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Pharmacol Ther. Author manuscript; available in PMC 2016 July 01.

Published in final edited form as:

Pharmacol Ther. 2015 July ; 151: 141–151. doi:10.1016/j.pharmthera.2015.04.002.

Targeting Cdc20 as a novel cancer therapeutic strategy

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Abstract

The Anaphase Promoting Complex (APC, also called APC/C) regulates cell cycle progression by forming two closely related, but functionally distinct E3 ubiquitin ligase sub-complexes, APC^{Cdc20} and APC^{Cdh1}, respectively. Emerging evidence has begun to reveal that Cdc20 and Cdh1 have opposing functions in tumorigenesis. Specifically, Cdh1 functions largely as a tumor suppressor, whereas Cdc20 exhibits an oncogenic function, suggesting that Cdc20 could be a promising therapeutic target for combating human cancer. However, the exact underlying molecular mechanisms accounting for their differences in tumorigenesis remain largely unknown. Therefore, in this review, we summarize the downstream substrates of Cdc20 and the critical functions of Cdc20 in cell cycle progression, apoptosis, ciliary disassembly and brain development. Moreover, we briefly describe the upstream regulators of Cdc20 and the oncogenic role of Cdc20 in a variety of human malignancies. Furthermore, we summarize multiple pharmacological Cdc20 inhibitors including TAME and Apcin, and their potential clinical benefits. Taken together, development of specific Cdc20 inhibitors could be a novel strategy for the treatment of human cancers with elevated Cdc20 expression.

Keywords

Cancer; Cdc20; SCF; E3 ligase; Drug; Target

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Conflict of Interest: The authors declare no conflict of interest.

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1. Introduction

Ubiquitination has been characterized to play a critical role in regulating diverse cellular processes including cell cycle progression, cell proliferation, apoptosis, DNA damage, migration and invasion (Hoeller et al., 2006; Nakayama & Nakayama, 2006). It has been well accepted that ubiquitination by the ubiquitin proteasome system (UPS) is a post-translational modification that controls protein degradation thereby the abundance of essential proteins involved in a plethora of cellular processes (Bassermann et al., 2014; Lipkowitz & Weissman, 2011; Wang, Z. et al., 2014). A wealth of evidence has emerged that two related, multi-subunit E3 ubiquitin ligase enzymes, the Anaphase Promoting Complex (APC) and the Skp1-Cullin1-F-box complex (SCF) have been considered as the major driving forces governing cell cycle progression (Lau et al., 2012; Wang et al., 2012; Wang, Z. et al., 2014; Zhang, J. et al., 2014). Notably, APC is the most complex E3 ubiquitin ligase that consists of at least 14 subunits (namely, APC1/TSG24, APC2, APC3/Cdc27, APC4, APC5, APC6/Cdc6, APC7, APC8/Cdc23, APC10/Doc1, APC11, APC13/SWM1, APC15/Mnd2, APC16, and Cdc26) and either one of two co-activators, Cdh1 or Cdc20 (Chang & Barford, 2014; Foe & Toczyski, 2011; Schreiber et al., 2011). Due to its large size and complex nature, the structure of the full APC holoenzyme remained poorly understood until recently, when its structure was elucidated by the Cryo-electron microscopy technology (Chang & Barford, 2014; Chang et al., 2014; Kulkarni et al., 2013). These structural insights support the model that the APC consists of a scaffolding subunit (including APC1, APC4, APC5), a catalytic and substrate recognition subunit (APC2, APC11, APC10), a tetratricopeptide repeat arm (APC3, APC6, APC8), and an accessory subunit (APC13, Cdc26, APC16) (Figure 1) (McLean et al., 2011; Vodermaier et al., 2003). Without a doubt, it is necessary to further determine the architectural details of the APC to aid in further understanding its biological functions.

To exert its biological functions, the APC core is associated with two activators, Cdc20 (cell division cycle 20 homologue, also called Fizzy) and Cdh1 (Cdc20 homologue 1, also known as Fizzy-related protein 1, FZR1), respectively, leading to two distinct E3 ubiquitin ligase complexes, APC^{Cdc20} and APC^{Cdh1} (Penas et al., 2011; Wang, Z. et al., 2013). Cdc20 contains seven WD40 repeats that are necessary for mediating protein-protein interactions (Hartwell et al., 1973). Emerging evidence has also revealed that Cdc20 and Cdh1 control the substrate specificity of the APC core-complex to bind and ubiquitinate target proteins for subsequent degradation. Notably, it has been demonstrated that Cdc20 and Cdh1 recruit their substrates via different motifs. For example, APC^{Cdc20} typically targets its substrates which contain a Destruction-box (D-box) (Clute & Pines, 1999; Michaelis et al., 1997; Nasmyth, 2001), TEK (Jin et al., 2008) or the newly identified ABBA (Di Fiore et al., 2015) motifs (Table 1). On the other hand, APC^{Cdh1} largely recruits substrates with either KEN-box (McGarry & Kirschner, 1998; Petersen et al., 2000), D-box (Bashir et al., 2004; den Elzen & Pines, 2001; Geley et al., 2001; Lindon & Pines, 2004; McGarry & Kirschner, 1998; Petersen et al., 2000; Wei et al., 2004), A-box (Littlepage & Ruderman, 2002), O-box (Araki et al., 2003), CRY box (Reis et al., 2006), LLK (Gao et al., 2009) or GxEN box (Castro et al., 2003) motifs (Table 1). It is still not fully understood how APC^{Cdc20} and APC^{Cdh1} mechanistically recruit their substrates with different motifs, but it provides a

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possible molecular explanation for their distinct roles in tumorigenesis that might stem from their abilities in targeting a different spectrum of substrates for destruction.

Consistent with this notion, although both Cdc20 and Cdh1 can activate the APC E3 ligase, they have distinct biological functions (Clijsters et al., 2013; Yu, 2002). For example, APC^{Cdc20} exerts its function during the metaphase to anaphase transition through destruction of critical cell cycle regulators (Kim & Yu, 2011; Yu, 2007), whereasAPC^{Cdh1} plays a key role in the late M and G1 phases (Hu et al., 2011; Qiao et al., 2010). Moreover, Cdh1 is considered as a tumor suppressor, while Cdc20 exhibits its oncogenic function (Penas et al., 2011; Wang, Z. et al., 2013). It is known that Cdc20 is an essential developmental gene, whose disruption in mice caused embryonic lethality and displayed condensed chromosomes, in part due to aberrant mitotic arrest (Li et al., 2007). Consistently, ablation of endogenous Cdc20 blocks *in vivo* tumorigenesis in a skin-tumor mouse model induced by a two-stage carcinogenesis protocol, largely due to elevated cellular apoptosis (Manchado et al., 2010). Furthermore, depleting endogenous Cdc20 in various cancer cell lines also led to a mitotic arrest followed by cell death. Together, these studies suggest that inhibition of APC^{Cdc20} enzymatic activity might lead to an elevated cellular apoptosis. Although the exact molecular mechanism underlying Cdc20 loss-induced apoptosis remains unknown, these studies strongly argue for Cdc20 as a novel anti-cancer therapeutic drug target. Indeed, inactivating APC by an IR-mimetic inhibitor, pro-TAME, which targets both APC^{Cdc20} and APC^{Cdh1}, also induced cell death in multiple cancer cell lines (Zeng et al., 2010). Therefore, in this article, we summarize the oncogenic role of Cdc20 in a variety of human cancers including pancreatic cancer, breast cancer, prostate cancer, colorectal cancer, lung cancer, glioblastomas, bladder, hepatocellular carcinoma and other cancers. Moreover, we discuss how aberrant overexpression of Cdc20 in various types of human cancers could be used to guide the development and use of Cdc20 inhibitors for treating human cancers. Finally, we describe several Cdc20 inhibitors and their potential clinical benefits.

2. Cdc20 exerts its biological functions largely by targeting its downstream substrates for ubiquitination and subsequent degradation

In recent years, many downstream targets of Cdc20 have been identified by various groups (Table 2). The initial role of Cdc20 was elucidated primarily in regulating cell cycle progression after it was discovered nearly half a century ago (Hartwell et al., 1970). Cells with Cdc20 mutants blocked cell division and stopped cell cycle progression toward anaphase and chromosome segregation (Hartwell et al., 1970). Mechanistically, many identified substrates of Cdc20 are involved in mitotic procession including Securin (Zur & Brandeis, 2001), Cyclin B1 (Lim et al., 1998; Shirayama et al., 1999), Cyclin A (Geley et al., 2001; Ohtoshi et al., 2000), Nek2A (Hames et al., 2001), Cenp-F (Gurden et al., 2010) and p21 (Amador et al., 2007). Further studies implicated Cdc20 in governing cellular apoptosis through regulating the stability of Mcl-1 (Harley et al., 2010) and Bim (Wan et al., 2014). Interestingly, Cdc20 has also been reported to play a key role in ciliary disassembly (Wang, W. et al., 2014) and brain development (Yang et al., 2007; Yang et al., 2009). In the following sections, we will summarize the different biological functions of Cdc20 in cell cycle progression, apoptosis, ciliary disassembly and brain development.

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2.1. Regulation of cell cycle

Different from APC^{Cdh1} with major functions in late M and G1 phases, APC^{Cdc20} plays an indispensable role during the metaphase to anaphase transition by targeting critical cell cycle regulators including Securin (Michaelis et al., 1997; Nasmyth, 2001) and Cyclin B (Clute & Pines, 1999) for ubiquitination-mediated destruction. It has been also identified that Cdc20 binds p21 in a D-box motif-dependent manner to promote the timely degradation of p21 in prometaphase, whereas Skp2 degrades p21 during the G1/S transition (Amador et al., 2007). Another study also proposed that Cdc20-mediated degradation of conductin governs Wnt/β-catenin signaling and controls the cell cycle (Hadjihannas et al., 2012). In line with this finding, Cdc20-resistant conductin blocked Wnt signaling and inhibited colony formation of colorectal cancer cells (Hadjihannas et al., 2012). More recently, one elegant study revealed that APC^{Cdc20} controls cell cycle through temporal degradation of Nek2A and Kif18A (Sedgwick et al., 2013). Notably, Cdc20-mediated Kif18A degradation depends on a C-terminal LR motif, whereas degradation of Nek2A by APC^{Cdc20} depends on an MR motif (Sedgwick et al., 2013). Additionally, Lim *et al.* found that the ubiquitination and degradation of the histone-demethylase PHF8 is also regulated by APC^{Cdc20}, whereas depletion of endogenous PHF8 led to prolonged G2 phase and defective mitosis. Interestingly, PHF8 contains a unique LXPKXLF motif that is required for binding to Cdc20 (Lim et al., 2013), but further studies are required to demonstrate whether other Cdc20 substrates also possess this degron. Moreover, Song *et al.* found that the APC^{Cdc20} complex promoted the degradation of four proteins that are required for spindle assembly including Bard1, HURP, NuSAP, and Hmmr (Song & Rape, 2010), further expanding the role of Cdc20 in spindle checkpoint assembly. On the other hand, Cho *et al.* discovered that APC^{Cdc20} controls mitotic progression via targeting RAP80 (receptor-associated protein 80) (Cho et al., 2012). Given a critical role of RAP80 in DNA damage repair pathway, it suggests a possible role of Cdc20 in DNA damage repair pathway by linking mitotic regulation with chromosome stability control. To this end, it has also been recently demonstrated that degradation of TRRAP (TRansformation/TRanscription domain-Associated Protein) by APC^{Cdc20} is required for a proper condensation of chromatin and chromosome segregation to govern the faithful segregation of duplicated DNA strands (Ichim et al., 2014). Taken together, identifying additional ubiquitin substrates will help us to better appreciate the molecular basis of the essential role of APC^{Cdc20} in timely regulation of cell cycle progression.

2.2. Regulation of apoptosis

In addition to regulating mitotic progression, Cdc20 has been implicated in the regulation of other cellular processes such as apoptosis through targeting Mcl-1 (Harley et al., 2010) and Bim (Wan et al., 2014). It is well known that the Bcl-2 protein family plays a critical role in the apoptotic signaling pathway (Cory & Adams, 2002). The Bcl-2 family of proteins are classified into anti-apoptotic group and pro-apoptotic members (Cragg et al., 2009). The anti-apoptotic members contain Bcl-2, Bcl-xL, Bcl-w and Mcl-1, while the pro-apoptotic Bcl-2 members include Bax, Bak and BH3-only proteins such as Bim (Cory & Adams, 2002). Initiating the spindle assembly checkpoint by Taxol or Nocodazole, largely through Mad2-dependent suppression of APC^{Cdc20} has been used as anti-cancer treatments (Janssen

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& Medema, 2011). Consistently, depleting Cdc20 or pharmacological inhibition of APC leads to elevated cellular apoptosis (Manchado et al., 2010), but the underlying molecular mechanism remains unclear. To this end, we recently reported that Cdc20 governs apoptosis largely through controlling the ubiquitination and stability of the pro-apoptotic protein, Bim (Wan et al., 2014). Notably, the pro-apoptotic protein Bim has attracted increasing attention as a pivotal regulator of apoptosis. The Bim expression level is controlled at both transcriptional and post-transcriptional levels in a cell- and tissuespecific manner (Akiyama et al., 2009). Moreover, Bim knockout mice exhibit a systematic lupus erythematosus-like autoimmune disease along with an abnormal accumulation of hematopoietic cells, suggesting that Bim is involved in the regulation of hematopoietic cells and the immune system (Akiyama & Tanaka, 2011). Further studies have also revealed critical roles for Bim in bone homeostasis and tumorigenesis, in part due to its critical contribution to trigger apoptosis (Akiyama & Tanaka, 2011).

Several E3 ligases have been reported to negatively regulate Bim stability. For example, c-Cbl and the ElonginB/C-Cullin2-CIS E3 ligase have been found to promote Bim degradation (Thien et al., 2010; Zhang et al., 2008). However, controversial results have been reported by different groups, thereby refuting a possible physiological role of either c-Cbl or CIS in Bim ubiquitination (Akiyama et al., 2003; Wiggins et al., 2007). Recently, it has been reported that Bim is degraded by β-TRCP (Moustafa-Kamal et al., 2013). However, as β-TRCP-mediated destruction of Bim required prior-phosphorylation, which is restricted to late G1 phase and is not relevant to the chemo-resistance associated with Taxol treatment (Dehan et al., 2009). On the other hand, we reported that APC^{Cdc20} is a physiological E3 ligase that governs the ubiquitination and destruction of Bim (Wan et al., 2014). In support of this concept, we provided evidence to validate Bim as an APC^{Cdc20} ubiquitin substrate. First, Bim abundance displayed a dramatic reduction during mitosis when APC^{Cdc20} is most active. Second, depletion of Cdc20 in various cell lines led to a significant upregulation of Bim abundance. Third, Bim was stabilized in Cdc20-depleted M phase cells. Fourth, Cdc20 specifically interacts with Bim through its C-terminal WD40 repeats motifs. Fifth, Bim contains two evolutionarily conserved D boxes. As such, Cdc20 promotes Bim ubiquitination and subsequent degradation in a D-box-dependent manner. More importantly, we pinpointed the physiological function of Cdc20-mediated Bim degradation. Specifically, we found that hyperactive Cdc20 contributes to chemoresistance by promoting Bim destruction in HTLV-1 positive adult T-cell leukemia cells. Notably, depletion of Cdc20 induced chemoradiation sensitization in head and neck cancer cells (Wan et al., 2014). These studies therefore reveal that Cdc20 plays a crucial role in promoting the survival of cancer cells through inhibiting apoptosis and that Cdc20 allows the acquisition of chemo- or radioresistance partly by enhancing Bim destruction to evade apoptosis triggered by chemotherapeutic agents and/or irradiation. It has been suggested that anti-mitotic reagents that were used as anti-cancer agents activate the Spindle Assembly Checkpoint (SAC) primarily by suppressing APC^{Cdc20}, and induce apoptosis after prolonged-treatment (Huang et al., 2009; Wan et al., 2014). Interestingly, one excellent study recently demonstrated that the mitotic checkpoint complex binds a second Cdc20 to inhibit active APC and this is essential for the SAC (Izawa & Pines, 2015). It is important to note that Cdc20 could induce apoptosis through promoting the degradation of anti-apoptotic

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protein Mcl-1 (Harley et al., 2010). Another study suggests that Cdc20 governs apoptosis via mediating the degradation of PC-PLC (phosphatidylcholine specific phospholipase C) in hepatocellular carcinoma cells (Chen et al., 2010). Altogether, it is eager to further mechanistically define the role of Cdc20 involved in the regulation of apoptosis in human cancer cells.

2.3 Brain development and other functions

Yang *et al.* identified that APC^{Cdc20} triggered presynaptic differentiation through degradation of NeuroD2, suggesting a potential role for Cdc20 in neuronal connectivity and plasticity in the brain (Yang et al., 2007; Yang et al., 2009). Consistently, Kim *et al.* found that Cdc20 has an essential function in dendrite morphogenesis in postmitotic neurons (Kim, A. H. et al., 2009). Remarkably, HDAC6 (histone deacetylase 6) promoted the polyubiquitination of Cdc20, activated Cdc20 activity, and enhanced the differentiation of dendrites, suggesting that Cdc20 is involved in neuronal connectivity and plasticity (Kim, A. H. et al., 2009). In addition to its role in neuronal differentiation, Wang *et al.* discovered a novel role for APC^{Cdc20} beyond cell cycle control and apoptosis, and implicated its function in ciliary disassembly in part through regulating Nek1 stability (Wang, W. et al., 2014). Furthermore, Chun *et al.* discovered that overexpression of Cdc20 enhanced polyubiquitination and proteosomal degradation of REV1, which is a specialized DNA polymerase for DNA repair (Chun et al., 2013). Notably, Sp100, which participates in viral resistance, transcriptional regulation, and apoptosis, was degraded by APC^{Cdc20} (Wang et al., 2011). These recent studies thus support the notion that beyond its canonical role in cell cycle regulation, Cdc20 may participate in many other important cellular processes including neuronal differentiation, ciliary formation or DNA damage repair. However, further in-depth studies are required to understand whether these new functions depend on its E3 ligase role or not (Wan et al., 2011), and whether they are intrinsically linked to its pivotal cell cycle role.

3. Cdc20 is regulated by multiple upstream factors

In addition to the extensive research efforts in determining the downstream substrates of Cdc20, recent studies have begun to define the upstream regulators of Cdc20 (Fang et al., 1998; Kidokoro et al., 2008; Reimann, Freed, et al., 2001). Here, we summarize the upstream regulators of Cdc20, allowing the readers to fully appreciate the complicated Cdc20 regulatory network system. Notably, it has been reported that p53 negatively regulates Cdc20 expression, which is supported by the demonstration that overexpression of p53 inhibited Cdc20, whereas depletion of p53 induced Cdc20 expression (Kidokoro et al., 2008). Specifically, Cdc20 expression was inhibited by genotoxic stresses in a p53-dependent manner largely through CDE-CHR elements present in the Cdc20 promoter. Moreover, this study also indicates that p53 exerted its anti-tumor activity via the indirect regulation of Cdc20 (Kidokoro et al., 2008). Strikingly, DNA damage-induced p53 downregulated Cdc20 through direct binding to its promoter, leading to chromatin remodeling (Banerjee et al., 2009). Furthermore, the function of p53 in HSF1 (heat shock factor 1)-mediated mitotic regulation and genomic instability could also be through regulation of the interaction between Cdc20 and HSF1 (Kim, H. S. et al., 2009).

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Additionally, the spindle checkpoint protein Mad2 was found to inhibit APC^{Cdc20} activity through forming a Mad2-Cdc20-APC complex to control anaphase initiation (Fang et al., 1998). Moreover, the F-box protein Emi1 (early mitotic inhibitor 1) controls mitosis via binding to APC^{Cdc20} as a pseudo-Cdc20-substrate and inhibiting its E3 ligase activity (Reimann, Freed, et al., 2001; Reimann, Gardner, et al., 2001). Another study showed that Bub1 and Bub3 blocked mitosis through suppression of APC^{Cdc20}-mediated degradation of Pds1 and Cyclin B (Fraschini et al., 1999). Interestingly, USP44 (ubiquitin-specific protease 44) deubiquitinated Cdc20 and blocked premature activation of APC via stabilization of the APC-inhibitory Mad2-Cdc20 complex (Stegmeier et al., 2007). Notably, tumor suppressor RASSF1A was reported to also inhibit APC^{Cdc20} and block degradation of Cyclins A and B at the spindle poles (Song et al., 2004). In addition to these regulatory mechanisms mediated by protein components, miR-449 and miR-494 have been discovered to regulate Cdc20 mRNA levels (Lize et al., 2010; Yamanaka et al., 2012). Hence, we are of the opinion that further in-depth exploration is required to determine more Cdc20 upstream regulators for understanding how Cdc20 function is misregulated in tumorigenesis.

4. Role of Cdc20 in human malignancies

Mounting evidence has revealed that Cdc20 plays an oncogenic role in human tumorigenesis. Overexpression of Cdc20 was observed in a variety of human tumors. Moreover, higher expression of Cdc20 is associated with clinicopathological parameters in various types of human cancers. Therefore, in the following sections, we will summarize the critical role of Cdc20 in a wide range of human cancers.

4.1. Pancreatic cancer

Pancreatic cancer, one of the most common malignancies, is the fourth leading cause of cancer-related death in the United States (Siegel et al., 2015). It is estimated that 48,960 Americans are expected to be diagnosed with pancreatic cancer and 40,560 people will die from this disease in the US in 2015 (Siegel et al., 2015). Currently, the 5-year relative survival rate for pancreatic cancer is only 7%. Pancreatic cancer has been shown to display Cdc20 overexpression at high frequencies. For example, over-expression of Cdc20 was detected in pancreatic tumor tissues compared with normal adjacent tissues from pancreatic cancer patients (Li et al., 2003). Interestingly, STK15, identified as a Cdc20-associated protein, was also overexpressed in 58% of pancreatic tumor tissues. However, STK15 level was not correlated with tumor size, differentiation, and metastasis (Li et al., 2003). In line with this finding, Chang *et al.* performed an excellent retrospective study and identified Cdc20 expression as a useful biomarker in pancreatic cancer prognosis (Chang et al., 2012). Specifically, Cdc20 expression is significantly higher in pancreatic tumor tissues than in chronic pancreatitis tissue and normal pancreatic tissues (Chang et al., 2012). Notably, high expression of Cdc20 was associated with poor differentiation and a lower 5-year recurrence-free survival rate (Chang et al., 2012). Consistently, depletion of Cdc20 suppressed cell growth in human pancreatic cancer cells and induced G2/M cell cycle arrest (Taniguchi et al., 2008). More importantly, depletion of Cdc20 led to enhanced cytotoxicity upon paclitaxel treatment, and increased effects of gamma-irradiation against pancreatic cancer

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cells (Taniguchi et al., 2008). Taken together, Cdc20 could be a useful marker of pancreatic cancer progression and a novel therapeutic target for the treatment of this deadly disease.

4.2. Breast cancer

Breast cancer is the most commonly diagnosed cancer and the second most lethal malignancy in women in the US. About 60,290 cases of female breast carcinoma are expected to be diagnosed in 2015 (Siegel et al., 2015). This disease is the leading cause of cancer death in women aged 20-59 years. These data suggest that early diagnosis and prevention are required to reduce mortality associated with this disease. To this end, some studies have demonstrated that Cdc20 plays an essential role in breast cancer progression. Specifically, Yuan *et al.* reported that the mRNA and protein levels of Cdc20 were significantly higher in breast cancer cells and high-grade primary breast cancer tissues (Yuan et al., 2006). In support of this notion, another independent study screened Cdc20 expression in 445 breast cancer patients with up to 20 years of follow-up and validated that Cdc20 is highly expressed in breast cancer patients (Karra et al., 2014). Importantly, overexpression of Cdc20 was associated with an aggressive course of breast cancer. Consistently, high expression of Cdc20 and securin are correlated to extremely poor outcome of breast cancer patients (Karra et al., 2014). However, it is important to recognize that further study is necessary to determine the oncogenic role of Cdc20 in breast tumorigenesis.

4.3. Prostate Cancer

Prostate cancer is the most frequently diagnosed tumors in men and the leading cause of cancer-related death followed by cancers of lung and bronchus in the US (Siegel et al., 2015). The treatment of prostate cancer has been improved due to the use of widespread prostate-specific antigen (PSA) testing for early detection of asymptomatic prostate cancer (Siegel et al., 2015), but there is an urgent need to develop new treatments for patients with castration resistance in late stage prostate cancers. To this end, several studies have identified that Cdc20 is involved in prostate tumorigenesis. One study has shown that Daxx, an APC inhibitor, interacts with Cdc20 and inhibits the degradation of APC^{Cdc20} substrates, leading to a transient delay in mitotic progression and chromosome instability (Kwan et al., 2013). Consistently, Daxx is overexpressed in prostate cancer tissues and positively correlated with the Gleason score and metastasis (Kwan et al., 2013). Moreover, LATs (L-type amino acid transporters) protein was expressed at all stages of prostate cancer. Inhibition of LAT suppressed tumor growth, cell cycle progression, and metastasis partly through downregulation of M-phase cell cycle genes including Cdc20 and mTORC1 in prostate cancer (Wang, Q. et al., 2013). Another study has suggested that knockdown of COX-2 by shRNA or using pharmacological COX inhibitors inhibited prostate cancer cell proliferation and arrested cell cycle progression via suppressing several key proteins in the kinetochore/centromere assembly including Cdc20 (Bieniek et al., 2014). Surprisingly, there is no direct evidence to show the physiological role of Cdc20 in prostate cancer development and progression, which awaits further studies.

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4.4. Lung cancer

Lung cancer is the most common cause of cancer death, which kills more than 158,000 people in the US this year (Siegel et al., 2015). The 5-year relative survival rate is currently 18% for lung cancer patients. Using spiral computed tomography for screening for early signs of lung cancer, the survival rate of lung cancer has been improved (Siegel et al., 2015). It has been documented that lung cancer can be categorized into two types including non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Multiple studies have indicated that Cdc20 is highly expressed, and could be a potential prognostic marker in human NSCLC (Kato et al., 2012). Notably, relatively higher expression of Cdc20 was associated with pleural invasion, and shorter 5-year overall survival in NSCLC in a gender-specific manner (male specificity) (Kato et al., 2012). In line with this notion, deregulation of Cdc20 was observed in lung adenocarcinoma tissue samples (Zhang, W. et al., 2014). Notably, knockdown of Cdc20 inhibited cell growth, induced G2/M cell cycle arrest, and retarded colony formation of lung cancer cells (Kidokoro et al., 2008). However, the exact molecular mechanism of Cdc20-mediated lung tumorigenesis is still elusive and needs to be further explored.

4.5. Colorectal cancer

Colorectal cancer is the second leading cause of cancer death in the United States (Siegel et al., 2015). Nearly 49,700 Americans are anticipated to die from this deadly disease in 2015 (Siegel et al., 2015). Colorectal cancer has been known to contain numerous genetic and physiological alterations, leading to enhanced cell growth and survival (Kuipers et al., 2013). Emerging evidence has revealed that Cdc20 is overexpressed in colorectal cancer cell lines and primary cancer tissues compared with normal colon epithelial cells and adjacent noncancerous tissue samples, respectively (Wu et al., 2013). Remarkably, Cdc20 expression was associated with clinical stage, metastasis, and shorter overall survival, suggesting Cdc20 could serve as an independent prognostic biomarker for human colorectal cancer (Wu et al., 2013). In support of this finding, another independent study also identified that expression of Cdc20 was increased by more than five-fold in 77% of colorectal cancer tissues (Kidokoro et al., 2008). In line with this finding, depletion of Cdc20 inhibited Wnt signaling via upregulation of conductin. Moreover, Cdc20-resistant conductin suppressed Wnt signaling and subsequently attenuated colony formation of colorectal cancer cells (Hadjihannas et al., 2012). Interestingly, in a separate study, it has also been observed that expression of Cdc20 was decreased in the human colorectal cancer tissues compared with the proximal tissues (Storcelova et al., 2013), suggesting that further in-depth investigation is needed to define the exact role of Cdc20 in colorectal cancer.

4.6. Hepatocellular carcinoma

Hepatocellular carcinoma is a common malignant cancer worldwide, especially in Asian countries including China. Cdc20 has been validated to be critically involved in hepatocellular carcinoma. Li *et al.* reported that overexpression of Cdc20 was observed in 68% hepatocellular carcinoma tissues compared to adjacent non-tumor liver tissues. Strikingly, high levels of Cdc20 were positively correlated with gender, tumor differentiation, and TNM stage (Li et al., 2014). In further support of this concept, depletion

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of endogenous Cdc20 decreased cell proliferation and induced G2/M cell cycle phase in hepatocellular carcinoma cells (Li et al., 2014). Moreover, one recent study demonstrated that SIRT2 regulated APC E3 ligase activity partly through deacetylating Cdc20, and that *Sirt2*-deficient male mice developed hepatocellular carcinoma (Kim et al., 2011). Therefore, Cdc20 could be a promising therapeutic target for human hepatocellular carcinoma, while additional studies are warranted to validate the clinical benefits of Cdc20 inhibition in treating liver cancers.

4.7. Glioblastoma

Glioblastoma is the most common type of malignant brain tumor. The expression of Cdc20 was up-regulated in glioblastoma and down-regulated in low-grade gliomas (Marucci et al., 2008). Overexpression of Cdc20 is highly related to glioblastoma (Marucci et al., 2008). Notably, Dai *et al.* further found that Cdc20 plays a critical role in FoxM1-triggered cell survival in human glioblastoma (Dai et al., 2013). Furthermore, MIIP (migration and invasion inhibitor protein) interacts with Cdc20 and inhibits APC-mediated degradation of Cyclin B1, thereby inhibiting of glioma development and progression (Ji et al., 2010), while the physiological and pathological contribution of Cdc20 to glioblastoma needs further validation and investigation.

4.8. Gastric cancer

Gastric carcinoma is a common and lethal malignancy in the world. Importantly, Cdc20 was found to have a pivotal role in governing the progression of gastric cancer. Specifically, the expression of Cdc20 was upregulated in various gastric cell lines and tumor tissue samples (Kim et al., 2005). Furthermore, another independent study confirmed that Cdc20 expression was significantly higher in gastric cancer tissues than in corresponding noncancerous tissues (Ding et al., 2014). Notably, overexpression of Cdc20 was positively associated with tumor size, TNM stage, histological grade, and lymph node metastasis. More importantly, upregulation of Cdc20 was closely correlated with poor overall survival (Ding et al., 2014). Taken together, Cdc20 may be an independent marker for predicting clinical outcomes of gastric cancer and therefore, inhibiting Cdc20 might be a promising anti-gastric cancer approach at least for patients with elevated Cdc20 expression levels.

4.9. Other types of human cancers

Additional studies have validated the oncogenic function of Cdc20 in other cancers such as bladder cancer, oral cancer and cervical cancer. For instance, high expression of Cdc20 was observed in urothelial carcinoma of the human bladder (Choi et al., 2013; Kidokoro et al., 2008). This finding was confirmed by microarrays in urinanry bladder cancer tissues (Zaravinos et al., 2011). Notably, upregulation of Cdc20 was correlated with advanced age and stage, high grade, distant metastasis, shorter recurrence-free survival and poorer overall survival in bladder cancer patients (Choi et al., 2013). Furthermore, Mondal *et al.* observed up-regulation of Cdc20 in several oral squamous cell carcinoma cell lines and primary head and neck tumors (Mondal et al., 2007; Thirthagiri et al., 2007). Moreover, overexpression of Cdc20 in OSCC cell lines led to aneuploidy due to deregulating the activity of APC in promoting premature anaphase (Mondal et al., 2007). Furthermore, high levels of Cdc20

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protein expression were observed in 37% of oral squamous cell carcinoma tissues. Strikingly, higher expression of Cdc20 was associated with shorter cancer-specific survival rate in oral cancer. These results thus indicate that Cdc20 may serve as an independent prognostic factor and a therapeutic target for oral cancer (Moura et al., 2014). In addition, Cdc20 was overexpressed in the high-grade squamous intraepithelial lesions and invasive squamous cell carcinoma in cervical cancer (Kim et al., 2014). Consistently, another independent study also defined that Cdc20 was up-regulated in invasive cervical cancers (Rajkumar et al., 2011). Recently, inhibition of Cdc20 was also reported to lead to G2/M cell cycle arrest and tumor growth inhibition in melanoma (Majumder et al., 2014). These results suggest that Cdc20 could function as a common oncoprotein in a majority of human cancers, therefore advocating for additional scientific investigation as well as translational studies to validate the clinical benefits of Cdc20 pharmacological inhibitors in treating various types of human cancers that are driven by Cdc20 overexpression.

5. Targeting Cdc20 for cancer therapies

Given the important oncogenic role of Cdc20 in tumorigenesis, its inhibitors could provide a therapeutic window in a range of human malignancies. It has been known that proteasome inhibitors can block ubiquitination-dependent proteolysis. Thus, many scientists have developed multiple proteasome inhibitors for treating human cancers (Adams, 2004; Allegra et al., 2014; Skaar et al., 2014). Remarkably, proteasome inhibitor bortezomib (Velcade™ Millennium Pharmaceuticals, Inc) and Carfizomib have been approved for treating multiple myeloma (Andreu-Vieyra & Berenson, 2014; Caravita et al., 2006; Mahindra et al., 2012). Moreover, several other proteasome inhibitors including Oprozomib, Delanzomib, and Marizomib have been used in clinical trials (Dou & Zonder, 2014). Due to their inhibitory effects on degradation of many key protein, these proteasome inhibitors have been shown to be toxic to normal cells, leading to numerous side effects including fever, anemia, diarrhea and nausea in patients (Dou & Zonder, 2014). These undesirable effects could be overcome by targeting specific components of the UPS system. To this end, discovering and developing small molecule inhibitors specifically targeting the Cdc20 oncoprotein could possibly be a novel strategy for the treatment of many types of human cancers.

5.1. TAME and pro-TAME

Studies from the King group have elegantly revealed that a small molecule, named as TAME (tosyl-L-arginine methyl ester), could bind APC and suppress its activation by Cdc20 and Cdh1 (Figure 2). A previous study from this group showed that TAME is an inhibitor of cyclin proteolysis in mitotic Xenopus egg extract (Verma et al., 2004). Moreover, they found that TAME reduced Cdc20 association with the APC and subsequent inhibited APC E3 ligase activity (Zeng et al., 2010). It is to be noted that TAME also inhibited the binding of Cdh1 to APC and reduced APC activation (Zeng et al., 2010). Given that TAME is not cell permeable, a TAME prodrug (pro-TAME), which can be processed by intracellular esterases to yield the active form of TAME, was synthesized. Further evidence revealed that pro-TAME disrupted the APC-Cdc20/Cdh1 interaction to reduce APC activation. Moreover, pro-TAME was found to induce mitotic arrest in the absence of spindle damage (Zeng et al., 2010). Intriguingly, pro-TAME in combination with

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microtubule inhibitors could have greater effects on enhancing cell death (Zeng et al., 2010). Strikingly, this group identified the precise mechanism by which TAME suppressed APC activation. TAME inhibits the binding of free Cdc20 to the APC. On the other hand, in the absence of APC substrates, TAME can promote Cdc20 dissociation from the APC by induction of Cdc20 auto-ubiquitination in its N-terminal region (Zeng & King, 2012). This process is suppressed by the binding of APC substrates such as cyclin B1, indicating that TAME stabilizes cyclin B1 by prematurely terminating its ubiquitination (Zeng & King, 2012). Further investigation is required to determine whether TAME and pro-TAME are clinically useful to retard tumorigenesis *in vivo*.

5.2. Apcin

Recently, another elegant study from the King laboratory validated that another small molecule, apcin (APC inhibitor), binds Cdc20 and prevents substrate recognition, thereby leading to competitively inhibition of the ubiquitination of Cdc20 substrates (Sackton et al., 2014). This group further explored the crystal structure of the apcin-Cdc20 complex and validated that apcin occupies the D-box-binding pocket within the WD40 domain (Sackton et al., 2014). It has been known that substrates can promote cooperative Cdc20 binding to the APC via a co-receptor interaction. To this end, further analysis has validated that apcin also blocks substrate-induced Cdc20 loading onto the APC core complex (Sackton et al., 2014). Interestingly, apcin is highly specific for stabilization of substrates that interact with APC through a D-box motif including cyclin B1 and securin, while TAME inhibited the degradation of all APC substrates due to its ability in directly blocking the recruitment of Cdc20 to the APC core complex (Sackton et al., 2014). Since apcin and TAME have distinct mechanisms to inhibit APC activation, the combination of apcin and TAME caused a synergistic stabilization of APC substrates such as cyclin B1, securin, and cyclin A2. Notably, apcin and pro-TAME synergized to increase the mitotic fraction in human cancer cell lines (Sackton et al., 2014). Taken together, the function of apcin can be dramatically enhanced by the combination of TAME, arguing that simultaneous inhibition of multiple protein-protein interactions by multiple compounds could represent a novel approach for the therapeutic targeting of protein complexes.

5.3. Withaferin A

Withaferin A, a bioactive component from Withania somnifera, has been confirmed to exhibit its anti-tumor activity against various types of human cancers including leukemia, pancreatic cancer, breast cancer and colorectal cancer. One recent study showed that withaferin A decreased STAT3 and induced cell death in neuroblastoma and multiple myeloma (Yco et al., 2014). Li *et al.* reported that withaferin A enhanced oxaliplatin-induced growth suppression and apoptosis via reactive oxygen species-mediated inactivation of the PI3K/Akt pathway in pancreatic cancer cells (Li et al., 2015). Moreover, withaferin A was found to inhibit canonical and constitutive NF-κB activities, resulting in induction of cellular apoptosis in lymphoma lines (Jackson et al., 2014). Strikingly, withaferin A eliminates cancer stem cells in ovarian cancer cells, leading to reduction in tumor growth and inhibition of metastasis in an ovarian orthotopic mouse model (Kakar et al., 2014). Notably, one elegant study demonstrated that withaferin A exerts its anti-tumor effects on breast cancer through regulation of ERK (extracellular signal-regulated kinase)/RSK

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(ribosomal S6 kinase) and DR5 (death receptor 5) as well as Elk1 (ETS-like transcription factor 1) and CHOP (C-EBP homologous protein) (Nagalingam et al., 2014). Furthermore, withaferin A treatment led to G2/M phase arrest and apoptosis in colorectal cancer cell lines (Das et al., 2014). Importantly, withaferin A exerts its anti-cancer activity through enhanced degradation of Cdc20 and Mad2, and also blocks SAC function, leading to mitotic delay, indicating that suppressing Cdc20 activity could be one molecular mechanism underlying the anti-cancer nature of Withaferin A (Das et al., 2014).

5.4. NAHA

It has been reported that NAHA, a N-alkylated amino acid-derived sulfonamide hydroxamate, has been shown to inhibit the expression of Cdc20 in breast cancer cells, but the underlying molecular mechanisms remains largely unclear (Jiang et al., 2012). Specifically, NAHA was found to enhance cell proliferation inhibition potency (Stanger et al., 2006). Further study revealed that NAHA inhibited proliferation and colony formation together with decreased Cdc20 levels. Moreover, NAHA retarded cell adhesion, invasion, and migration partly through inhibiting secretion of uPA(urokinase-type plasminogen activator) (Jiang et al., 2012). Notably, NAHA also inhibited breast cancer cell-mediated angiogenesis in part via down-regulation of VEGF (vascular endothelial growth factor). Consistent with this finding, NAHA decreased tumor volume and tumor weight as well as angiogenesis in mouse xenograft model of breast cancer (Jiang et al., 2012). However, further in-depth study is required to determine mechanistically how NAHA regulates the expression of Cdc20 in human cancer cells.

5.5. Ganodermanontriol

Ganodermanontriol (GDNT), a ganoderma alcohol from medicinal mushroom, has been discovered to inhibit cell proliferation via targeting Cdc20 in breast cancer cells (Jiang et al., 2011). Mechanistically, GDNT treatment led to the inhibition of cell growth, colony formation and invasion partly through down-regulation of Cdc20, uPA and uPAR (urokinase-type plasminogen activator receptor) expression (Jiang et al., 2011). Moreover, MycoPhyto® Complex (MC), a novel medicinal mushroom blend, was also identified to inhibit the expression of multiple cell cycle regulatory genes including Cdc20, leading to inhibition of cell proliferation and induction of cell cycle arrest as well as suppressing cell invasiveness in breast cancer cells (Jiang & Sliva, 2010). Altogether, GDNT and MC could be non-specific Cdc20 inhibitors.

5.6. Genistein

Genistein, a phytoestrogenic isoflavonid, has been believed to have pleiotropic biological effects in human malignancies, with relatively low toxicity to normal cells (Banerjee et al., 2008). A growing body of data implicates that genistein as a protein tyrosine kinase inhibitor that inhibits cell growth, migration, invasion, angiogenesis, and metastasis, and induces apoptosis and cell cycle arrest through regulation of multiple cellular signaling pathways (Sarkar et al., 2010). Emerging evidence also demonstrated that genistein deregulates Akt, NF-κB, Wnt and Hedgehog signaling pathways in human cancers (Sarkar et al., 2006). Further study has demonstrated that genistein exhibits its anti-carcinogenic properties through down-regulation of core regulatory genes including Cdc20 in primary glioblastoma,

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rhabdomyosarcoma, hepatocellular carcinoma and human embryonic carcinoma cells (Regenbrecht et al., 2008). In addition, recent study has also indicated that genistein governed the expression of Cdc20, leading to control of cell cycle in breast cancer cells (Zhang et al., 2015). Interestingly, emerging evidence has shown that genistein upregulated many genes involved in cell cycle such as Cdc20 in breast cancer patients (Shike et al., 2014). Therefore, it is necessary to further determine the role of genistein in breast cancer and whether inhibition of Cdc20 is the major signaling pathway through which genistein exerts its anti-cancer effects.

5.7. CFM-4 and BCHHD

CARP-1 is a peri-nuclear phosphoprotein that regulates cell growth and apoptosis. Studies have revealed that CARP-1 is a part of the NF-κB proteome and β-catenin signaling pathways (Jamal et al., 2014). Moreover, CARP-1 is also an co-activator of steroid/thyroid nuclear receptors. Furthermore, CARP-1 was found to bind APC2 and Cdc20 as well as Cdh1 (Puliyappadamba et al., 2011). CFM-4 (CARP-1 functional mimetic 4) was discovered to disrupt the CARP-1/APC-2 binding. CFM-4 prevents the binding between CARP-1 and APC2, leading to cell cycle arrest and apoptosis. CFM-4-induced apoptosis is involved in down-regulation of Cdc20 in breast cancer cells (Puliyappadamba et al., 2011). Additionally, CFM-4 inhibited cell growth and invasion in malignant pleural mesothelioma (Jamal et al., 2014). Moreover, the 6-brominated coumarin hydrazide-hydrazone derivative (BCHHD) 7c was recently found to inhibit Cdc20 expression in drug-resistant pancreatic cancer cells, suggesting that BCHHD 7c could be a potent anti-tumor drug to overcome drug resistance in pancreatic cancer (Nasr et al., 2014), but the contribution of the Cdc20 pathway in this process needs additional in-depth investigation.

6. Conclusions and future perspectives

In conclusion, as Cdc20 is critically involved in human tumorigenesis, development of specific Cdc20 inhibitors could be a strategy for improving the treatment of human cancers. It is noteworthy that Apcin is a specific inhibitor with a direct action against the APC^{Cdc20} complex, while other inhibitors are not specific to target Cdc20. Therefore, more efforts are needed to discover other more specific Cdc20 inhibitors. We hope this article could stimulate more research efforts to develop specific Cdc20 inhibitors as anti-cancer agents. This is an important research direction given that increasing evidence suggest Cdh1 as a tumor suppressor while Cdc20 as an oncogene, therefore excluding the usage of pan-APC inhibitors, but advocating for specific Cdc20 inhibitors as a novel anti-cancer approach. One alternative approach may be to regulate Cdc20 upstream regulators including p53, Emi1, and USP44. On the other hand, it is also feasible to inhibit the E3 ubiquitin ligase activity of the APC^{Cdc20} complex to block Cdc20 oncogenic function for cancer therapy. Due to their non-toxic nature, inhibiting Cdc20 by natural agents such as withaferin, GDNT, genistein could be a safer approach for better treatment of human cancers. We believe that deeper investigation is required to explore the mechanisms of Cdc20-mediated tumorigenesis, which will provide the rationale for developing specific Cdc20 inhibitors as effective anti-cancer agents in the near future. For future studies to determine the role of Cdc20, identification of its additional ubiquitin substrates by novel methods is critical (Figure 3). It

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is also important to explore the novel role of Cdc20 outside cell cycle progression control and apoptosis. To fully understand the function of Cdc20 in tumorigenesis, generation of Cdc20 conditional knockout (KO) or knockin mouse models will be necessary to better appreciate the physiological role of Cdc20 in various human cancer settings (Figure 3). To achieve better treatment outcome, Cdc20 inhibitors with greater specificity and efficacy should be developed and validated by both *in vitro* cell culture based studies and *in vivo* mouse modeling studies.

Acknowledgments

This work was supported in part by the NIH grants to W.W. (GM094777 and CA177910). W.W. is an ACS research scholar and a LLS research scholar. This work was also supported by grant from NSFC (81172087) and a project funded by the priority academic program development of Jiangsu higher education institutions.

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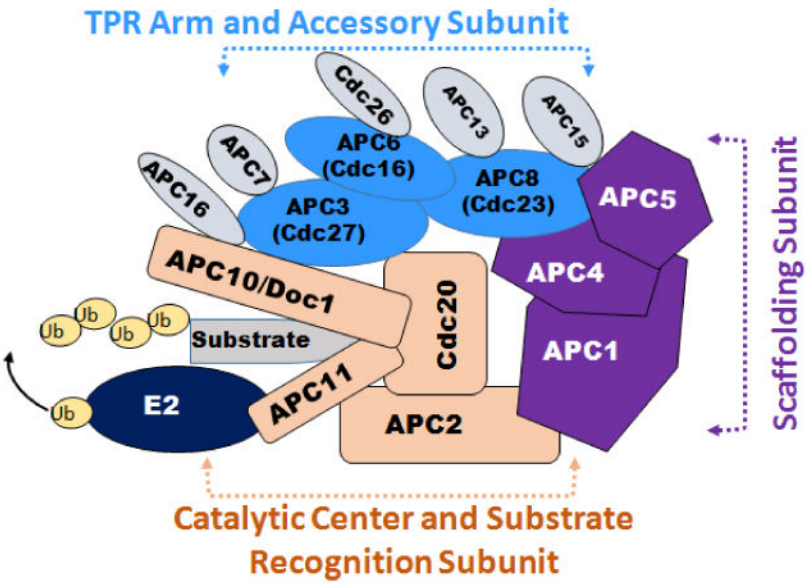

Figure 1. A schematic illustration of the APC ubiquitin E3 ligase complex

The APC core complex includes a scaffolding subunit (APC1, APC4, APC5), a catalytic and substrate recognition subunit (APC2, APC11, APC10), a tetratricopeptide repeat arm (APC3, APC6, APC8), and an accessory subunit (APC13, Cdc26, APC16).

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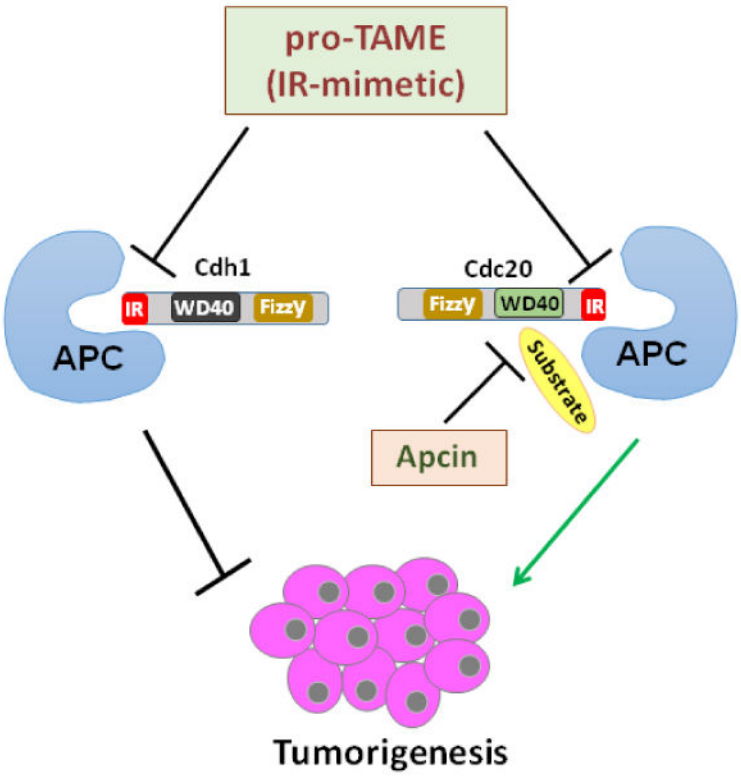

Figure 2. A schematic illustration of how pro-TAME and Apcin inhibit the APC E3 ligase activity

pro-TAME, an IR-mimetic, directly blocks the recruitment of APC coactivators including Cdc20 and Cdh1, to the APC core complex. On the other hand, Apcin occupies the D-box-binding pocket on the side of the WD40 domain of Cdc20 and therefore blocking substrate-induced Cdc20 loading onto the APC core complex.

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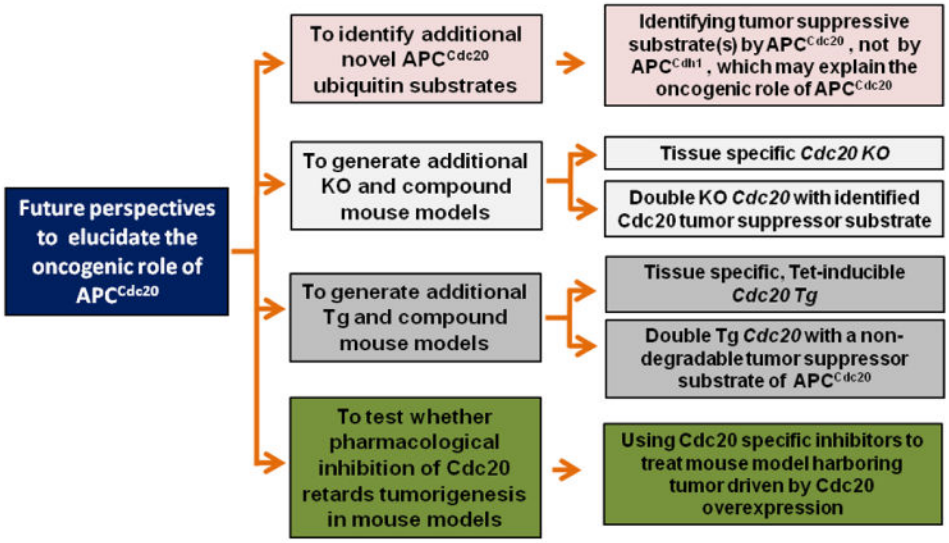

Figure 3. Future Perspectives

A schematic illustration of the future studies to determine the oncogenic role of Cdc20 in various cancer settings, as well as to further validate whether pharmacological inhibition of Cdc20 could achieve clinical benefits for cancer patients, at least those with elevated Cdc20 expression levels.

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Table 1

The different types degrons that are recognized by Cdh1 and Cdc20

APC^{Cdh1}				APC^{Cdc20}			
Degron	**Consensus**	**Representative Substrates**	**References**	**Degron**	**Consensus**	**Representative Substrates**	**References**
D-box	RxxLx(2-5)N/D/E	Cyclin A2, Plk1, Skp2, Geminin, Cdc6	(Bashir et al., 2004; den Elzen & Pines, 2001; Geley et al., 2001; Lindon & Pines, 2004; McGarry & Kirschner, 1998; Petersen et al., 2000; Wei et al., 2004)	**D-box**	RxxLx(2-5)N/D/E	Cyclin B1, Securin, etc.	(Clute & Pines, 1999; Michaelis et al., 1997; Nasmyth, 2001)
KEN box	KENxxD/Q/E/N	Geminin, Cdc6	(McGarry & Kirschner, 1998; Petersen et al., 2000)	**TEK**	TEK	Securin	(Jin et al., 2008)
A box	QRVL	Aurora A, Aurora B	(Littlepage & Ruderman, 2002)	**ABBA Motif**	KxxFxxYxDxxE	Cyclin A1, Cyclin A2	(Di Fiore et al., 2015)
O box	paspLtekNak	ORC1	(Araki et al., 2003)	**LXPKXLF Motif**	LXPKXLF	PHF8	(Lim et al., 2013)
GxEN	GxEN	X-kid	(Castro et al., 2003)	**LR Motif**	LR	Kif18A	(Sedgwick et al., 2013)
LLK	LLK	Claspin	(Gao et al., 2009)	**MR Motif**	MR	Nek2A	(Sedgwick et al., 2013)
CRY	CRY	Cdc20	(Reis et al., 2006)				

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Table 2

Summary of the identified ubiquitination substrates for APC^{Cdc20}

Substrates	Functions/signaling pathways of substrates	Coactivators	References
Bard1	Controls spindle pole formation	Cdc20/Cdh1	(*Song & Rape, 2010*)
Bim	Plays key roles in regulating apoptosis	Cdc20	(Wan et al., 2014)
Cenp-F	Functions in kinetochore and chromosome segregation in mitosis	Cdc20	(*Gurden et al., 2010*)
Conductin	Inhibits the Wnt signaling pathway	Cdc20	(*Hadjihannas et al., 2012*)
Cyclin A	Controls S phase and G2/M transition	Cdc20/Cdh1	(*den Elzen & Pines, 2001*; *Geley et al., 2001*)
Cyclin B	Activates Cdk1 and controls the G2/M transition	Cdc20/Cdh1	(*Clute & Pines, 1999*)
E2F1	Governs G1/S transition and apoptosis	Cdc20/Cdh1	(*Budhavarapu et al., 2012*)
Hmmr	Regulates the localization of Tpx2 at the spindle pole	Cdc20/Cdh1	(*Song & Rape, 2010*)
HURP	Nucleates and crosslinks microtubules in the vicinity of chromatin	Cdc20/Cdh1	(*Song & Rape, 2010*)
Id1	Inhibits dendrite growth	Cdc20	(*Kim, A. H. et al., 2009*)
Kif18A	Plays a role in chromosome congression	Cdc20	(*Sedgwick et al., 2013*)
Mcl-1	Anti-apoptotic protein	Cdc20	(*Harley et al., 2010*)
Mps1	Regulates the spindle assembly checkpoint and chromosome-microtubule attachments	Cdc20/Cdh1	(*Cui et al., 2010*)
Nek1	Functions in primary cilium formation	Cdc20	(*Shalom et al., 2008*)
Nek2A	Regulates centrosome separation and spindle formation	Cdc20	(*Hames et al., 2001*; *Hayes et al., 2006*)
NeuroD2	Inhibits presynaptic differentiation	Cdc20	(*Yang et al., 2009*)
Nlp	Functions in centrosome maturation	Cdc20/Cdh1	(*Wang & Zhan, 2007*)
NuSAP	Nucleates and crosslinks microtubules in the vicinity of chromatin	Cdc20/Cdh1	(*Song & Rape, 2010*)
PHF8	Activates gene transcription by demethylating histon H3 and H4	Cdc20	(*Lim et al., 2013*)
$p21^{Cip1}$	Inhibits cyclin-dependent kinase activity	Cdc20	(*Amador et al., 2007*)
RAP80	Recruits BRCA1 to DNA damage sites	Cdc20/Cdh1	(*Cho et al., 2012*)
REV1	Functions on replicating across DNA lesions at the stalled replication fork	Cdc20/Cdh1	(*Chun et al., 2013*)
Securin	Inhibits separase activity	Cdc20/Cdh1	(*Michaelis et al., 1997*; *Nasmyth, 2001*)
Sp100	Participates in viral resistance, transcriptional regulation and apoptosis	Cdc20	(*Wang et al., 2011*)
TRRAP	Histone acetyltransferase complex component	Cdc20/Cdh1	(*Ichim et al., 2013*)

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Table 3
The list of compounds targeting the Cdc20 activity

Compound	Target and function	Reference
TAME	Reduces Cdc20 association with the APC and subsequently inhibits APC activity.	(Zeng et al., 2010)
Pro-TAME	A TAME pro-drug, disrupts the APC-Cdc20 interaction and then reduces APC activation.	(Zeng et al., 2010)
Apcin	Occupies the D-box-binding pocket on the side of the WD40 domain, blocks substrate-induced Cdc20 loading onto the APC.	(Sackton et al., 2014)
Withaferin A	Enhances degradation of Cdc20, blocks SAC function, leading to mitotic delay.	(Das et al., 2014)
NAHA	Inhibits the expression of Cdc20 in breast cancer cells, retards cell proliferation and colony formation.	(Jiang et al., 2012)
Ganodermanontriol, Mycophyto complex	Down-regulates Cdc20 expression, inhibits cell proliferation and invasion in breast cancer cells.	(Jiang et al., 2011; Jiang & Sliva, 2010)
Genistein	Regulation of Cdc20 in various human cancers to exert its anti-tumor activity.	(Regenbrecht et al., 2008; Shike et al., 2014; Zhang et al., 2015)
CFM-4	Down-regulates Cdc20 in breast cancer cells, induces apoptosis.	(Puliyappadamba et al., 2011)
BCHHD 7c	Inhibits Cdc20 expression in drug resistant pancreatic cancer cells.	(Nasr et al., 2014)