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Emerging roles of the CIP2A–TopBP1 complex in genome integrity

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Abstract

CIP2A is an inhibitor of the tumour suppressor protein phosphatase 2A. Recently, CIP2A was identified as a synthetic lethal interactor of BRCA1 and BRCA2 and a driver of basal-like breast cancers. In addition, a joint role of TopBP1 (topoisomerase IIβ-binding protein 1) and CIP2A for maintaining genome integrity during mitosis was discovered. TopBP1 has multiple functions as it is a scaffold for proteins involved in DNA replication, transcriptional regulation, cell cycle regulation and DNA repair. Here, we briefly review details of the CIP2A–TopBP1 interaction, its role in maintaining genome integrity, its involvement in cancer and its potential as a therapeutic target.

Graphical abstract

CIP2A-TopBP1 complex

interaction details

CIP2A
TopBP1

role in genome integrity and cancer

recruitment in mitosis
attachment
preservation of genome integrity

Introduction

Topoisomerase IIβ-binding protein 1 (TopBP1) is involved in multiple cellular processes, including DNA replication, transcriptional regulation, cell cycle regulation and DNA repair [reviewed in (1)]. Its central role in DNA repair is to activate the DNA damage response kinase ataxia-telangiectasia mutated and RAD3-related (ATR) at single-stranded DNA coated by replication protein A during S/G2 phase (2,3). Recruitment of TopBP1 to sites of DNA damage may be promoted by the MRE11–RAD50–NBS1 complex (2,4). Subsequently, TopBP1 may recruit the 9-1-1 DNA repair clamp followed by ATR, which is then activated by TopBP1 (5). TopBP1 also has a particular role in maintaining genome integrity during mitosis (6–8). When DNA double-strand breaks (DSBs) are induced in mitosis, TopBP1 is recruited to DNA damage sites marked by phosphorylated H2AX (γH2AX) and this recruitment relies on the presence of the protein MDC1 (9). Depletion of MDC1 or disruption of TopBP1 recruitment leads to persisting DNA damage and increased formation of micronuclei containing acentric chromatin fragments (9).

The best-characterized role of the cancerous inhibitor of protein phosphatase 2A (CIP2A) is endogenous inhibition of the serine/threonine phosphatase PP2A (10,11). PP2A regulates a large number of protein phosphosites in multiple critical cellular pathways, including the DNA damage response pathway. PP2A inhibition is a critical driving step for many cancers (12). Furthermore, CIP2A is also involved in multiple key oncogenic signalling pathways and is found to be highly expressed in various types of tumours (10,13–14). Recently, CIP2A was identified as a synthetic lethal interactor of BRCA1 and BRCA2 and simultaneously as a driver of basal-like breast cancers (BLBCs). In both studies, analysis of DepMap data...
identified TopBP1 co-dependency in cancers, which led to the discovery of a CIP2A–TopBP1 axis (15,16).

**Cellular localization of CIP2A and TOPBP1**

During interphase, TopBP1 is distributed ubiquitously in the nucleus, whereas CIP2A is primarily detectable in the cytoplasm. After nuclear envelope breakdown at the onset of mitosis, both proteins form distinct co-localizing foci at centrosomes (7,15,17–19). CIP2A–TopBP1 foci are also observed at sites of DNA damage, as induction of DSBs by ionizing radiation (IR) increases the number of CIP2A–TopBP1 foci, which can take the shape of filamentous structures. In addition, these IR-induced CIP2A–TopBP1 foci and filaments co-localize with γH2AX and MDC1 (9,15,19). Upon irradiation of RPE-1 cells, MDC1 was essential for the recruitment of TopBP1 and CIP2A to mitotic chromatin (19). However, in untreated, homologous repair (HR)-defective BRCA2−/− cells derived from the DLD-1 cell line, MDC1 knockout eradicated only 70% of CIP2A–TopBP1 foci (15). The remaining 30% of CIP2A–TopBP1 foci point to the existence of an alternative pathway for the recruitment of both proteins to DNA damage during mitosis. Indeed, replication stress induction by aphidicolin also led to an increase in co-localizing CIP2A–TopBP1 foci/filaments in an MDC1-independent manner (15). Furthermore, in contrast to CIP2A, MDC1 knockout in BRCA2−/− cells was not lethal (15). Most likely, there are two pathways for CIP2A and TopBP1 recruitment to sites of DNA damage, one being MDC1-dependent and the other by an unknown mechanism. This second recruitment pathway could be mediated by other proteins found to be co-dependent on TopBP1 or CIP2A in cancer cell lines, e.g. POLQ (POL) and RHINO (15,16,20). On the other hand, TopBP1 might also bind directly to chromatin, as it has been shown to bind to damaged DNA in vitro (21,22).

The filamentous structures are thought to be higher order assemblies of CIP2A and TopBP1, but the dependency of filamentous structures on the type of DNA damage and cell line is unclear. In untreated BRCA2−/− cells, CIP2A foci were found to be more prominent and were MDC1-dependent, whereas the more rarely observed CIP2A filamentous structures were MDC1-independent (15). The most striking case of filament formation (>90% of structures) was observed in untreated BRCA1-defective MDA-MB-436 breast cancer cells (15). Besides the molecular context, the resolution limits during microscopy might be an additional reason for the different levels of filaments reported in different studies. In irradiated U2OS cells, super-resolution imaging revealed ~65% of TopBP1 structures to be filamentous, whereas these were not as easily detectable with standard microscopy techniques (9).

**CIP2A and TOPBP1 complex formation**

TopBP1 and CIP2A foci overlap during mitosis, suggesting the formation of a complex. The goal of multiple studies has been to characterize a direct interaction between TopBP1 and CIP2A and pinpoint the sites in the two proteins that mediate this interaction. TopBP1 is a large scaffold protein containing nine BRCA1 C-terminal (BRCT) domains and an ATR activating domain (Figure 1A). Two independent yeast two-hybrid screens identified a region between BRCT3 and BRCT6 in TopBP1 at amino acids 829–853 to be crucial for its interaction with CIP2A (15,16). Further evidence of the importance of this region came through a LacR/LacO interaction assay in U2OS cells and the ability of the overexpressed HA-TopBP1 740–899 fragment to pull down CIP2A in HEK293T cells (15,19). A follow-up yeast two-hybrid screening of single residue alanine substitutions identified F837, D838, V839, L843 and L846 to be crucial (15). In TopBP1-depleted DLD-1 cells expressing a mutant with amino acid substitutions F837A, D838A and V839A, termed TopBP1Δ3, no IR-induced mitotic CIP2A or TopBP1Δ3 foci could be observed (15). Interestingly, deletion of TopBP1 amino acids 774–798 also led to a significant decrease of TopBP1 and CIP2A focus formation in mitotic U2OS cells after IR, suggesting the presence of a second important site for CIP2A interaction (19). Intriguingly, these two sites are highly conserved in vertebrates but lack any apparent structure (Figure 1A).

The exact location of the TopBP1 interaction site in CIP2A has not been identified, but multiple approaches have excluded the C-terminal coiled-coil domain (15,19). Using AlphaFold2 multimer complex prediction in ColaFold, we pinpoint a potential TopBP1 interaction site between amino acids 150–270 containing multiple highly conserved alpha-helices (Figure 1B and C) (23). It will be exciting to experimentally address the significance of this region in CIP2A for TopBP1 interaction.

Another critical question is how the interaction of TopBP1 with CIP2A is confined to mitosis. Interestingly, CIP2A has been identified as an exportin 1 (XPO1, also known as CRM1) target (24). XPO1 mediates the nuclear export of proteins containing leucine-rich NESs. Inhibiting the NES-dependent protein transport by XPO1 with leptomycin B or selinexor led to increased nuclear localization of CIP2A in interphase U2OS cells (19). Additionally, deletion of a predicted NES site in CIP2A (amino acids 561–625) increased nuclear localization in interphase RPE-1 cells but not to the extent of XPO1 inhibition, indicating the presence of additional NES sites in CIP2A (19). Importantly, irradiated U2OS interphase cells formed nuclear CIP2A foci after leptomycin B treatment that co-localized with TopBP1 (19). This suggests that the physical separation of CIP2A and TopBP1 outside of M phase prevents CIP2A–TopBP1 complex formation. There is also evidence of a mechanism that actively transports CIP2A to the nucleus before the onset of mitosis. In cancer cells, the endoplasmic reticulum protein LRRCS9 and CIP2A were reported to interact and accumulate at the nuclear membrane during G1/S (25). At G2/M, both proteins entered the nucleus and CIP2A formed the characteristic foci (25). Intriguingly, depletion of LRRCS9 in PC-3 cells disrupted the formation of nuclear CIP2A foci at G2/M. However, depletion of LRRCS9 led to a decrease in both nuclear and cytoplasmic CIP2A, indicating an overall decrease in CIP2A levels and potentially explaining the disruption of focus formation. Thus, it is possible that nuclear envelope breakdown during prophase is sufficient for CIP2A migration to mitotic chromatin. In support of this, TopBP1 and CIP2A were found to form a complex in interphase micronuclei (26).

CIP2A is known to dimerize and is speculated to oligomerize via its coiled-coil domain (27,28). TopBP1 is able to oligomerize and form condensates, which raises the question whether and how higher order assemblies of TopBP1 and CIP2A interact with each other and whether this could explain the formation of filamentous structures (29–31). Adam et al. (15) speculated that higher order assemblies are likely important for genome integrity, as deletion of the CIP2A coiled-coil
of dramatic TopBP1-depleted single-guide but endogenous supplementation of the ATR activating domain (AAD), nuclear localization signal (NLS) and the CIP2A interaction site. (B) Schematic of CIP2A domains with AlphaFold pLDDT prediction confidence score and conservation score per residue. ECRs derived from the conservation score are marked by black bars. Highlighted are BRCO-8, the ATR activating domain (AAD), nuclear localization signal (NLS) and the CIP2A interaction site. (C) Cartoon structure of ColabFold multimer prediction of complex formation between CIP2A and TopBP1. CIP2A is shown in yellow and TopBP1 in blue. The predicted interaction site is circled and CIP2A is coloured red.

Consequences of disturbing CIP2A–TopBP1 interaction during mitosis

In U2OS and RPE-1 cell lines, depletion of either TopBP1 or CIP2A led to the disappearance of all TopBP1 and CIP2A foci in mitosis (15,19). However, as both CIP2A and TopBP1 have important functions outside of mitosis in preserving genome integrity, only a few studies have managed to dissect the direct impact of disrupting the CIP2A–TopBP1 interaction during mitosis. In one case, auxin-induced degradation of TopBP1 during mitosis increased the number of chromosome gaps/breaks, induced micronucleation and increased the frequency of 53BP1 nuclear bodies in the next G1 phase, evidencing severe consequences for the daughter cells caused by unprocessed DNA damage (7). Another approach was to supplement a CIP2A binding site-mutated TopBP1 to cells lacking endogenous TopBP1. In this set-up, removal of endogenous TopBP1 caused severe growth defects in BRCA2/−/− cells but not in parental DLD-1 cells (15). Supplementation of single-guide RNA-resistant, interaction-defective TopBP1ΔA in TopBP1-depleted DLD-1 cells failed to rescue this and led to a dramatic increase in micronuclei, highlighting the importance of the CIP2A–TopBP1 interaction in HR-defective cells.

Interestingly, overexpression of a polypeptide consisting of TopBP1 1756–1960 fused to a destabilizing tag for controlled stabilization in DLD-1 cells led to complete loss of mitotic CIP2A foci, providing an alternative system to disrupt the CIP2A–TopBP1 interaction without depleting endogenous TopBP1 (15). In this set-up, the disruption of CIP2A–TopBP1 interaction caused a severe proliferation defect, dramatically increased micronucleation and led to the accumulation of lagging acentric chromosomes in BRCA2/−/− DLD-1 cells but not in parental DLD-1 cells. A similar growth defect and increase in micronuclei could be observed in the BRCA1-defective cell line MDA-MB-436 when expressing the polypeptide that disrupts CIP2A–TopBP1 interaction (15). In conclusion, the intact CIP2A–TopBP1 complex is essential for the survival of HR-defective cells.

The above approach also allowed to dissect whether the complex formation is essential for DNA damage arising during interphase. Disruption of CIP2A–TopBP1 in BRCA2/−/− cells released into mitosis after being held in G2 for an extended time led to no growth defects or abnormal micronucleation (15). This indicated that the cells had enough time during the arrest to repair DNA damage arising in S phase, e.g. by non-homologous end joining (33). As a result, the DNA lesions responsible for the increase in micronucleation observed in BRCA2-deficient cells lacking CIP2A–TopBP1 complex formation likely originated in interphase.

Together, these findings implicate the CIP2A–TopBP1 complex in tethering broken chromosomes during mitosis and consequently preventing missegregation. However, the question remains how frequently the tethering persists throughout mitosis. TopBP1 foci accumulate during nuclear envelope breakdown and gradually disappear during mitosis, which indicates repair of the tethered chromosomes by mitotic DNA repair pathways (7). Indeed, TopBP1 foci were found to
Furthermore, for side mitosis to co-localize disrupted, anaphase. DNA mechanism. Figure 4 decreased it mitotic lesions. TopBP1 was enriched by either MDC1 or a yet unknown alternative mechanism. The CIP2A–TopBP1 complex tethers broken chromosomes and mitotic DNA damage repair pathways are activated to resolve any remaining DNA lesions. HR-defective cells accumulate DNA lesions that remain unresolved until mitosis. Mitotic DNA repair is not capable of repairing all remaining DNA lesions. As a result, broken chromosomes are held together by CIP2A–TopBP1, which enables them to be pulled to centrosomes during anaphase. The breaks can then be resolved by HR-independent DNA repair pathways in the next G1 phase. If CIP2A–TopBP1 complex formation is disrupted, broken chromosome fragments disperse and are not pulled to the centrosomes. Consequently, they cannot be repaired by mitotic DNA repair pathways and cause loss of genetic material and micronucleation.

**Co-localization**

The role of the CIP2A–TOPBP1 complex in cancer

Two recent studies uncovered a role for the CIP2A–TopBP1 complex in chromothriptic events (26,28). Due to errors in mitosis, chromosomes can be missegregated and subsequently entrapped in micronuclei. After the micronuclei undergo imperfect DNA repair and replication, they can cause chromothripsis during the subsequent mitosis. Classical chromothripsis is characterized by the presence of pulverized chromosomes and DNA copy number losses, which are frequently observed in tumours and thought to drive the evolution of cancer cells. Intriguingly, it appears that so-called balanced chromothriptic...
events that do not lead to DNA copy number losses can also occur. Yet, such events can also be potent drivers of cancer as they lead to improved survivability, but similar to classical chromothripsis still cause rearrangements.

The CIP2A–TopBP1 complex tethers the micronuclear chromosome fragments during mitosis and thereby ensures asymmetric inheritance by a single daughter cell in the subsequent cell division [26,28]. Depletion of CIP2A or TopBP1 led to the dispersion of acentric micronuclear chromosome fragments that were then either evenly inherited by both daughter cells or misaccumulated as cytoplasmic DNA. This implicates the CIP2A–TopBP1 complex in safeguarding against classical chromothripsis, thus preventing the loss of genetic material. However, this mechanism does not guarantee complete prevention of DNA copy number loss, as balanced chromothrip-tic events are rather rare [28]. Nevertheless, disrupting the CIP2A–TopBP1 interaction could be a potential treatment option, as DNA copy number loss decreases the fitness of HR-deficient cancers.

Not only are CIP2A and TopBP1 co-dependent genes in cancer cell lines, but their expression levels are also highly correlated across thousands of tumour samples [28]. MDC1 expression also correlates with both CIP2A and TopBP1. Interestingly, TopBP1 and CIP2A expression are high in cancers with genomic rearrangements, including chromothripsis. However, chromothripsitic cancers with low TopBP1 and CIP2A expression are enriched in deletions, an indicator of classical chromothripsis [28]. In contrast, MDC1 expression positively correlates with cancers rich in deletions, which could again suggest that MDC1 is not solely responsible for CIP2A–TopBP1 tethering.

High expression of TopBP1 or CIP2A has also been linked to chemotherapy resistance [37–39]. Intriguingly, CIP2A and TopBP1 depletion in various cancers increases sensitivity to a number of chemotherapeutics. In particular, depletion of either CIP2A or TopBP1 in gastric cancers decreased oxaliplatin resistance [40,41]. Similarly, doxorubicin and cisplatin sensitivity was also increased in cancer cells upon knockdown of CIP2A or TopBP1 [38,39,42–44]. However, there is still no evidence suggesting that chemotherapy resistance relies on the CIP2A–TopBP1 axis.

Among different breast cancer types, both CIP2A and TopBP1 messenger RNA expression levels are highest in the basal subtype [16]. Higher expression of CIP2A can drive the initiation of BLBCs in mice and is associated with poor overall survival in basal-like triple-negative breast cancer. The breast cancer cell lines with the highest CIP2A dependency are mostly HR-defective [16].

Multiple studies explored whether PP2A inhibition by CIP2A is important for the CIP2A–TopBP1-mediated genome stability in HR-defective cells. Disruption of CIP2A binding to PP2A did not rescue increased micronucleation when disturbing CIP2A–TopBP1 interaction [15]. Furthermore, no interaction between PP2A and CIP2A–TopBP1 could be detected [15]. However, treating a patient-derived triple-negative BLBC xenograft with DT-061, a small-molecule activator of PP2A (SMAP), decreased the tumour volume significantly. Surprisingly, this drug led to a decrease in overall CIP2A protein levels, which could potentially also interrupt the CIP2A–TopBP1 complex formation, explaining the lethality of HR-defective cancer cells [16]. However, based on these results, it is still unclear whether PP2A inhibition plays a role in the CIP2A–TopBP1 interaction-mediated synthetic lethality in HR-defective cells. Nevertheless, SMAPs may serve as potent therapeutics for PARP-resistant cancers in the future. In a different approach, over-expression of the TopBP1[756–1000] polypeptide, which disrupts the CIP2A–TopBP1 complex in xenografted BRCA2-deficient tumours in a mouse model, efficiently inhibited tumour growth [15].

Altogether, the CIP2A–TopBP1 axis emerges as a promising target for future strategies for the treatment of HR-deficient cancers.

Data availability
No new data were generated or analysed in support of this research.

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Conflict of interest statement
None declared.

References
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