expression correlates with poor patient prognosis in BL-TNBC [2].

Another scenario where the role of CIP2A as a molecular coordinator of phosphosignaling and DNA integrity could be important is in mitosis and in preventing chromosome shattering in micronuclei. Both processes are initiated by rearrangements of Lamin family proteins in the (micro)nuclear envelope, followed by nuclear entry of the CIP2A-TopBP1 complex (Figure 2). CIP2A and PP2A are important regulators of Lamin protein phosphorylation and nuclear envelope function [15,66], and it is therefore possible that CIP2A-mediated PP2A inhibition is functionally involved in ensuring micronuclear envelope permeability for the CIP2A-TopBP1 complex.

Current data indicate that PP2A activity does not regulate direct CIP2A-TopBP1 protein interaction [3,4]. However, except for physical scattering of pulverized chromosomes by the TopPB1-CIP2A complex [6,7], there is currently no convincing functional evidence to exclude the involvement of PP2A regulation in various phenotypes linked to the CIP2A-TopBP1 complex. PP2A complexes are known to regulate many aspects of the DDR. The CIP2A-regulated PP2A-B56 complex is involved in PALB2-dependent replacement of RPA by RAD51 on end-resected

Key Figure

CIP2A coordinates the hallmarks of HRD cancers

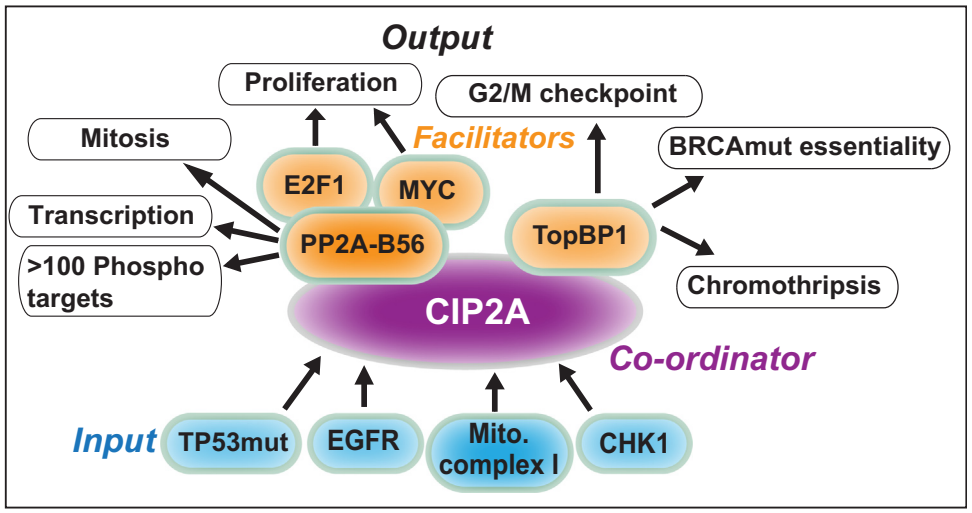

Figure 3.

For a Figure360 author presentation of Figure 3, see https://doi.org/10.1016/j.trecan.2023.09.001

CIP2A coordinately regulates the phosphoproteome, mitosis, proliferative MYC and E2F1 activity, the G2/M checkpoint, and the survival of BRCA mutant (mut) and HRD cells, as well as genomic integrity (including chromothripsis) (Outputs). Many of these properties are also hallmarks of HRD cancers (including BL-TNBC). Mechanisms that activate CIP2A expression also constitute hallmarks of HRD cancers [TP53 inhibition, constitutive CHK1 activity, increased oxidative phosphorylation by mitochondrial (Mito.) complex I] (Inputs). The overexpressed CIP2A functions as a coordinator to control the functional hallmarks of HRD cancers (Outputs). Whereas many of the output functions driven by CIP2A are mediated by inhibition of PP2A phosphatase activity, its involvement in the G2/M checkpoint, BRCA mutant cell essentiality, and chromosomal integrity is facilitated by its direct interaction with TopBP1. Abbreviations: BL-TNBC, basal-like triple-negative breast cancer; CIP2A, cancerous inhibitor of PP2A; HRD, PP2A, protein phosphatase 2A; TopBP1, topoisomerase II-binding protein 1.

DNA during HR repair [67]. Further, although the specific PP2A complex was not identified, PP2A activity was shown to regulate the TopBP1 deubiquitinase OTUD6, thereby leading to ATR-mediated CHK1 phosphorylation and radiation resistance [68]. Furthermore, phosphorylation of one of the critical DDR proteins in the TopBP1 complex, NBN, is regulated by CIP2A [2,15], and CDK2-mediated phosphorylation of NBN promotes survival following DNA damage [69]. In addition, it is well established that DDR is characterized by massive phosphorylation changes in the DNA repair and checkpoint complexes [47], including the TopBP1 complex [70]. Linking this to PP2A, both the G2/M checkpoint and various DNA repair [BRCA1, HR, and non-homologous end joining (NHEJ)] pathways were among the most significantly regulated pathways by PP2A activation in a recent phosphoproteome study also employing CIP2A targeting [15]. Last, based on current understanding, the interaction with B56 proteins is essential for CIP2A protein stability [26], and therefore high expression of CIP2A in mitotic and DNA-damaged nuclei indicates that it is also associated with B56 in this situation. It is very interesting that the N-terminal portion of CIP2A mediates both PP2A [26] and TopBP1 [3] interaction, indicating that these molecular functions could interact with each other. Therefore, both the PP2A-dependent and -independent functions of the CIP2A-TopBP1 complex may cooperate in the response to DNA damage and/or in progression through mitosis.

Concluding remarks

The recent findings that CIP2A is functionally related to several *bona fide* DDR proteins, its role in the G2/M checkpoint, its essentiality for HRD cancer cells, and its role in chromothripsis have opened up entirely new directions for cancer research. Importantly, all the main findings of these studies have been validated by different groups. However, many issues remain unresolved (see Outstanding questions). One of the most obvious concerns the molecular explanation of why the CIP2A-TopBP1 complex is essential for HRD cancer. There are many *CIP2A*-codependent DDR genes other than *TOPBP1* that might provide an answer to this question. One such interesting prospect is POLQ, which is involved in **microhomology-mediated end-joining (MMEJ)**. It has been reported that HRD tumors overexpress and are highly dependent on POLQ [71]. The fact that *POLQ* and *CIP2A* are coexpressed in BLBC and are codependent genes [2] indicates that POLQ might be relevant for the essential role of CIP2A in HRD cells. This view is further enhanced by results that both CIP2A [31] and POLQ are phosphorylated by PLK1, and that POLQ phosphorylation is important for the repair of DNA DSBs in mitosis [72].

The recently discovered role of the CIP2A-TopBP1 complex during balanced chromothripsis [61] is exciting, and ~5% of cancer cases display this chromothripsis phenotype [6]. Furthermore, the essential role of the CIP2A-TopBP1 complex in HRD-deficient cancer cells [2,3] is expected to be relevant for up to 30% of cancers [73]. Finally, as PP2A inhibition is a prerequisite for the development of human cancers [36,37], and CIP2A is overexpressed in up to 70% of all cancer cases [8,9,14], these numbers together make it a universally relevant oncoprotein and a very promising therapeutic target across cancer types. This view is further enhanced because it is dispensable for normal development [18].

Outstanding questions

What are the molecular mechanisms underlying the essentiality of CIP2A in HRD cancers?

What is the relationship between the PP2A-dependent and -independent functions of CIP2A in cancer initiation, development, DDR, and therapy resistance?

About 30% of human cancers do not express CIP2A. Therefore, how do these cancers develop without the contribution of CIP2A to phosphorylation-dependent signaling and ensuring mitotic fidelity?

Phosphatases have recently become druggable, and CIP2A is an obvious future cancer therapy target protein. What are the most promising approaches to inhibit CIP2A function in cancer?

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Declaration of interests

J.W. is a consultant and scientific advisory board member of Anavo Therapeutics B.V., and a founder and board member of Thestra Oy. S.N. declares no conflicts of interests.

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