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Short communication

Pan-Cdk inhibitor ZK304709 suppresses Cdc20 expression and potentiates the anticancer activity of apcin in HeLa cervical cancer cells

XIANGYANG LE[1,2]
QINGSONG CHEN[1,3,4]
SHUYANG CAO[1,3,4]
GAOYUN HU[1,3,4]
QIANBIN LI[1,3,4]
ZHUO CHEN[1,3,4,*]

[1] *Department of Medicinal Chemistry, Xiangya School of Pharmaceutical Sciences, Central South University, Changsha 410013, Hunan, China*

[2] *Department of Pharmacy, Yiyang Central Hospital, Yiyang 413000 Hunan, China*

[3] *Hunan Key Laboratory of Diagnostic and Therapeutic Drug Research for Chronic Diseases Changsha 410013, Hunan, China*

[4] *Hunan Key Laboratory of Organ Fibrosis, Changsha 410013, Hunan China*

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ABSTRACT

Cell division cycle 20 homologue (Cdc20), a key regulator of the anaphase-promoting complex/cyclosome (APC/C), is frequently overexpressed in human cancers and represents a promising therapeutic target. However, monotherapy targeting Cdc20 has shown limited efficacy, partly due to compensatory activation of cyclin-dependent kinase 1 (Cdk1). In this study, we investigated the combinatorial potential of the pan-Cdk inhibitor ZK304709 with the Cdc20 inhibitor apcin in HeLa cervical cancer cells. Transcriptomic analysis revealed that both *CDC20* and *CDK1* are upregulated in cervical cancer tissues. Mechanistically, apcin treatment induced cyclin B1 accumulation and enhanced Cdk1 phosphorylation at Thr161, suggesting feedback activation. In contrast, ZK304709 reduced *p*-Cdk1(T161) levels and suppressed Cdc20 expression at both protein and mRNA levels. Functionally, the combination of apcin and ZK304709 synergistically inhibited cell proliferation and induced G2/M phase arrest in HeLa cells. These findings demonstrate that dual inhibition of Cdk1 and Cdc20 disrupts compensatory signalling pathways and enhances antitumour efficacy in HeLa cells, providing a rational strategy for combination therapy in cervical cancer.

Keywords: cell division cycle 20 homologue, cyclin-dependent kinase 1, combination chemotherapy, synergistic effects, antitumour activity

INTRODUCTION

Cell division cycle 20 homologue (Cdc20) is a key regulator of mitotic progression and functions as an essential co-activator of the anaphase-promoting complex/cyclosome (APC/C), an E3 ubiquitin ligase that mediates timely degradation of mitotic substrates (1, 2). Aberrant overexpression of Cdc20 has been observed across a wide range of malignancies and is closely associated with tumour progression and poor prognosis (3–5). Data from the Human Protein Atlas show that Cdc20 expression is largely undetectable in normal tissues but markedly elevated in various cancers (6), highlighting its potential as a therapeutic target. Pharmacologic inhibition of Cdc20 impairs APC/C function, leading to mitotic arrest and subsequent apoptosis (5, 7).

* Correspondence; e-mail: cz_job@csu.edu.cn

Apcin, the first small-molecule inhibitor of Cdc20, binds to the destruction-box (D-box) receptor site of its WD40 domain, thereby interfering with substrate recognition and subsequent ubiquitin-mediated degradation (8). However, apcin exhibits limited anticancer efficacy as a single agent, prompting efforts to optimise its structure or develop alternative targeting strategies (9–11). Notably, bifunctional agents, such as dual Cdc20/tubulin inhibitors and Cdc20-directed proteolysis-targeting chimaeras (PROTACs), have shown enhanced antitumour effects, highlighting the therapeutic value of Cdc20-centric combination approaches (9, 12, 13). These findings suggest that multi-targeted interventions may offer a more robust approach than Cdc20 monotherapy.

In this context, elucidating potential compensatory mechanisms underlying Cdc20 inhibition becomes crucial for guiding rational combination strategies. Cancer cells frequently evade targeted therapies through activation of compensatory signalling pathways that promote continued proliferation and survival (14, 15). Cyclin B1, a well-characterised substrate of APC/C^{Cdc20}, also serves as an essential regulatory subunit of cyclin-dependent kinase 1 (Cdk1) (16). Inhibition of Cdc20 leads to cyclin B1 accumulation, which can result in enhanced Cdk1 activation. Indeed, previous reports have demonstrated that Cdc20 depletion leads to compensatory upregulation of Cdk1 activity (17). This functional feedback underscores the potential of co-targeting Cdk1 and Cdc20 to improve therapeutic efficacy.

Among agents targeting Cdk1, BEY1107 is the only inhibitor that has progressed to phase I/II clinical trials (NCT05093907 and NCT03579836). Several other pan- or multi-Cdk inhibitors currently under investigation also exhibit potent Cdk1-inhibitory activity, though their clinical applicability is often constrained by dose-limiting toxicity (18, 19). Combining such inhibitors with other agents at subtoxic doses may enhance efficacy while minimising adverse effects. ZK304709, a pan-Cdk inhibitor originally developed for solid tumours, has demonstrated broad-spectrum antitumour activity *in vitro* and *in vivo* (20–22). While its clinical use is restricted by poor dose linearity beyond 90 mg (21, 23), low-dose combinations represent a rational strategy for repositioning this compound.

In cervical cancer, both Cdc20 and Cdk1 are markedly overexpressed compared with normal cervical tissues, and their aberrant activation has been implicated in tumour progression (4, 24). Pharmacologic inhibition of Cdc20, exemplified by apcin and its analogues, effectively suppresses cervical cancer cell growth, and blockade of Cdk1 induces G_2/M-phase arrest and apoptosis, highlighting their potential as therapeutic targets (12, 25). These findings collectively suggest that simultaneous inhibition of Cdc20 and Cdk1 may provide enhanced therapeutic benefits. Based on this rationale, the present study was designed to investigate the therapeutic potential of combining ZK304709 with the Cdc20 inhibitor apcin in HeLa cervical cancer cells. We aimed to determine whether co-inhibition of Cdk1 and Cdc20 could overcome compensatory survival mechanisms, enhance antitumour efficacy, and provide mechanistic insight into the interaction between these two mitotic regulators.

EXPERIMENTAL

Chemicals and reagents

Apcin and ZK304709, synthesised and characterised in-house, were used in this study. All other reagents were of analytical grade and obtained from reputable suppliers.

Bioinformatics analysis

The mRNA expression levels of CDC20 and CDK1 in various cancer types were analysed using the UALCAN online platform (http://ualcan.path.uab.edu).

Cell culture

The HeLa human cancer cell line was obtained from the American Type Culture Collection (ATCC) and cultured at the Experimental Center of Xiangya School of Pharmaceutical Sciences. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Thermo Fisher Scientific, USA) supplemented with 10 % fetal bovine serum (FBS, Gibco) and 1 % penicillin-streptomycin. All cultures were incubated at 37 °C in a 5 % CO_2-humidified atmosphere.

Cell counting kit-8 (CCK-8) assay

HeLa cells were seeded in 96-well plates at a density of 3,000 cells per well and treated with compounds at various concentrations for 48 h. Afterwards, the medium was replaced with 10 % CCK-8 solution (diluted in basal medium, TargetMol, China) and incubated for 0.5 h. Absorbance at 450 nm was measured using a Cytation™ 5 Cell Imaging Multi-Mode Reader (BioTek, USA).

Western blot analysis

HeLa cells were seeded into six-well plates and treated with compounds at different concentrations for 12 h. Total protein extraction was performed using radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime Biotechnology, China), and protein concentration was determined using the bicinchoninic acid (BCA) Protein Assay Kit (Beyotime Biotechnology). Proteins were separated *via* sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes. Blocking was done with 5 % skim milk in phosphate-buffered saline with Tween-20 (PBST) at room temperature for 2 h. The membranes were then incubated overnight at 4 °C with primary antibodies, including cyclin B1 (1:1000, Cell Signaling Technology, USA), Cdk1 (1:10000, Abcam, UK), *p*-Cdk1(T161) (1:1000, Abcam), Cdc20 (1:800, Abcam), and β-actin (1:5000, ZENBIO Biotechnology, China). Following washing, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Abbkine, China) for 2 h at room temperature. Protein bands were visualised using an enhanced chemiluminescence (ECL) detection kit and imaged with a Bio-Rad ChemiDoc XRS+ system.

5-Ethynyl-2'-deoxyuridine (EdU) assay

HeLa cells were seeded in 96-well plates at a density of 3,000 per well. After cell attachment, the cells were treated with the respective compounds and incubated for 48 h. EdU labelling was performed using the BeyoClick™ EdU Cell Proliferation Kit with Alexa Fluor 488 (Beyotime Biotechnology), followed by fixation, permeabilisation, click-iT reaction, and nuclear staining according to the manufacturer's protocol. Fluorescence imaging was performed using a Cytation™ 5 Cell Imaging Multi-Mode Reader. EdU-positive cells were quantified by calculating the proportion of EdU-positive nuclei relative to total nuclei (Hoechst staining) in randomly selected microscopic fields using ImageJ software.

Flow cytometry assay

HeLa cells were seeded in six-well plates and treated with compound-containing medium for 24 h. Cells were harvested using 0.25 % trypsin (EDTA-free), washed twice with cold phosphate-buffered saline (PBS), and fixed in 70 % ethanol for 12 h. After rinsing with PBS, cells were stained with propidium iodide (PI) using the Cell Cycle Staining Kit (Multi Sciences, China). Flow cytometry was performed using a CytoFLEX cytometer (Beckman Coulter, USA).

Real-time quantitative PCR (RT-qPCR)

HeLa cells were seeded in 6-well plates and treated with the indicated concentrations of compounds for 12 h. Total RNA was extracted using 1 mL of Trizol reagent (Beyotime Biotechnology) according to the manufacturer's instructions. Briefly, after lysis, chloroform was added for phase separation, followed by precipitation with isopropanol and washing with 75 % ethanol. The purified RNA was dissolved in RNase-free water, and the concentration and purity were determined spectrophotometrically. For reverse transcription, 500 ng of total RNA was converted to cDNA using a commercial reverse transcription kit (PrimeScript™ RT Master Mix, Takara, China) with the following conditions: 37 °C for 15 min and 85 °C for 5 s.

RT-qPCR was performed using TB Green® Premix Ex Taq™ II (Takara) on a Bio-Rad CFX96 Real-Time PCR system. Each 20 μL reaction contained 10 μL 2× SYBR Premix, 1 μL of each primer (10 μmol L^{-1}), 2 μL cDNA, and nuclease-free water to volume. The amplification protocol consisted of 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s, and a melting curve analysis from 65 to 95 °C to verify specificity. The expression level of CDC20 mRNA was quantified by RT-qPCR using the primers 5′-GCACAGTTCGCGTTCGAGA-3′ (forward) and 5′-CTGGATTTGCCAGGAGTTCGG-3′ (reverse). β-Actin was employed as the reference gene, with primers 5′-GCACAGAGCCTCGCCTTT-3′ (forward) and 5′-TACATGGCTGGGGTGTTGA-3′ (reverse). The relative expression of *CDC20* mRNA was calculated using the 2-ΔΔCt method.

Small interfering RNA (siRNA) transfection

HeLa cells were seeded in 6-well plates and transfected with Cdk1-specific siRNAs (si#1: 5′-GGAACTTCGTCATCCAAAT-3′, si#2: 5′-GTTATATCTCATCTTTGA-3′, si#3: 5′-GTACTGCAATTCGGGAAAT-3′) or a negative control siRNA according to the manufacturer's instructions (RIBOBIO, China). siRNAs and Lipofectamine™ 3000 (Invitrogen, Thermo Fisher Scientific) were separately diluted in 125 μL Opti-MEM (Gibco, USA), then combined, mixed gently, and incubated for 15 min at room temperature to allow complex formation. Prior to transfection, cells were washed with PBS and maintained in 1.5 mL antibiotic-free complete medium. The siRNA-lipid complexes (250 μL/well) were added dropwise, while blank control wells received Opti-MEM alone. After incubation, cells were harvested for Western blotting analysis.

Statistical analysis

Experimental data are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism 9.5 (GraphPad Software, USA). Group comparisons among more than two groups were conducted using one-way ANOVA followed by Tukey's *post hoc* test. Correlation between variables was assessed using simple

linear regression analysis. Statistical significance was defined as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), whereas $p \geq 0.05$ was considered not significant (ns).

RESULTS AND DISCUSSION

Overexpression of CDC20 and CDK1 mRNA in tumour tissues identified by TCGA data

Both Cdc20 and Cdk1 are critical oncogenic regulators that are frequently upregulated across various cancer types (26–28). Comprehensive analysis of mRNA expression data from The Cancer Genome Atlas (TCGA) revealed a consistent overexpression of both *CDC20* and *CDK1* in the majority of tumour samples compared to adjacent normal tissues. Notably, the mRNA levels of *CDC20* and *CDK1* were significantly higher in tumour tissues, with this upregulation being especially pronounced in cervical squamous cell carcinoma relative to adjacent normal tissues (Fig. 1a,b). These findings provide compelling

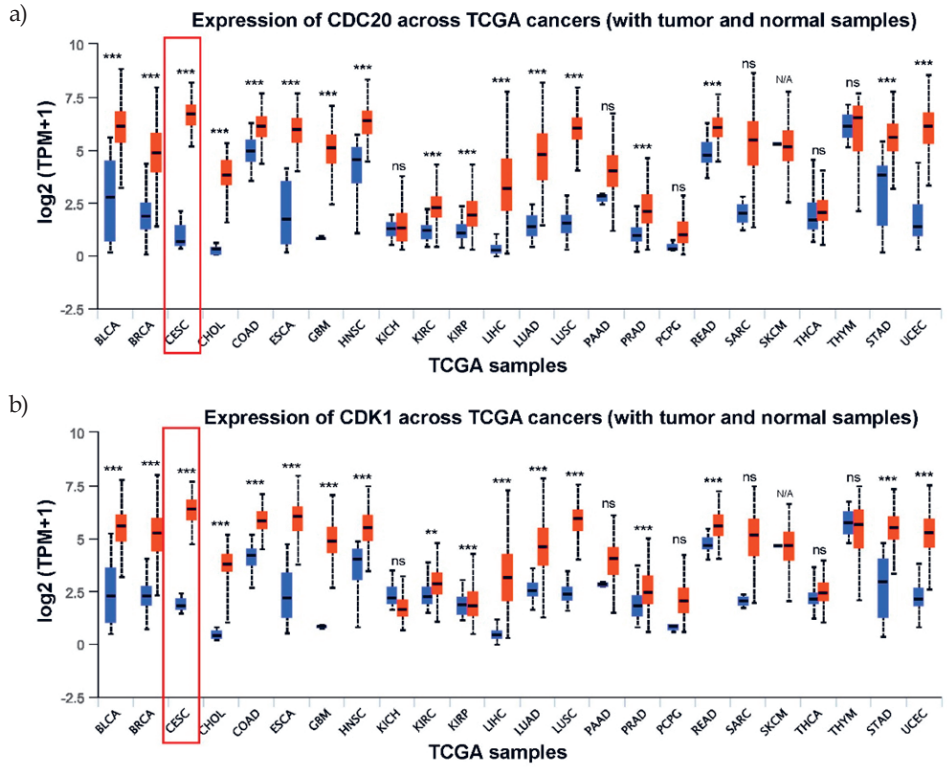

Fig. 1. Differential expression of *CDC20* and *CDK1* mRNA in cancer *vs.* normal tissues from TCGA data. a) *CDC20* and b) *CDK1* mRNA expression levels across various tumour types were analysed using the UALCAN online platform (http://ualcan.path.uab.edu) based on TCGA transcriptomic data. Blue boxes represent normal tissues, and red boxes represent tumour tissues. Statistical significance (**$p < 0.01$, ***$p < 0.001$; ns – not significant; N/A – not applicable) was calculated automatically by the UALCAN platform.

evidence that Cdc20 and Cdk1 could serve as potential therapeutic targets for cervical cancer, offering a promising avenue for targeted treatment strategies.

Cdc20 inhibitor apcin activates Cdk1 activity

Apcin, the first small-molecule inhibitor of Cdc20, demonstrated only modest antitumour efficacy in HeLa cells (Fig. 2a). Prior studies suggest that inhibition or silencing of Cdc20 may trigger compensatory activation of Cdk1, potentially limiting the effectiveness of Cdc20-targeted monotherapies (17). Given that Cdk1 activation is regulated by its association with cyclin B1 and specific phosphorylation events, including phosphorylation at Thr161 and dephosphorylation at Thr14 and Tyr15, we assessed whether apcin influenced these pathways. As shown in Fig. 2b, apcin treatment led to a dose-dependent accumulation of cyclin B1. Although total Cdk1 levels remained constant, apcin significantly increased phosphorylation at Thr161 (*p*-Cdk1(T161)), indicating enhanced Cdk1 activity (Fig. 2c). These results support the notion that apcin activates Cdk1 signalling in cervical cancer cells *via* cyclin B1 accumulation.

ZK304709 inhibits Cdk1 activity and downregulates Cdc20 expression

ZK304709 is a pan-Cdk inhibitor originally developed for solid tumours and has been reported to suppress Cdk1 activity in *vitro* (20–22). In our study, ZK304709 treatment did

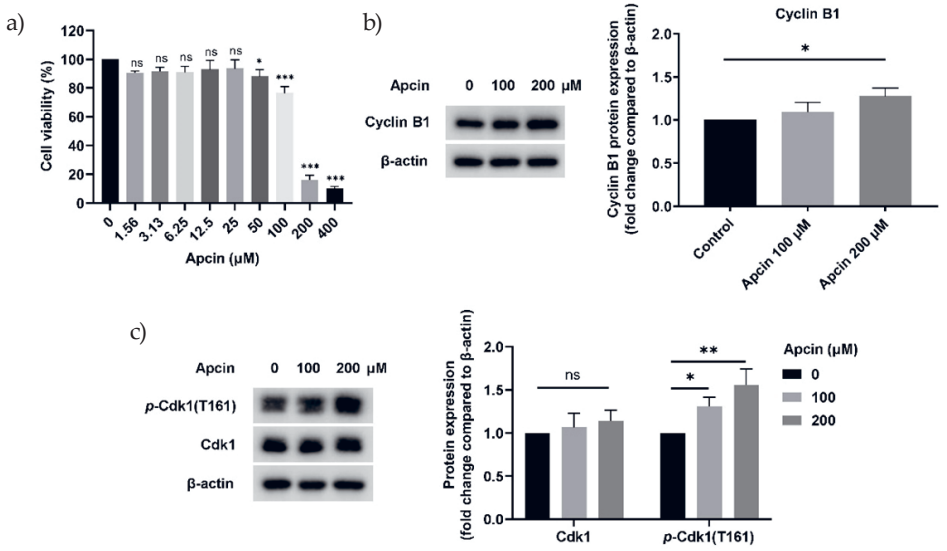

Fig. 2. Apcin treatment enhances Cdk1 activity in HeLa cells. a) Cell viability of HeLa cells treated with increasing concentrations of apcin for 48 h, as determined by the CCK-8 assay; b) Western blot analysis and densitometric quantification of cyclin B1 levels following 12 h apcin treatment; c) Western blot analysis and densitometric quantification of total Cdk1 and *p*-Cdk1(T161) after apcin treatment (0, 100, or 200 μmol L^{-1}, 12 h). Data are presented as mean ± SEM ($n = 3$). Statistical analysis was performed using one-way ANOVA followed by Tukey's *post hoc* test. *$p < 0.05$, **$p < 0.01$, ns – not significant *vs.* control (0 μmol L^{-1}).

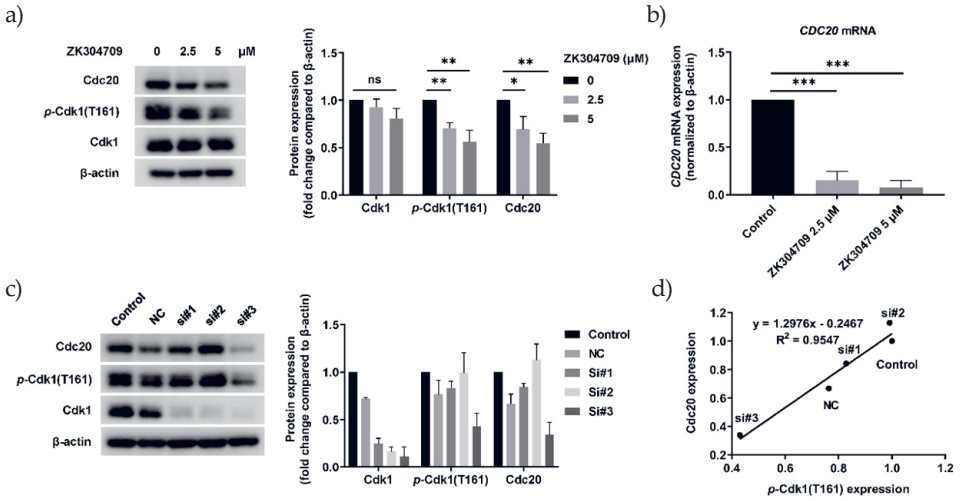

Fig. 3. ZK304709 inhibits Cdk1 phosphorylation and downregulates Cdc20 expression. a) Western blot analysis and densitometric quantification of total Cdk1, *p*-Cdk1(T161), and Cdc20 in HeLa cells treated with ZK304709 (0, 2.5, or 5 μmol L^{-1}) for 12 h; b) *CDC20* mRNA expression measured by RT-qPCR after ZK304709 treatment for 12 h; c) Western blot analysis and densitometric quantification of Cdk1, *p*-Cdk1(T161), and Cdc20 after siRNA-mediated Cdk1 knockdown (si#1, si#2, si#3); d) simple linear regression analysis of the correlation between *p*-Cdk1(T161) and Cdc20 expression levels. Data are presented as mean ± SEM ($n = 3$). Statistical analysis was performed using one-way ANOVA followed by Tukey's *post hoc* test for group comparisons, and simple linear regression for correlation analysis. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns – not significant.

not affect total Cdk1 protein levels but markedly decreased phosphorylation at Thr161, indicating inhibition of Cdk1 activation in HeLa cells (Fig. 3a). Given the co-overexpression of *CDK1* and *CDC20* in various cancers, we next investigated whether ZK304709 also affects Cdc20 expression. Western blot analysis revealed a dose-dependent reduction in Cdc20 protein following ZK304709 treatment (Fig. 3a), which was further confirmed at the mRNA level by RT-qPCR (Fig. 3b).

To further explore the link between Cdk1 activity and Cdc20 expression, we performed siRNA-mediated knockdown of Cdk1 using three independent sequences (si#1, si#2, si#3). Although all siRNAs effectively reduced total Cdk1 expression, their effects on *p*-Cdk1(T161) and Cdc20 levels varied. si#1 had no impact on *p*-Cdk1 or Cdc20, si#2 caused a modest increase in both, while si#3 significantly reduced *p*-Cdk1(T161) and concurrently suppressed Cdc20 expression (Fig. 3c). Correlation analysis confirmed a positive association between *p*-Cdk1(T161) and Cdc20 protein levels (Fig. 3d), suggesting that Cdk1 activation may contribute to the regulation of Cdc20 expression. Together, these findings indicate that ZK304709 downregulates Cdc20, at least in part, *via* inhibition of Cdk1 activation.

ZK304709 synergises with apcin to enhance anticervical cancer activity

To evaluate whether Cdk1 inhibition augments the antitumour activity of apcin, we first identified a sub-cytotoxic concentration of ZK304709 (≤ 2.5 μmol L^{-1}) that had no sig-

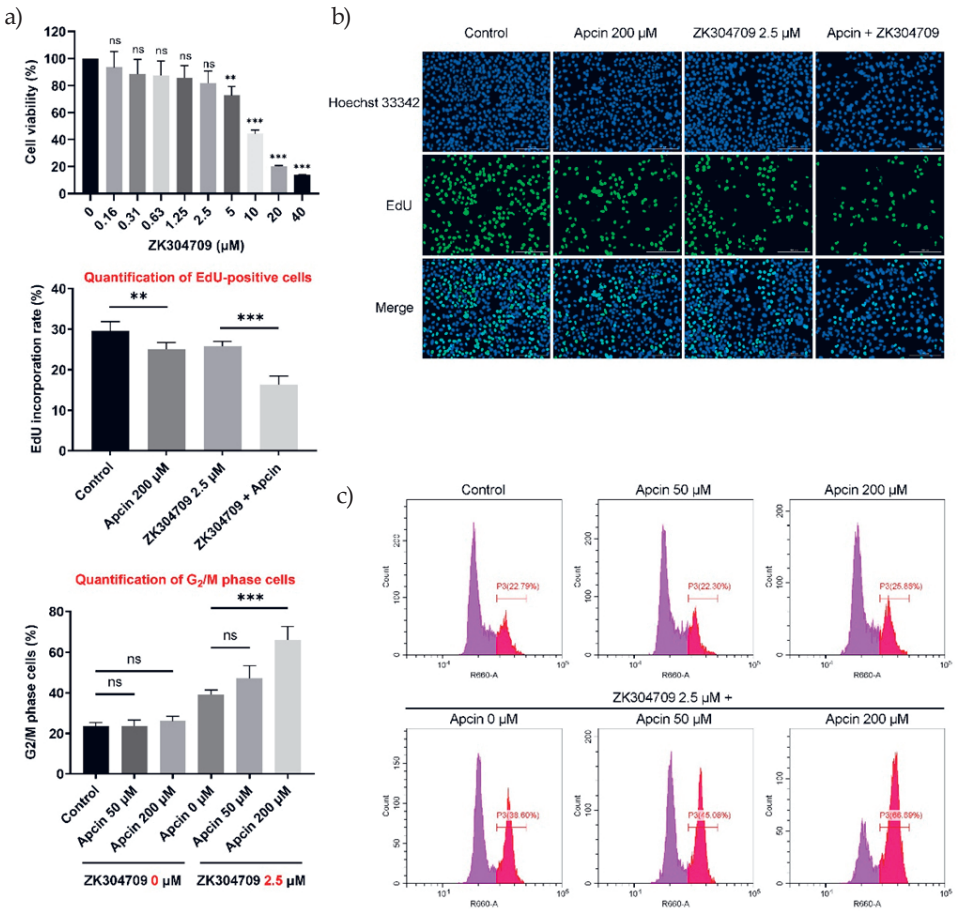

Fig. 4. ZK304709 enhances the antiproliferative and cell cycle effects of apcin. a) HeLa cell viability after 48 h treatment with various concentrations of ZK304709; b) representative fluorescence images of EdU incorporation following 48 h treatment with vehicle, apcin (200 μmol L^{-1}), ZK304709 (2.5 μmol L^{-1}), or their combination, with quantification of EdU-positive cells. Nuclei were counterstained with Hoechst 33342. Scale bar: 200 μm; c) flow cytometric analysis of the G2/M phase after 24 h treatment with vehicle, apcin (50 or 200 μmol L^{-1}), ZK304709 (2.5 μmol L^{-1}), or their combination, with quantification of the G2/M population. Data are presented as mean ± SEM (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's *post hoc* test. $^{**}p < 0.01$, $^{***}p < 0.001$, ns – not significant.

nificant effect on HeLa cell viability as determined by CCK-8 assay (Fig. 4a). EdU incorporation assays revealed that 200 μmol L^{-1} apcin modestly reduced DNA synthesis, lowering the proportion of EdU-positive cells from 29.52 to 25.04 %. Notably, co-treatment with 2.5 μmol L^{-1} ZK304709 further decreased EdU positivity to 16.34 %, representing a significantly greater reduction than either agent alone (Fig. 4b). These results suggest that ZK304709 enhances the antiproliferative effect of apcin in a synergistic manner.

We next examined whether this enhanced antiproliferative activity was associated with cell cycle arrest. Flow cytometry analysis showed that apcin alone had minimal impact on cell cycle distribution, whereas 2.5 μmol L^{-1} ZK304709 alone increased the G2/M population to 39.14 %. Importantly, the combination of 200 μmol L^{-1} apcin and 2.5 μmol L^{-1} ZK304709 markedly elevated the G2/M population to 66.04 %, indicating strong mitotic arrest induced by the dual treatment (Fig. 4c). At a lower concentration (50 μmol L^{-1}), combining apcin with 2.5 μmol L^{-1} ZK304709 reproducibly increased the G2/M population, with the average rising from 39.14 to 47.17 %. This modest trend, although not statistically significant, suggests a weaker synergistic effect compared with the pronounced response observed at 200 μmol L^{-1} (Fig. 4c). These findings demonstrate that ZK304709 synergistically enhances apcin-induced cell cycle arrest and support the therapeutic rationale for co-targeting Cdc20 and Cdk1 in cervical cancer.

Mechanistic insights and therapeutic implications

Our data support a mechanistic model in which apcin-induced inhibition of Cdc20 leads to compensatory activation of Cdk1, likely mediated by cyclin B1 accumulation and increased phosphorylation of Cdk1 at Thr161. This feedback activation may partially offset the antiproliferative effects of apcin. Co-treatment with the pan-Cdk inhibitor ZK304709 disrupts this compensatory loop by concurrently suppressing Cdk1 activity and downregulating Cdc20 expression, thereby enhancing mitotic arrest and antitumour efficacy (Fig. 5).

These findings parallel reports on the mouse double minute 2 homolog (Mdm2) inhibitor nutlin-3a, which stabilises p53 and downregulates Cdc20, thereby synergising with apcin in hematologic malignancies (29). The similarity between nutlin-3a and ZK304709 in converging on Cdc20 suppression highlights a broader therapeutic concept: modulation of upstream regulators can enhance the efficacy of Cdc20 inhibitors. Given the close association between Cdk1 activity and p53 signalling, and the established role of p53 as a nega-

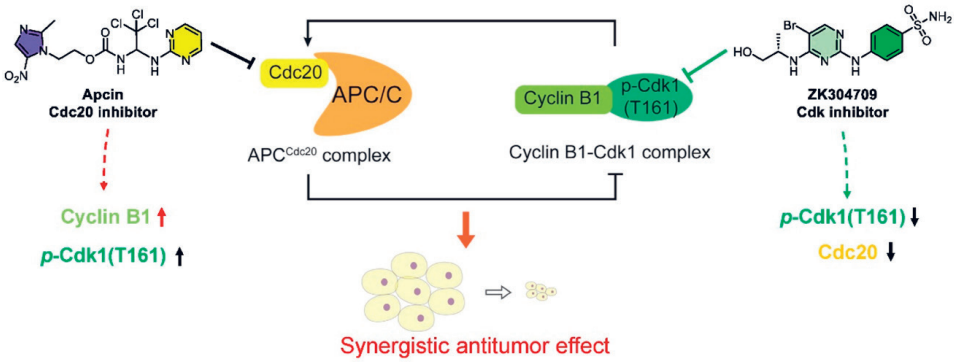

Fig. 5. Proposed mechanism underlying the synergy between ZK304709 and apcin. Apcin treatment leads to feedback activation of Cdk1 *via* cyclin B1 accumulation and increased Thr161 phosphorylation, partially limiting its efficacy. Co-treatment with ZK304709 suppresses this feedback loop by inhibiting Cdk1 activity and reducing Cdc20 expression, resulting in enhanced mitotic arrest and antiproliferative effects.

tive regulator of Cdc20, it is plausible that ZK304709 suppresses Cdc20 not only through direct inhibition of mitotic kinases but also via p53-mediated transcriptional repression (30–32). This dual mechanism may account for the enhanced suppression of Cdc20 observed in our study.

Furthermore, as interest in pan-Cdk and multi-Cdk inhibitors continues to grow, our study provides mechanistic and functional evidence supporting their strategic use in rational combination regimens. From a therapeutic perspective, targeting feedback or escape pathways such as Cdk1 activation may represent a viable strategy to enhance the efficacy of mitotic checkpoint-based therapies. Our findings provide a preclinical rationale for combining low-dose Cdk1 inhibitors with Cdc20-targeted agents as a synergistic and potentially less toxic approach for cervical cancer treatment.

Study limitations

Several limitations of this study should be acknowledged. First, all experiments were performed exclusively in HeLa cells, which, although a well-characterised cervical cancer model, may not fully represent the diversity of cervical cancer subtypes. The responses to Cdc20 inhibition may vary in other subtypes. Future studies incorporating multiple cervical cancer cell lines will be necessary to assess the generalizability of our findings across HPV subtypes.

Second, although we observed a clear correlation between Cdk1 activity and Cdc20 expression, the mechanistic basis of this relationship remains to be fully elucidated. It is unclear whether Cdk1 directly regulates Cdc20 expression or acts through intermediary signalling pathways. Further experiments involving targeted inhibition or overexpression of Cdk1 and Cdc20 in diverse cellular contexts will be essential to dissect this feedback mechanism more precisely.

CONCLUSIONS

In summary, this study highlights the therapeutic potential of combining the pan--Cdk inhibitor ZK304709 with the Cdc20 inhibitor apcin for the treatment of cervical cancer. By simultaneously suppressing Cdk1 activity and Cdc20 function, this combination effectively disrupts compensatory signalling pathways and enhances mitotic arrest. These findings provide preclinical evidence supporting a rational dual-targeting strategy that may improve the efficacy of antimitotic agents.

Abbreviations, acronyms, symbols. – APC/C, anaphase-promoting complex/cyclosome; ATCC, American Type Culture Collection; BCA, bicinchoninic acid; CCK-8, Cell Counting Kit-8; Cdc20, cell division cycle 20 homologue; Cdk1, cyclin-dependent kinase 1; DMEM, Dulbecco's Modified Eagle Medium; ECL, enhanced chemiluminescence; EdU, 5-ethynyl-2′-deoxyuridine; FBS, fetal bovine serum; HRP, horseradish peroxidase; Mdm2, mouse double minute 2 homolog; PBS, phosphate-buffered saline; PBST, phosphate-buffered saline with Tween-20; PI, propidium iodide; PROTAC, proteolysis targeting chimera; PVDF, polyvinylidene fluoride; RIPA, radioimmunoprecipitation assay; RT-qPCR, real-time quantitative polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SEM, standard error of the mean; siRNA, small interfering RNA; TCGA, The Cancer Genome Atlas.

Data sharing statement. – The original contributions presented in this study are included in the article material. Further inquiries can be directed to the corresponding author.

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Conflicts of interest. – The authors report no conflicts of interest.

Authors contributions. – Conceptualisation, X.L., Q.L., and Z.C.; data curation, X.L., Q.C., and S.C.; formal analysis, X.L. and S.C.; investigation, X.L., Q.C., and S.C.; methodology, X.L.; validation, Q.C.; writing, original draft preparation, X.L.; writing, review and editing, Q.L. and Z.C.; supervision, G.H. and Z.C.; project administration, Q.L.; funding acquisition, X.L. and Z.C. All authors have read and agreed to the published version of the manuscript.

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