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Review Article

CDC20: a novel therapeutic target in cancer

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Abstract: Cell division cycle protein 20 (Cdc20) is a member of the cell cyclin family. In the early stage of mitosis, it activates the anaphase-promoting complex (APC) and forms the E3 ubiquitin ligase complex APC^{Cdc20}, which destroys key regulators of the cell cycle and promotes mitosis. Cdc20 serves as a target for the spindle checkpoint, ensuring proper chromosome segregation. As an oncoprotein, Cdc20 is highly expressed in a variety of malignant tumors, and Cdc20 overexpression is associated with poor prognosis of these tumors. This review aims to dissect the tumorigenic role of Cdc20 in human malignancies and its targeting strategies.

Keywords: Cell division cycle protein 20, E3 ligase, hepatocellular carcinoma, breast cancer, osteosarcoma, cancer, apcin

Introduction

The ubiquitin-proteasome system (UPS) is responsible for the degradation and transformation of abnormal or redundant proteins. UPS regulates cell proliferation, differentiation, and metabolism, neural network formation, autophagy, and other physiological or pathological processes [1]. UPS is strictly controlled and the system usually consists of ubiquitin (Ub), 26S proteasomes, deubiquitinating enzymes (DUBs), ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin-protein ligase (E3) [2]. APC is a huge multi-subunit protein complex with at least 13 subunits that can control key substrates of the cell cycle by ubiquitination. APC localizes them to the 26S proteasome, initiating anaphase and leading to mitotic withdrawal through further degradation [3]. Two structurally homologous auxiliary subunits, Cdc20 and Cdc20 homolog 1 (Cdh1), are generally considered as 'APC coactivators'. Cdc20 and Cdh1 are responsible for ligating substrates and activating the ubiquitin ligase activity of APC, forming two different E3 ubiquitin ligase complexes, APC^{Cdc20} and APC^{Cdh1} [4]. Cdc20 mainly plays an inhibitory role in the division and early G1 phases, hindering division by degrading Securin and mitotic cycle proteins, respectively. Therefore, abnormal function of Cdc20 may considerably affect cell growth and tumorigenesis [5, 6]. Many studies have shown that Cdc20 is generally an oncoprotein and its downregulation is associated with DNA damage, impaired repair process, and apoptosis. Studies have confirmed that Cdc20 is overexpressed in various cancer stem cells and malignant tumors such as breast cancer, ovarian cancer, osteosarcoma, and hepatocellular carcinoma. As a prognostic marker for many types of cancer, Cdc20 overexpression predicts high grades, advanced stages, and poor prognosis [7].

This article explains the molecular structure and biologic function of Cdc20 and its role in the development of many types of malignant tumors. The article also dissects the upstream regulators of Cdc20 and discusses different types of Cdc20 inhibitors that may serve as targeted therapy for cancer patients.

Structure of Cdc20

Mutations of Cdc20 were originally identified in Saccharomyces cerevisiae. Cells with Cdc20 mutations had abnormal microtubule functions [8-10]. Cdc20 homologues, including $Slp1^{+}$ in fission yeast, Fzy in Drosophila, X-Fzy in

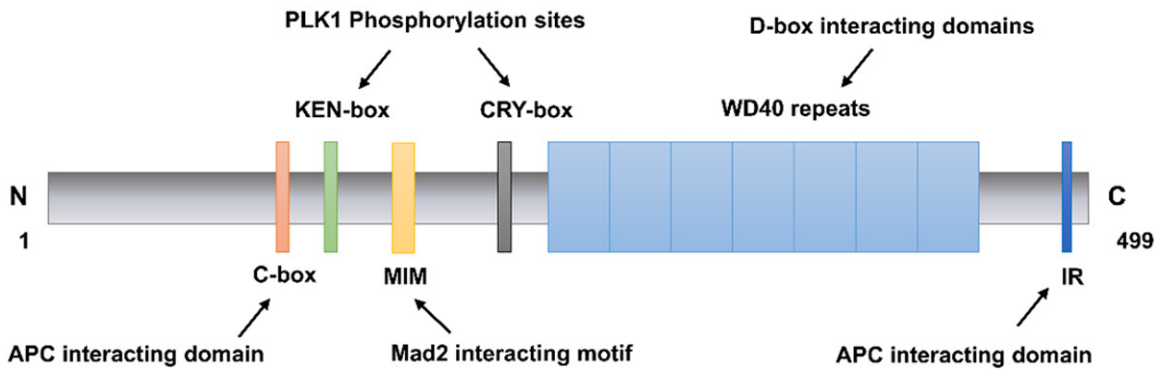

Figure 1. A schematic illustration of the structure of Cdc20. The structure of Cdc20 includes C-box, KEN-box, MIM, CRY-box, WD40 repeats, and IR domains, all of which bind to specific target motifs to regulate biological processes.

Xenopus, and p55Cdc in mammals, were successively discovered with strong homology in their WD repeat sequences [11-14]. The Cdc20 gene, including 11 exons and 10 introns, is located in the chromosome 1p34.1 region. Cdc20 has 499 amino acids, an N-terminal region with low structural complexity, and a C-terminal region with WD40 folding. The N-terminal disorder region consists of a C-box, KEN-box, Mad2 interacting motif (MIM), and CRY-box motif, while the highly conserved C-terminal region contains seven WD40 repeats and the Ile-Arg (IR) motif [15]. Different binding domains specifically perform their duties (**Figure 1**). The C-terminal region is highly conserved and contains seven WD40 repeat sequences and each WD40 repeat sequence comprises four β strands. Together, WD40 repeat sequences form a seven-bladed β-propeller structure, which is used for protein binding and substrate recognition [15]. It is worth noting that Cdc20 and Cdh1 recruit substrates through different motifs. Cdc20 can recruit substrates with a D-box, and Cdh1 can recruit substrates with D-box and/or KEN-box for ubiquitination [16]. However, the mechanism by which APC^{Cdc20} and APC^{Cdh1} recruit substrates for the corresponding motifs is unclear. The C-terminal of Cdc20 degrades several substrates, including Securin [17], Cyclin B1 [18], Cyclin A [19], Nek2A [20], p21 [21], and Mcl-1 [22], to precisely regulate the cell cycle (**Table 1**). In Drosophila, Cdc20 homologous gene, Fzy, was necessary for Cyclin A/B degradation [11].

Biological function of Cdc20

Cell cycle progression depends on periodic fluctuations in cyclin-dependent protein kinase (CDK) activity. During mitosis, Cdk1 inactivation is a prerequisite for the end of mitosis. Degradation of the positive regulatory subunits, CyclinA and CyclinB, through the ubiquitin-proteasome signaling pathway is pivotal for Cdk1 inactivation. Initially, it was found that Cdc20 mutation can cause the abnormal arrest of mitosis and lead to embryonic death [23]. Currently, it is believed that by activating APC, Cdc20 forms an E3 ubiquitin ligase complex APC^{Cdc20}, which degrades downstream substrates, regulates mitosis, promotes cell apoptosis, and regulates brain development.

Regulation of cell mitotic cycle

Accurate separation of genetic material during mitosis is essential for generating normal offspring cells. This process is initiated by the cyclin/cdk complex and terminated by ubiquitin-dependent degradation of cyclin subunits and other inhibitors of the M/G1 transition. The ubiquitin-proteasome machinery is responsible for the degradation of cyclin subunits. Cdc20 has different expression and localization in different stages of the cell cycle and regulates cell cycle progression. During the G2 phase, the core subunit of APC is phosphorylated by Cdk1 and other kinases, allowing Cdc20 to efficiently bind to APC [24]. At this time, Cdh1 phosphorylation by Cdks and binding to nucleoporins prevent APC^{Cdh1} activation, ensuring that APC^{Cdc20} is the main active form of APC in mitosis. Early mitotic inhibitor 1 (EMI1) and early mitotic spindle checkpoints further limit APC^{Cdc20} activity, ensuring that all chromosomes are connected to the mitotic spindle poles. APC^{Cdc20} degrades Cyclin A and Nek2A in the prometaphase. In the metaphase and anaphase, APC^{Cdc20} degrades

Table 1. Functions of the identified substrates for APC^{Cdc20}

Compound	Function	Reference
Securin	Inhibits the activation of separase, thereby inhibiting the premature segregation of chromosomes.	[17]
Cyclin B1	Activates CDK1, promoting the transition of cells from G2 to M phase.	[18]
Cyclin A	Promotes cell cycle G1/S and G2/M transitions.	[19]
Nek2A	Regulates microtubule assembly and centrosome segregation.	[20]
p21	Inhibits cyclin-dependent kinases in the G1, S and G2 phases of the cell cycle.	[21]
Mcl-1	Anti-apoptotic protein.	[22]
Bim	Exerts a pro-apoptotic effect in cells.	[32]
NeuroD2	Inhibits presynaptic differentiation and controls neural differentiation.	[43]
FMRP	Regulates dendritic pruning and synaptic elimination and the development of a variety of cocaine-induced behaviors.	[46, 47]
SMAR1	Regulates DNA damage and repair pathways.	[52-54]
PHD3	Catalyzes the hydroxylation of two proline residues in HIF1a.	[58, 59]
NUSAP1	Binds to and stabilizes microtubules and participates in cell division.	[63, 64]
Foxo1	Regulates transcription in response to external signal stimulation by binding to DNA-binding elements within the promoters of target genes.	[73]

Securin, attenuating the partial inhibition of the isolated enzyme. At this stage, APC^{Cdc20} initiates CyclinB degradation, thus activating phosphorylation-mediated separation enzymes. Activated separase cleaves the adhesin complex for proper segregation of chromosomes, which initiates the anaphase [25]. Anaphase is associated with decreased function of Cdks, activating APC^{Cdh1} [25]. However, some studies indicated that abnormal spindle morphology and abnormal chromosome segregation detected in mec1 or rad53 mutants were not associated with APC. They also reported that two microtubule-associated proteins, Cin8 and Stu2, are additional targets for Mec1 and Rad53 at S phase checkpoints [26]. However, the detailed molecular mechanism of APC-independent function of Cdc20 has not yet been uncovered.

Spindle assembly checkpoints (SAC) monitor the connection between microtubules and motility, prevent the premature transition of the cell cycle from metaphase to anaphase, and maintain genome stability. Among them, the mitotic checkpoint complex (MCC) is an essential SAC consisting of two sub-complexes, Cdc20-Mad2 and Bubr1-Bub3 [27]. BubR1 is a centromere-responsive multidomain protein kinase. BubR1 possesses an N-terminal, homologous to the yeast Mad3 protein. The N-terminal identifies the KEN box motif of Cdc20 and is required for all functions, whereas the C-terminal kinase domain is not [28]. In the presence of chromosome localization and/or spindle microtubule attachment errors, SAC induces a conformational change of Mad2, thereby activating MCC [28] (**Figure 2**). MCC binds to APC^{Cdc20}, and forms a functionally inactive APC^{MCC} complex. Interestingly, PLK1 is required for correct connections between sister centromeres. PLK1 phosphorylates Cdc20 and accelerates its interaction with MCC to bidirectionally align sister centromeres [15]. In the presence of any defects in microtubule-kinetochore attachment or spindle tension, checkpoint proteins induce metaphase arrest [29, 30].

Regulation of cell apoptosis

In addition to regulating cell cycle progression, Cdc20 also regulates apoptosis (**Figure 3**). Cdc20 regulates apoptosis by targeting Mcl-1 and Bim. Cdc20 knockout decreased the activity of the anti-apoptotic protein Mcl-1 and inhibited Chk1 phosphorylation, thereby inhibiting DNA homologous recombination repair protein Rad51 and inducing cell apoptosis [31]. Cdc20 can target Bim, a pro-apoptotic protein, in a D-box-dependent manner, promote ubiquitin-mediated destruction of Bim, and regulate cell apoptosis [32]. Studies have shown that Cdc20 knockout promotes apoptosis by preventing Bim degradation. Cdc20 knockout promoted temozolomide and radiation-induced DNA dam-

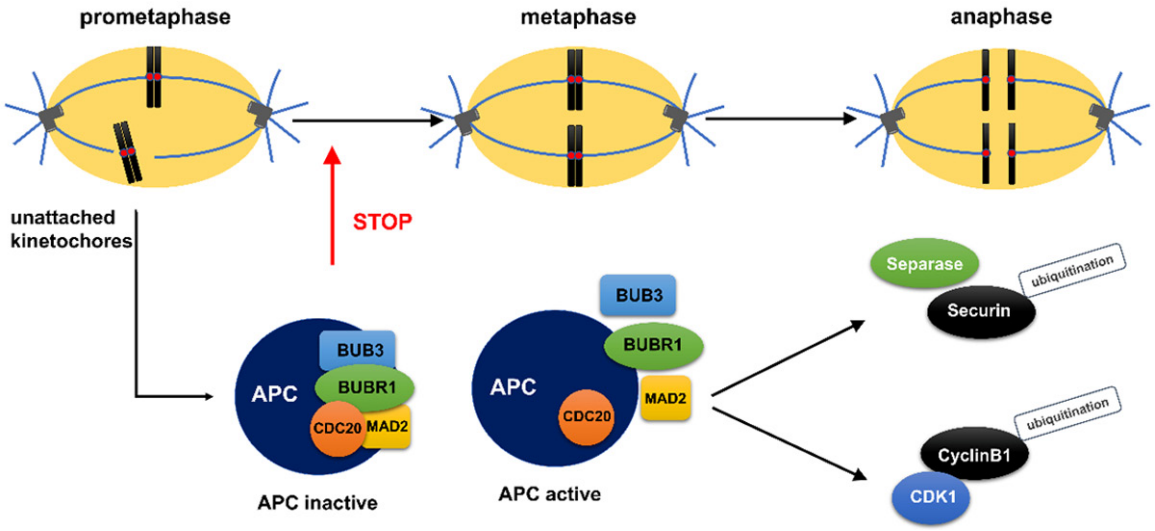

Figure 2. A schematic illustration of how Cdc20 participates in regulating the cell division process. The metaphase-to-anaphase checkpoint is activated when SAC proteins detect kinetosomes that are not attached to spindle microtubules. Cdc20 is sequestered by MAD2 and BUBR1/BUB3 in the form of MCC, which no longer activates APC and inhibits the initiation of chromosome segregation. Until all centromeres are properly attached to the spindle poles, MCC dissociates from CDC20, APC reactivates and degrades Securin and CyclinB1, releasing separase.

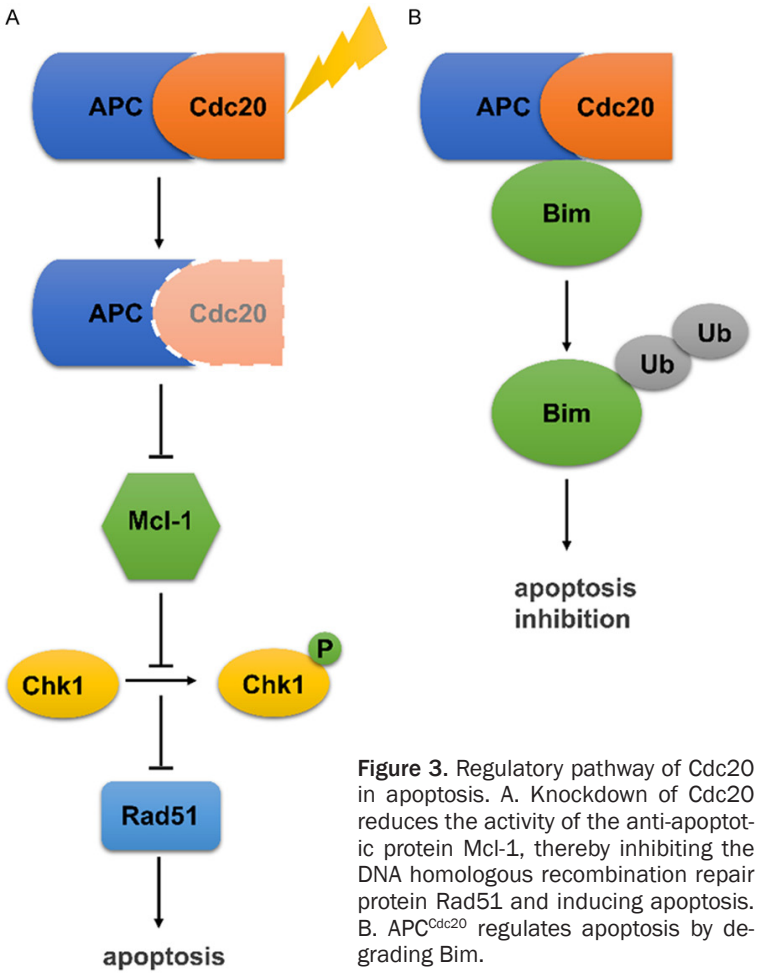

Figure 3. Regulatory pathway of Cdc20 in apoptosis. A. Knockdown of Cdc20 reduces the activity of the anti-apoptotic protein Mcl-1, thereby inhibiting the DNA homologous recombination repair protein Rad51 and inducing apoptosis. B. APC^{Cdc20} regulates apoptosis by degrading Bim.

age and death in glioblastoma stem cells [33]. It has also been reported that Cdc20 can ameliorate chemoresistance by upregulating pro-apoptotic factors [34].

Regulation of brain development

The normal development of the mammalian cerebral cortex requires radial migration [35]. The migration of neurons mainly has two steps: the precursor near the cell body expands, and then other intracellular structures, such as the centrosomes, move to the expanded part and form a new cell body [36-39]. A unique swelling structure, called a dilator, forms in migrating neurons, and is required to move the centrosomes and nucleus. Coiled-coil protein associated with myosin II and DISC1 (CAMDI) is a novel protein interacting with disrupted in schizophrenia 1 [40]. CAMDI regulates cortical radial migra-

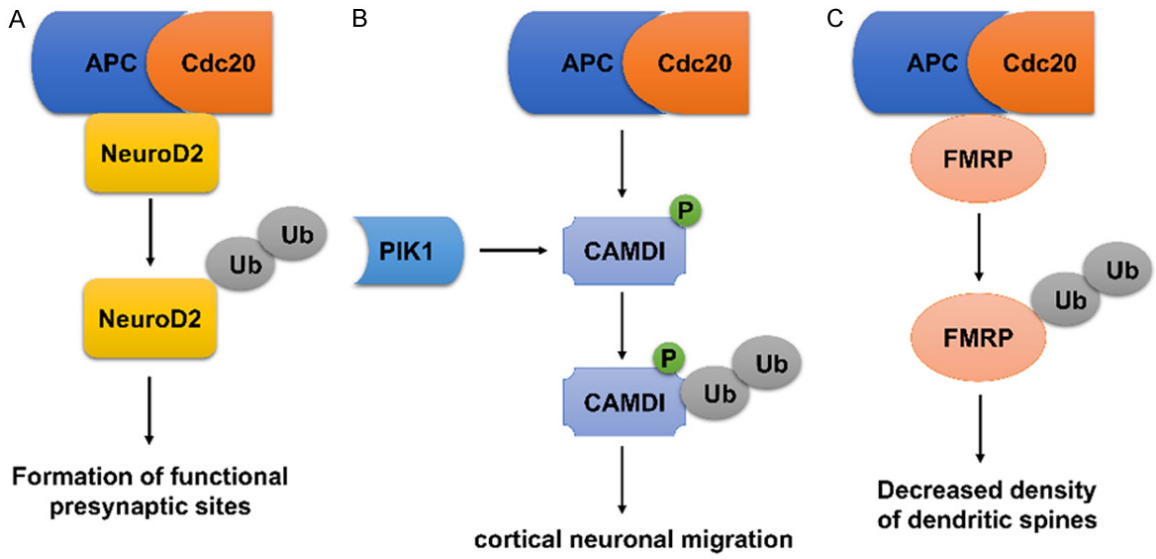

Figure 4. Regulatory pathways of Cdc20 in neural development. A. APC^{Cdc20} promotes the formation of functional presynaptic sites by degrading NeuroD2. B. APC^{Cdc20} promotes the expansion formation of cortical migration by degrading CAMDI. C. APC^{Cdc20} reduces dendritic spine density by degrading FMRP.

tion and promotes centrosome maturation by antagonizing the function of histone deacetylase 6 (HDAC6) [41]. During mouse brain development, APC^{Cdc20} degrades CAMDI after centrosome translocation into the dilated area. The oscillatory regulation of CAMDI protein is needed for proper cortical migration [42]. Furthermore, the Cdc20-APC ubiquitin signalling pathway promotes functional presynaptic site formation through targeted degradation of NeuroD2 [43]. Cdc20 plays a direct role in controlling the initiation of glial cell migration [44]. Some studies have shown that Cdc20 is expressed in post-mitotic neurons during brain development, and its protein level increases with the maturation of neurons [45]. Cdc20 knockout severely impaired the dendrite formation of granular neurons in the cerebellar cortex. Fragile X mental retardation protein (FMRP) is involved in behavioural sensitization and cocaine-induced synaptic plasticity of adult nucleus accumbens (NAC) [46]. Wang *et al*. [47] observed that Cdc20 upregulation downregulated FMRP and reduced the density of dendritic spines. These findings confirm that the APC^{Cdc20} ubiquitin signaling pathway plays an essential role in many biological processes such as neuronal connection, dendritic morphogenesis, and plasticity in the mammalian brain (**Figure 4**).

Role of Cdc20 in malignant tumors

Previously, it has been found that Cdc20 is involved in the development of malignant tumors and the expression level of Cdc20 is associated with the pathologic grade of the tumor. Cdc20 overexpression is associated with a higher grade and a worse prognosis of cancer. The role of Cdc20 in several malignant tumors will be discussed in the following sections (**Table 2**).

Breast cancer

About 75% of breast cancers are estrogen receptor-positive (ER+). Hormone therapy is the main treatment for ER+ tumors, which significantly improves survival. Wang *et al*. [48] used METABRIC and immunohistochemical methods to evaluate the association of Cdc20 expression with the long-term outcome of ER+ breast cancer. In patients with ER+ breast cancer, Cdc20 overexpression was positively correlated with adverse clinical outcome. Cdc20, alone or in combination with other markers, can be used to select patients for hormone therapy. The 25-year (mean 10.0 years) follow-up of 445 patients with breast cancer indicated that Cdc20 and Securin overexpression were related to the severity of DNA aneuploidy [49]. Furthermore, immune overexpression of Cdc20

Table 2. Function of Cdc20 protein in different cancer types

Cancer	Interactor	Function	Reference
Breast cancer	Rec8	Reverses the tumor suppressor effect of Rec8.	[50]
	SMAR1	Binds and ubiquitinates the SMAR1 proteasome in a D-box motif-dependent manner.	[52-54]
Hepatocellular carcinoma	PHD3	Activates HIF-1 signaling through ubiquitin degradation of PHD3.	[58, 59]
	Bcl2/Bax	Regulates the proliferation ability of hepatocellular carcinoma through the Bcl2/Bax pathway.	[60]
Osteosarcoma	NUSAP1	Cdc20 Knockdown reverses abnormal cell proliferation induced by NUSAP1.	[63, 64]
	Bim/p21	Supports cellular oncogenicity by regulating the expression of Bim and p21.	[65]
Gastric cancer	CBX4	Drives cell growth and tumor sphere formation.	[69]
	MYBL2	Cooperates with MYBL2 to induce GC cell proliferation and inhibit cell apoptosis.	[71]
Bladder cancer	Foxo1	Enhances the radiation resistance of bladder cancer cells by regulating Foxo1.	[73]
Prostate cancer	Axin	Enhances the self-renewal capacity of CD44+ prostate cancer stem cells.	[75]

alone or combined with immune overexpression of Securin was associated with advanced disease and a 6.8-fold increased risk of mortality. Rec8 is a mucin component specifically expressed during meiosis. It controls the correct separation of centromeres and chromosomes. Cdc20 overexpression significantly reversed the inhibitory effect of Rec8 overexpression on proliferation, invasion, and infiltration of breast cancer cell line Mcf-7 and inhibited apoptosis of Mcf-7 cells. Nevertheless, the molecular mechanism underlying Rec8-mediated inhibition of Cdc20 expression is unclear [50]. Triple-negative breast cancer (TNBC) is the most invasive subtype of breast cancer, which can easily metastasize to other organs. Using the NCBI database, Song *et al*. [51] studied mitosis-related genes and proteins related to TNBC. They found that Cdc20 was significantly upregulated in TNBC, and its transcriptional level was positively correlated with patient survival in the absence of metastasis and recurrence. Further studies showed that Cdc20 deletion reduced the proliferation and migration of four TNBC cell lines. Scaffold matrix attachment region 1 binding protein (SMAR1) is a DNA regulatory element, that can activate p53 and induce cell cycle arrest in the G1 and G2/M phases [52, 53]. SMAR1 also regulates DNA damage and plays a vital role in tumor suppression [53]. Some studies in breast cancer cell lines and patient samples have reported that Cdc20 promotes SMAR1 degradation through K48-linked specific polyubiquitin [54]. This suggests that Cdc20 is a negative regulator of SMAR1 in advanced cancers. These results indicate that using small molecules to block SMAR1-Cdc20 interaction may be a novel chemotherapy strategy.

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the main type of primary liver cancer with a high mortality rate. Cdc20 is highly expressed in HCC tissues compared to paired non-tumor adjacent tissue [55]. Cdc20 knockdown by double-stranded small interference RNA (siRNA) decreased HCC cell growth and led to G2/M arrest [55]. Thus, Cdc20 overexpression promotes the development of HCC. E3 ubiquitin ligases play both carcinogenic and anti-tumour roles in cancer. Some E3 ubiquitin ligases are related to chemotherapy resistance and may become targets for treating cancer. The immune microenvironment is an important part of the tumor microenvironment, which is composed of T cells, macrophages, natural killer cells, and other immune cells.

The immune microenvironment markedly affects the prognosis of cancer [56]. Cdc20 expression was positively correlated with the number of B cells, CD8+ T cells, CD4+ T cells,

and macrophages in HCC [57]. Cdc20 knockout upregulated oxygen-dependent endogenous proline hydroxylase 3 (PHD3), accelerated hypoxia-inducible factor-1α (HIF-1α) degradation, and impaired vascular endothelial growth factor (VEGF) secretion in hepatoma cells [58, 59]. Mechanistically, Cdc20-mediated PHD3 ubiquitination attenuated the interaction between von Hippel-Lindau (VHL) and HIF-1α, thereby preventing VHL-mediated degradation and increasing the stability and function of HIF-1α protein [59]. Further studies revealed that apcin, a Cdc20 inhibitor, may have therapeutic potential in HCC by inhibiting PHD3 degradation. ZHAO *et al*. [60] found that Cdc20 regulates the proliferation and radiosensitivity of p53-mutant HCC cells through Bcl2/Bax pathway. In addition, Cdc20 combined with radiation more robustly inhibited the proliferation of liver cancer cells, aggravated DNA damage, led to G2/M phase arrest, and increased the cell death rate and radiosensitivity [60]. These results indicate that Cdc20 inhibition has a significant radiosensitizing effect.

Osteosarcoma

Osteosarcoma (OS) is a malignant tumor with mesenchymal origin. It mainly affects the long bones of children and young adults. Currently, patients with low-grade OS only undergo surgical resection. However, extensive surgical resection and adjuvant chemotherapy are needed for advanced lesions. Sackton *et al*. [61] have reported that apcin directly prevents Cdc20 from binding to its substrates, thereby inhibiting mitosis in human cancer cells. Some studies found that apcin upregulates Bim in OS cells, which exhibits anti-tumor activity in OS cells [62]. Apcin has therapeutic potential in human OS by disrupting APC-Cdc20 interaction. In addition, Cdc20 may be a promising target for chemotherapy. Nucleolar and spindle-associated protein 1 (NUSAP1) is an essential regulator of mitosis. Previous studies have shown that selective downregulation of NUSAP1 during cell proliferation leads to various abnormal cellular events such as decreased number of central spindle microtubules, abnormal chromosome segregation and spindle assembly, G2/M phase arrest, and chromosomal translocation [63]. NUSAP1 upregulation in OS cells can accelerate proliferation and cell cycle progression by combining with Cdc20 and CCNA2. On the contrary, silencing Cdc20 or CCNA2 gene can significantly prevent abnormal cell proliferation induced by NUSAP1 [64]. In addition, another study reported that Cdc20 knockdown upregulates Bim and p21 in OS cells, thereby leading to cell cycle arrest and apoptosis [65]. Therefore, Cdc20-mediated degradation of Bim and p21 promotes carcinogenesis in OS cells. In addition, the combination of apcin and cisplatin showed better anti-cancer efficacy than cisplatin alone, which shows that targeting Cdc20 may be a new solution for treating cisplatin-resistant OS. It was found that mitotic genes related to metaphase to anaphase transition, such as Cdc20, Securin, Cyclin A2, and Cyclin B2, were overexpressed in OS cell lines, and their expression increased with tumor progression [66].

Gastric cancer

Gastric cancer (GC) is the third leading cause of cancer-related mortality worldwide. Genetic, environmental, and epigenetic factors are involved in the pathogenesis of GC. Polycomb repressive complex (PRC) 1/2 can regulate histone modifications [67, 68]. As an essential component of PRC1, chromobox 4 (CBX4) plays a crucial role in the epigenetic modifications of many tumors. In GC, CBX4 can significantly enhance proliferation, migration, and metastasis by regulating Cdc20 transcription. CBX4 inhibition can suppress Cdc20-mediated growth of GC [69].

The clinicopathologic assessment of 131 cases of GC tissues by Ding *et al*. [70] uncovered that the transcription level of Cdc20 was significantly higher in GC than in paracancerous tissues. In addition, Cdc20 expression level was positively correlated with the malignant behavior of tumor cells, invasion, and TNM stage. Deng *et al*. [71] demonstrated that V-Myb avian myeloblastosis viral oncogene homolog-like 2 (MYBL2) was significantly upregulated in GC cells. MYBL2 knockdown inhibited HGC-27 cell proliferation and promoted apoptosis, and these effects were reversed by Cdc20. Therefore, the synergistic effect of MYBL2 and Cdc20 can induce GC cell proliferation and inhibit cell apoptosis, which may involve the Wnt/β-catenin signaling pathway.

Other tumors

Bioinformatic analysis and experimental studies have shown that Cdc20 is a vital regulator

Table 3. Functions of Cdc20 inhibitors

Compound	Function	Reference
Apcin	Occupies the D-box binding pocket of the WD40 domain, competitively prevents recognition of its substrate and disrupts protein-protein interactions within the Cdc20-substrate complex.	[61, 94, 95]
PTE, PPAC	Downregulates Cdc20 expression in breast cancer cells.	[96]
Diosgenin	Reduce the expression of Cdc20 in OS cells to exert its anticancer effect.	[97-99]
GDNT	Downregulates Cdc20 expression and subsequently inhibits the growth and invasiveness of breast cancer cells.	[100]
Curcumin	Inhibits Cdc20 expression in a variety of human cancers to exert its antitumor properties.	[101, 102]
Withaferin A	Disrupts the MAD2-Cdc20 complex in colorectal cancer cells to regulate the spindle assembly checkpoint.	[103]
Genistein	Downregulates the expression of related regulatory factors including Cdc20 and inhibits the occurrence and development of breast cancer.	[104, 105]
TAME	Mimics the IR-Tail structure of Cdc20/Cdh1 and competes with IR-Tail for the same binding site on APC, reducing Cdc20/Cdh1 binding to APC.	[106, 107]
Pro-TAME	A cell-permeable TAME precursor drug, blocks the binding of Cdc20 or Cdh1 to APC and inhibits APC activity.	[106]
NAHA	Inhibits the expression of Cdc20 protein in breast cancer cells and inhibits cell adhesion and invasion.	[109, 110]
BCHHD 7c	Down-regulated Cdc20 gene expression in breast cancer drug-resistant cells, induced cell apoptosis and cell cycle arrest in G2/M phase.	[111]
CFM-4	Regulates the function of APC^{Cdc20} to interfere with G2/M progression.	[112]
HPIP	Utilizes its D-box and IR domains to play dual roles as a substrate and inhibitor of the APC^{Cdc20} complex, respectively.	[113, 114]
BI-D1870	Prevents the binding of Cdc20 to APC, increases the binding of inhibitor MAD2 to Cdc20 and thus prevents mitotic exit.	[115]

of mitosis and cell cycle in non-small cell lung cancer [72]. Furthermore, Cdc20 knockdown inhibited the growth of lung adenocarcinoma cells in vitro, suggesting that Cdc20 overexpression may be an indicator of poor prognosis in patients with lung adenocarcinoma. Furthermore, it has been indicated that Cdc20 is an upstream regulator of Foxo1 in bladder cancer and is overexpressed in radiation-resistant bladder cancer cells [73]. Cdc20-knockout cells cultured in acidic environments showed chromosomal instability and better viability [74]. In addition, increased extracellular acidity and decreased oxygen consumption by Cdc20 knockout cells induced tumorigenesis in non-tumor cells. These results suggest that chromosomal instability and acidic microenvironment caused by Cdc20 gene knockout can promote tumorigenesis by downregulating autophagy and apoptosis. Cdc20 also enhanced the self-renewal of CD44+ prostate cancer stem cells by promoting nuclear translocation and transactivation of β-catenin [75]. Cdc20 combined with CD44 or β-catenin can be used as a prognostic marker for prostate cancer.

Upstream regulators of Cdc20

Various upstream regulators of Cdc20 have been identified by various methods (**Table 3**). However, how they regulate cell survival by controlling the expression of Cdc20 has not been revealed. p53 is a critical regulator of the cell cycle activated by cellular stress or DNA damage [76]. Currently, there are three accepted approaches for p53-mediated direct inhibition: first, binding-site overlap; second, p53 squelching of transcriptional activators; and third, p53-mediated recruitment of histone deacetylases (HDACs) [76]. It has been demonstrated that p53 activated by DNA damage directly binds to the Cdc20 promoter. p53 recruits corepressors mSin3A and HDAC1, and Cdc20 promoter chromatin undergoes histone methylation [77]. High intracellular concentrations of p53 can repress the promoter of Cdc20 through the CDE/CHR element [77]. p53 Mu-

Table 4. Functions of the upstream regulators of Cdc20

Compound	Function	Reference
p53	Tumor suppressor.	[76-78, 81]
MicroRNAs (MiR-449, MiR-494, MiR-4459, MiR1321/MiR-7515)	Regulates gene expression at the post-transcriptional level by binding to the 3'-untranslated region (3'-UTR) of target mRNAs.	[82-86]
RASSF1A	RAS negative effect regulator.	[87-89]
SIRT2	A class of highly conserved nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylases, which play different roles by deacetylating different substrates.	[90]
MAD2	Monitors and identifies the precise connection of spindle microtubules to chromosomal kinetochores, and abnormalities identified may cause cell cycle arrest.	[91]
EMI1	Inhibits APC activation by binding to Cdc20, accumulates Cyclin B, promotes cell mitosis to enter M phase, and can also inhibit APC ubiquitination and regulate cells to enter mitotic S phase.	[92]
EMI2	An APC ubiquitin ligase inhibitor that regulates cell cycle progression.	[93]
Cdh1	Exerts cell cycle regulation function mainly in early G1/S phase by forming APC^{Cdh1} complex.	[25]

tations can attenuate p53-mediated apoptosis, making cancer treatment more difficult [78]. P53 knockdown significantly upregulates Cdc20, and DNA damage inhibits Cdc20 expression in a p53-dependent manner [79]. Murine double minute 2 (MDM2) is an E3 ubiquitin ligase overexpressed in different types of cancer [80]. Cdc20 is an essential downstream regulator of the MDM2-p53 signalling pathway in diffuse large B-cell lymphoma [81]. MicroRNAs (miRNAs) are endogenous non-coding RNAs that negatively regulate genes by binding to the 3'-untranslated region (3'-UTR) of target mRNAs to induce their degradation [82]. The miR-449 family is encoded by a locus in the second intron of Cdc20B on chromosome 5. Therefore, the expression of miR-449 and its host gene Cdc20B are cooperatively regulated [83]. MiR494 can induce G2/M arrest by inhibiting Cdc20 expression [84]. MiR-4459 upregulation can downregulate Cdc20B and ATG13 in human embryonic stem cells. Therefore, miR-4459 can regulate stemness through the cell cycle and autophagy [85]. Recently, Hu *et al.* [86] unfolded that miR-1321/miR-7515 is an upstream target of Cdc20. MiR-1321/miR-7515 can reduce the migration and invasion of lung cancer cells by inhibiting Cdc20. RASSF1A is an important tumor suppressor gene, and hypermethylation of CpG islands in its promoter leads to its inactivation [87]. In early mitosis, RASSF1A inhibits APC^{Cdc20} in a D-box motif-dependent manner. RASSF1A phosphorylation by Aurora A and Aurora B facilitates its degradation by APC^{Cdc20} [88]. Consistently, previous studies indicated that the RASSF1A-APC^{Cdc20} regulatory axis tightly controls prometaphase [89]. In addition, SIRT2, as a positive regulator of Cdc20, activates APC by deacetylating Cdc20. SIRT2 knockout mice exhibit reduced APC activity, resulting in genomic instability [90]. Abnormal kinetochore segregation activates SAC and transforms O-MAD2 into C-MAD2. C-MAD2 subsequently binds to Cdc20 to form the MCC complex and inhibit APC^{Cdc20} and chromosome segregation [91]. EMI1 is a member of the F-box protein family, which strictly regulates mitosis and the S phase. EMI1 inhibits APC activation by binding to Cdc20, leads to cyclin B accumulation, and induces mitosis and M phase [92]. Another study found that the zinc-binding region domain of EMI2 inhibits APC by binding to Cdc20 and induces abnormal cell division [93]. Cdc20 plays a role in the G1 phase and M phase. In the late M phase, Cdh1 degrades Cdc20 by forming the APC^{Cdh1} complex and regulates the cell cycle in the G1 phase.

Cdc20 inhibitors

Cdc20 has a potential tumorigenic role in humans. Therefore, Cdc20 inhibition by small-molecule inhibitors is a new avenue to treat tumors. Next, specific and non-specific Cdc20 inhibitors will be discussed to help the development of Cdc20 inhibition (**Table 4**).

Apcin

Initially, a research group identified apcin in Xenopus egg extracts [94]. Recent studies have shown that apcin is the only specific inhibitor of Cdc20. It occupies the break-frame binding pocket of the WD40 domain, competitively inhibiting Cdc20 ubiquitination [61]. Apcin dose-dependently binds to endogenous Cdc2, and adding substrate can increase the Cdc20 load on APC in a concentration- and D-box-dependent manner [61]. Among different APC substrates, apcin effectively stabilized cycB1-NT and securin, but not cyclinB1. Unlike apcin, TAME inhibited APC-mediated degradation of all substrates [61]. Since apcin and TAME can differently inhibit APC, their combination not only synergistically stabilizes APC substrates, but also expedite cell death. Apcin-mediated inhibition of APC decreased the adhesion and proliferation ability of endometrial cells [95].

Natural inhibitors of Cdc20

A triterpene mixture extracted from the mushroom Poria cocos (PTE), purified triterpenes dehydropachymic acid, and polyporenic acid C (PPAC) dose-dependently downregulated Cdc-20 transcription in pancreatic cancer cells [96]. Diosgenin, a steroidal saponin from Fenugreek, has antitumor activity [97, 98]. Diosgenin can inhibit the proliferation of different human cancer cells by activating p53 and caspase-3 [97]. It was found that diosgenin dose-dependently decreased Cdc20 transcription in OS cells [99]. Jiang *et al.* [100] revealed that ganodermanontriol (GDNT) inhibits Cdc20 expression, thereby inhibiting the proliferation and invasion of breast cancer cells. Curcumin is a golden pigment extracted from turmeric. It exhibits antitumor properties in various human cancers [101]. Curcumin inhibits Cdc20 transcription and decreases pancreatic cancer cell survival [102]. Withaferin A can degrade the MAD2-Cdc20 complex to regulate spindle assembly checkpoint in colorectal cancer cells [103]. Genistein is the simplest isoflavonoid, which extensively exists in Leguminosae. It is often used as a cancer chemoprevention drug [104]. Zhang *et al.* [105] reported that genistein can downregulate Cdc20 and prevent the development of breast cancer.

TAME and Pro-TAME

Tosyl-L-arginine methyl ester (TAME) is a small molecule compound that structurally mimics the IR tail of Cdc20/Cdh1 and competes with the IR tail for the same binding site on APC. It inhibits the interaction between Cdc20 and APC in mitotically inhibited xenopus egg extracts, but cannot prevent Cdc20 binding in somatic cells [106]. However, the absence of TAME antagonizes the interaction between C-box and APC. In the absence of APC substrates, TAME inhibits the binding of free Cdc20 to APC and releases the pre-bound Cdc20 by inducing automatic ubiquitination of Cdc20 [107]. TAME can block Cdh1- and Cdc20-mediated activation of APC. Cdh1 is a tumor suppressor, and APC is activated by Cdh1 to form the APC^{Cdh1} complex, which inhibits tumor development. Pro-TAME is a cell-permeable TAME precursor drug. Pro-TAME can be hydrolyzed to form TAME, which competitively binds the APC core complex and blocks Cdc20- or Cdh1-mediated activation of APC [106]. Although pro-TAME can inhibit APC^{Cdh1} activation, it has little effect on interphase progression. Pro-TAME induces metaphase arrest without interfering with mitotic spindle morphology or function [108]. The synergistic effect of apcin and pro-TAME increases the rate of mitosis in human cancer cell lines [61].

Other Cdc20 inhibitors

2-[Benzyl-(2-nitro-benzenesulfonyl)-amino]-N-hydroxy-3-methyl-N-propyl-butyramide (NAHA) has been proven to effectively inhibit the proliferation of breast cancer cells [109]. Jiang *et al.* [110] established a mouse model of breast cancer and indicated that NAHA significantly downregulated Cdk2 and Cdc20, inhibited cell growth, and reduced tumor size. NAHA inhibited colony formation and cancer cell adhesion by reducing the expression of urokinase-type plasminogen activator. NAHA also inhibited angiogenesis by downregulating vascular endothelial growth factor [110]. However, the specific regulatory mechanism of NAHA is still unclear. Nasr *et al*. [111] found that BCHHD 7c can suppress Cdc20 expression in drug-resistant pancreatic cancer cells. The C-terminal region of CARP-1 can incorporate Cdc20 or Cdh1 into APC2, which may regulate the function of APC^{Cdc20} and lead to G2/M arrest [112]. Hematopoietic PBX-interacting protein (HPIP) is an oncoprotein overexpressed in several types of cancer [113]. HPIP can attenuate APC^{Cdc20}-mediated protein degradation, and HPIP mutations are resistant to APC^{Cdc20}-mediated protein

degradation during mitosis. HPIP plays a dual role. Its D-box and IR domains are the substrate and inhibitor of APC^{Cdc20} complex, respectively. They maintain the temporal stability of cyclins during cell cycle transition to ensure the smooth progression of mitosis [114]. BI-D1870 prevents Cdc20 incorporation into APC but increases MAD2 incorporation into Cdc20, disrupting the withdrawal of mitosis [115].

Conclusions and prospects

Cdc20 is a major regulator of the cell cycle and apoptosis. It participates in different signaling pathways and regulates cell proliferation, growth, and apoptosis. Cdc20 activates the anaphase-promoting complex E3 ubiquitin ligase, thereby degrading downstream substrates. Cdc20 precisely controls genetic material distribution and mitosis termination. Reduced Cdc20 expression impairs DNA repair, thereby inducing cell apoptosis. Abnormal expression of Cdc20 has been detected in various types of cancer. Cdc20 overexpression is associated with high grades of tumors and poor prognosis. Apcin is a specific Cdc20 inhibitor that binds to Cdc20 and inhibits APC activity. However, other inhibitors are non-specific. TAME and pro-TAME can block Cdc20 or Cdh1 incorporation into APC and inhibit APC, but they have poor selectivity. Therefore, developing specific Cdc20 inhibitors may be a new strategy for treating cancer. It is necessary to explore Cdc20-mediated tumorigenesis in a more comprehensive and in-depth manner and develop Cdc20 inhibitors with higher specificity and potency.

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Disclosure of conflict of interest

None.

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