Journal of Cancer Research and Clinical Oncology (2019) 145:2413–2422
https://doi.org/10.1007/s00432-019-02994-0

REVIEW – CANCER RESEARCH

PLK4: a promising target for cancer therapy

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Received: 7 April 2019 / Accepted: 5 August 2019 / Published online: 6 September 2019

Abstract

Purpose Polo-like kinase 4 (PLK4) is a serine/threonine protein kinase that regulates centriole duplication. PLK4 deregulation causes centrosome number abnormalities, mitotic defects, chromosomal instability and, consequently, tumorigenesis. Therefore, PLK4 has emerged as a therapeutic target for the treatment of multiple cancers. In this review, we summarize the critical role of centrosome amplification and PLK4 in cancer. We also highlight recent advances in the development of PLK4 inhibitors and discuss potential combination therapies for cancer.

Methods The relevant literature from PubMed is reviewed in this article. The ClinicalTrials.gov database was searched for clinical trials related to the specific topic.

Results PLK4 is aberrantly expressed in multiple cancers and has prognostic value. Targeting PLK4 with inhibitors suppresses tumor growth in vitro and in vivo.

Conclusions PLK4 plays an important role in centrosome amplification and tumor progression. PLK4 inhibitors used alone or in combination with other drugs have shown significant anticancer efficacy, suggesting a potential therapeutic strategy for cancer. The results of relevant clinical trials await evaluation.

Keywords PLK4 · Centrosomes · PLK4 inhibitor · CFI-400945 · Cancer

Introduction

Centrosomes are nonmembranous organelles that act as microtubule organizing centers (MTOCs) in most animal cells. They are critical for regulating cell shape, motility and division (Gemble and Basto 2018; Gonczy 2015; Wu et al. 2017). Hence, centrioles are accurately controlled to duplicate once per cell cycle to ensure the fidelity of genome segregation (Tsou and Stearns 2006). Centrosome amplification (CA) is a centrosome abnormality arising from the deregulation of centrosome duplication. CA specifically induces abnormal chromosome segregation and correlates with chromosomal instability (CIN), as well as with tumor initiation and progression, and poor clinical outcomes (Chan 2011; Denu et al. 2016; Levine et al. 2017). Given the essential roles of centrosomes, deregulation of centriole duplication is proposed to play a crucial role in tumor progression.

The Polo-like kinases (PLKs) are a family of serine/threonine kinases that play a critical role in cell cycle regulation and cellular responses under stress (Helmke et al. 2016; Zitouni et al. 2014). Mammalian cells express five PLK family members (PLK1–5). All PLKs share a similar structure, with an N-terminal kinase catalytic domain and C-terminal Polo-box domains (PBDs) (Archambault et al. 2015). Polo-like kinase 4 (PLK4), also known as SAK, is a regulator of centriole duplication (Habedanck et al. 2005; Kleylein-Sohn et al. 2007). In proliferating tissues, PLK4 is expressed as a low-abundance enzyme under normal conditions and is required for centriole biogenesis via phosphorylation and interaction with centriolar proteins (Habedanck et al. 2005; Maniswami et al. 2018). Overexpression of PLK4 results in centriole amplification and further genomic instability and tumorigenesis (Holland et al. 2010). Indeed, aberrant PLK4 expression has been reported to be involved in several

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common human cancers (Marina and Saavedra 2014; Shinmura et al. 2014). Thus, strong evidence supports the critical role of PLK4 in carcinogenesis and therapeutic invention. Here, we survey the progress in the current understanding of the roles of PLK4 in cancer and therapeutic approaches for targeting PLK4.

Overview of centrosome amplification in cancer

The centrosome, comprising two centrioles and the surrounding pericentriolar material, is the major MTOC in animal cells (Wu and Akhmanova 2017). Any dysfunction of centrosomes, including aberrancies in microtubule organization or cell division, motility and signaling, is potentially linked with tumorigenesis. Unsurprisingly, cancer cells have been found to display centrosome abnormalities. CA, characterized by the presence of more than two centrosomes at the beginning of mitosis, is the most commonly reported abnormality in cancer (Godinho et al. 2014).

Over a century ago, supernumerary centrosomes were observed and surmised to cause cancer; soon after, the relationship between CA and cancer was proposed (Boveri 2008). Chan et al. concluded that CA was frequently detected in solid tumors and hematological malignancies, and was usually associated with advanced tumor grade and poor clinical outcomes (Chan 2011). Denu et al. analyzed 362 breast cancer samples, 305 of which showed higher mean centrosome values than normal breast samples (Denu et al. 2016). Mittal et al. found a greater proportion of CA in pancreatic ductal adenocarcinoma cell lines and tissues than in normal pancreas (Mittal et al. 2015). Notably. CA has been reported to occur in preneoplastic lesions during the early stage of tumorigenesis (Pihan et al. 2003). A recent study on Barrett's esophagus patients revealed that CA was detected as early as premalignant stages and increased during malignant transformation (Lopes et al. 2018). These findings highlight the important implications of CA in tumor initiation and progression.

To gain a deeper understanding of whether there is a causal relationship between CA and cancer, researchers exploited PLK4 overexpression to promote the formation of extra centrosomes. Studies in a mouse model revealed that PLK4-induced CA resulted in skin differentiation defects and chromosome segregation errors but was insufficient for promoting cell proliferation and carcinogenesis (Kulukian et al. 2015). In contrast, Levine et al. demonstrated that elevated PLK4 expression caused CA and aneuploidy in vivo, and accelerated the onset of intestinal tumors (Levine et al. 2017). Moreover, p53 deficiency facilitated carcinogenesis in the presence of PLK4 overexpression, accompanied by inactivation of the p53-dependent pathway (Coelho et al. 2015; Sercin et al. 2016). Collectively, these findings indicate that targeting the function and regulation of PLK4 may be a therapeutic strategy against CA-induced tumor progression.

Structure and regulation of PLK4

Members of the PLK family contain a conserved N-terminal kinase catalytic domain and C-terminal PBDs (Lowery et al. 2005; Zitouni et al. 2016). The catalytic domain containing the ATP-binding site is a target of PLK inhibition via small molecule ATP-competitive inhibitors (Rudolph et al. 2009; Spaniol et al. 2011; Steegmaier et al. 2007; Valsasina et al. 2012). PBDs are critical for PLK localization and kinase activity, and have been established as another target for PLK inhibition (Scharow et al. 2016; Zitouni et al. 2014). PLK4 is structurally distinct from other Polo family members. Its divergent C-terminal region contains a single Polo-box and a central domain called the "cryptic Polo-box" (CPB). The CPB consists of two architecturally distinct PBDs, PB1–PB2, that form an interdomain interaction and have a dimerization interface (Slevin et al. 2012). PB1 and PB2 of PLK4 form a homodimer for target binding and centrosome localization. In addition, the single PBD self-associates as an intermolecular, domain-swapped homodimer that is required for PLK4 kinase activity and autoinhibition (Jana et al. 2012; Klebba et al. 2015).

PLK4 is inherently unstable and its kinase activity determines its own stability via autophosphorylation and subsequent ubiquitin-mediated proteolysis mediated by the Skp/Cullin/F box E3 ubiquitin ligase (SCF^{bTrCP}) complex (Cunha-Ferreira et al. 2009; Holland et al. 2010). When the kinase activity reaches a certain threshold, PLK4 autophosphorylates within a 24-amino acid phosphodegron containing a β-TrCP-binding site, promoting the recruitment of the E3 ligase and further degradation of PLK4. This autoregulatory pattern ensures the stability of PLK4 and proper centriole duplication.

In addition to ubiquitin-mediated proteasomal degradation, several mechanisms have been described to regulate PLK4 kinase activity. In contrast to direct binding to the promoter, PLK4 is transcriptionally repressed by p53 through an indirect mechanism. Histone deacetylase (HDAC) repressors are a group of involved factors since p53-dependent downregulation of PLK4 was largely reversed in a dose-dependent manner followed by treatment with an HDAC inhibitor (Li et al. 2005). Depletion of PLK4 contributed to p53-induced apoptosis, whereas overexpression of PLK4 attenuated p53-mediated apoptosis.

Drug (e.g., doxorubicin)-induced DNA damage inhibits the transcription of PLK4 and decreases its expression. In HCT116 $p53^{+/+}$ colon cancer cells, the transcript

levels of PLK4 were decreased by approximately fourfold upon doxorubicin-induced DNA damage. In HCT116 $p53^{-/-}$ and HCT116 $p21^{-/-}$ cells, this effect was basically abrogated, indicating that PLK4 was regulated by p53 and p21 (Fischer et al. 2014). The DNA damage response has been reported to activate p53 and p21, leading to the formation of the DP, RB-like, E2F4 and MuvB (DREAM) complex, which binds to the promoter of cell cycle genes to induce transcriptional repression (Quaas et al. 2012). Cell cycle-dependent elements (CDEs) and cell cycle gene homology regions (CHRs) were identified as putative transcriptional binding sites in the PLK4 promoter region. Mutations in these sites have been shown to lead to an increase in PLK4 promoter activity in the early stages of the cell cycle. In G_0 cells, DREAM complex components were shown to bind to the PLK4 promoter through CDEs/CHRs and downregulate PLK4 expression. Furthermore, the human papilloma virus E7 oncoprotein abrogates p53-mediated PLK4 repression by disrupting DREAM complex function (Fischer et al. 2014). These data suggest that PLK4 is indirectly repressed by p53 through the p53–p21–DREAM–CDE/CHR pathway, which has been verified as a principal mechanism underlying p53-dependent suppression of cell cycle genes (Fischer et al. 2016).

The nuclear factor kappa B (NFκB) family is a family of transcriptional factors that affect many cellular processes through regulating the expression of multiple genes, including cell cycle genes. In U2OS osteosarcoma cells, depletion of each NFκB subunit resulted in the downregulation of PLK4, implying that PLK4 is an NFκB-regulated kinase. Indeed, putative NFκB binding sites have been identified in the PLK4 promoter and all subunits have been shown to bind to the PLK4 promoter, inducing PLK4 expression (Ledoux et al. 2013).

Studies on promoter methylation of PLKs in malignancy have shown that PLK4 is subject to epigenetic modification, especially under oxidative stress (Ward and Hudson 2014). Previously, haploinsufficiency of PLK4 was reported to be linked to hepatocellular carcinogenesis. For example, researchers detected increased PLK4 methylation with downregulation of expression during the development of hepatocellular carcinoma (HCC) in PLK4 heterozygous mice (Ward et al. 2011). In HCC-derived cell lines, epigenetic downregulation of PLK4 was induced following exposure to hypoxia and reactive oxygen species and this modification may be p53 dependent (Ward and Hudson 2014). Additionally, bone marrow aspirates from patients with hematological malignancies exhibited PLK4 hypermethylation with decreased protein levels (Ward et al. 2015). Considering that aberrant PLK4 methylation may contribute to carcinogenesis, treatment with hypomethylating drugs has been envisioned as a potential cancer therapy in combination with traditional agents (Ward and Hudson 2014).

Another study by Fournier and colleagues found that acetylation of PLK4 diminishes its kinase activity (Fournier et al. 2016). Lysine acetylation is a posttranslational modification that modulates the function of histone and nonhistone proteins. PLK4 was identified as a novel substrate for the acetylation at Lys45 and Lys46 by lysine acetyltransferases (KATs) (Fournier et al. 2016). KAT2A and KAT2B acetylate the kinase domain of PLK4, and dampen its kinase activity by stabilizing the inactive conformation. Accordingly, acetylation of PLK4 prevents CA and maintains genome stability.

The role of PLK4 in cancer

Almost all tumors have complex pathogenesis with the participation of multiple factors. The above-mentioned studies provide enough evidence to support the correlation between CA and several cancers. Because PLK4 is the master regulator of centrosome number, PLK4 overexpression induces CA, which indicates a critical role for PLK4 in cancer. Indeed, a series of experiments have demonstrated that PLK4 is aberrantly expressed in human cancer, participating in tumorigenesis, cancer metastasis and the response to chemotherapy (Kazazian et al. 2017; Li et al. 2016; Rosario et al. 2015; Tian et al. 2018).

PLK4 expression in malignant cells and tissues

PLK4 expression levels vary among different cancer types. PLK4 expression has been observed to be upregulated in a broad spectrum of human tumors. Shinmura et al. showed a significant increase in PLK4 mRNA levels in 57.1% (4/7) of gastric cancer cell lines and in 50.0% (24/48) of primary gastric cancers (Shinmura et al. 2014). Notably, the expression of PLK4, as determined immunohistochemically, was found to be commonly upregulated in breast cancer; only 2.6% of samples were negative. Overexpression of PLK4 had prognostic significance and was associated with shortened patient survival times ($P=0.003$) (Li et al. 2016). Kawakami et al. analyzed PLK4 mRNA expression profiles in lung cancer using the TCGA database; PLK4 was overexpressed in lung adenocarcinomas compared with normal lung tissues, and this overexpression was associated with inferior overall and progression-free survival ($P<0.05$) (Kawakami et al. 2018a). Moreover, expression of PLK4 has also been shown to be upregulated in melanoma and brain tumors (Denu et al. 2018; Sredni et al. 2017a, b; Zhang et al. 2019).

In contrast, the expression of PLK4 was found to be reduced in human HCC and hematological malignancies. A gradual decline in PLK4 expression was found from nontumorous liver tissue to HCC tissue via PCR and western blot

analysis. Researchers also analyzed the genomic status of PLK4 and found that 45.3% of HCC exhibited loss of heterozygosity at the PLK4 locus, consistent with its reduced expression levels (Pellegrino et al. 2010). A survival analysis of HCC patients indicated an association of PLK4 downregulation with shorter overall survival ($P=0.002$) and disease-free survival ($P=0.012$) times (Liu et al. 2012).

A similar trend was observed in hematological malignancies: PLK4 protein levels were decreased in lymphoproliferative neoplasms (by 35%) as well as in myelodysplastic syndromes (MDS)/leukemia compared with those in normal tissues ($P<0.05$). The evidence indicating PLK4 downregulation by the finding of epigenetic mechanisms in blood neoplasms was supported by PLK4 promoter hypermethylation in 82.0% of lymphoma and 80.5% of MDS/leukemia samples (Ward et al. 2015). However, Li et al. analyzed different datasets for relapsed acute lymphoblastic leukemia (ALL) in the Gene Expression Omnibus (GEO) database and identified PLK4 as one of the co-upregulated genes, indicating its role as a diagnostic marker for relapsed ALL (Li et al. 2018). In addition, genetic analysis of classical Hodgkin lymphoma (cHL) revealed PLK4 amplification in four of five cancer cell lines via whole-exome sequencing, explaining the phenomenon of the high degree of aneuploidy with genetic instability in cHL (Hudnall et al. 2016).

In summary, the expression of PLK4 is upregulated in most solid tumors and downregulated in HCC and most hematological malignancies. However, since several studies included limited number of clinical samples, the expression levels of PLK4 in cancers need more investigations. Generally, PLK4 deregulation has clinical applications and the role of PLK4 as an oncogene or a tumor suppressor depends on the specific cancer type.

PLK4 as a driver of tumorigenesis and metastasis

The above examples show that PLK4-induced CA contributes to CIN and promotes tumorigenesis. However, control of centriole duplication may not be the only function of PLK4 associated with tumorigenicity. In addition to its impact on quantitative changes in centrosomes, PLK4 deregulation is involved in abnormal cytokinesis related to tumor development. In a previous study, Ko et al. demonstrated that mice with PLK4 haploinsufficiency exhibited a significantly increased incidence of spontaneous liver and lung cancers (Ko et al. 2005). Later, Rosario et al. speculated that PLK4 haploinsufficiency promoted tumorigenesis through defective cytokinesis. Under normal conditions, PLK4 is localized to the midbody and phosphorylates the RhoA guanine nucleotide exchange factor Ect2. $Plk4^{+/-}$ murine embryonic fibroblasts (MEFs) with defective Ect2 localization and disrupted RhoGTPase function were predisposed to undergo aberrant cytokinesis, not caused by CA, culminating in aneuploidy and tumorigenesis (Rosario et al. 2010). Conversely, one other study refuted the direct involvement of PLK4 in cytokinesis since the reduction in PLK4 expression levels was unclear in $Plk4^{+/-}$ cells (Holland et al. 2012). Those researchers indicated that PLK4 heterozygosity resulted in a slight decrease in protein levels with no induction of cytokinesis failure, whereas PLK4 depletion led to abnormal cell division and delayed mitosis. PLK4 localization was restricted to the centrosome rather than the spindle midbody during M phase, supporting the hypothesis that PLK4 may indirectly affect cytokinesis through its role in centriole duplication. However, a recent study introduced a new possibility that the cleaved fragment of phosphorylated PLK4 (the active form of PLK4) undergoes redistribution to the spindle midzone and midbody, which strongly supports the functional role of PLK4 in cytokinesis (Press et al. 2019).

In addition to mediating abnormal centriole duplication and cytokinesis, PLK4 facilitates malignant transformation by regulating cell proliferation, motility and migration. Bao et al. reported that PLK4-mediated activation of ataxia telangiectasia and Rad3-related (ATR)/checkpoint kinase 1(CHEK1) signaling promoted oncogenic behavior in HCC cells. This group further showed a direct interaction between PLK4 and ATR. In addition, microRNA-126 plays a tumor-suppressive role in HCC and negatively regulates the expression of PLK4 (Bao et al. 2018). A recent study reported that PLK4–IKBKE signaling is involved in the proliferation of glioblastoma cells. In that study, PLK4 was shown to interact with and phosphorylate IKBKE and thereby induce NF-κB transactivation, which results in the induction of antiapoptosis genes, thereby enhancing proliferation and chemoresistance (Zhang et al. 2019).

Additionally, PLK4 has been hypothesized to regulate cell motility and migration through the Rho GTPase signaling pathway. Rosario et al. reported that haploid levels of PLK4 impaired cell motility and that PLK4 hyperactivity enhanced cell migration in fibroblasts and cancer cells. PLK4 induced actin rearrangement and protrusion formation to promote cell migration through RhoA activation. PLK4 was also associated with the expression of matrix metallopeptidase-3 (MMP-3), MMP-13 and other promotility genes (Rosario et al. 2015).

Further experiments have been performed to validate the effects of PLK4 on metastasis. Kazazian et al. identified an interaction between the PLK4 PB1–PB2 domain and the actin-nucleating Arp2/3 complex. PLK4 phosphorylated and activated Arp2, an Arp2/3 subunit critical for actin polymerization and branching, and consequently promoted invasive and metastatic progression in breast cancer (Kazazian et al. 2017). A recent study identified PLK4 as a tumor promoter in neuroblastoma (NB) (Rosario et al.

2015, 2018). Epithelial–mesenchymal transition (EMT) is commonly considered a key contributor to tumor progression and metastasis. Elevated expression of PLK4 in NB tissues promotes EMT via activation of the phosphoinositide 3-kinase (PI3 K)/protein kinase B (AKT) signaling pathway and thereby promotes tumor development.

Reciprocal function between p53 and PLK4 in cancer

The tumor suppressor p53 functions as a transcription factor by regulating multiple downstream effectors and triggering growth arrest and apoptosis. During mitosis, p53 plays a critical role in the prevention of CA, aneuploidy and chromosome missegregation, consequently preserving genomic stability. PLK4, as a driver of CA and tumorigenesis, is believed to cooperate with p53 inactivation in cancer development. Indeed, previous studies indicated that in the context of p53 dysfunction, transient PLK4 overexpression promoted the formation of squamous cell carcinomas of the skin in mice (Sercin et al. 2016). Coelho et al. reached a similar conclusion that elevated expression of PLK4 accelerated lymphoma and sarcoma tumorigenesis in the absence of p53 (Coelho et al. 2015). Since PLK4 overexpression led to CA and p53-dependent cell cycle arrest, these observations supported the hypothesis that cells with PLK4 overexpression and p53 deficiency were prone to form tumors.

PLK4 has been reported to be transcriptionally repressed by p53 through HDAC transcriptional repressors or the p53–p21–DREAM–CDE/CHR pathway (Fischer et al. 2014; Li et al. 2005). Nakamura et al. showed that activation of p53 suppressed PLK4 expression and decreased the risk of PLK4-induced CA under stress (Nakamura et al. 2013). Conversely, in thymic tumors from PLK4-overexpressing mice, the p53 pathway was shown to be partly inactivated. These data imply a potential feedback loop between p53 and PLK4 in tumor development (Levine et al. 2017).

Targeting PLK4 for cancer treatment

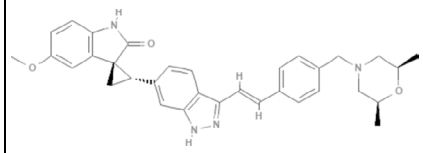
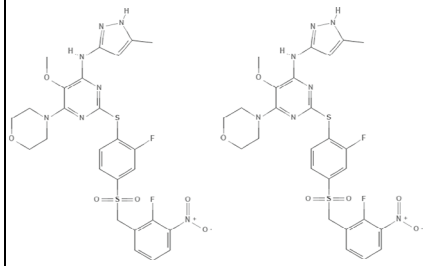
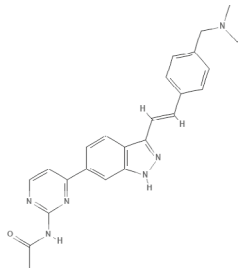
PLK4 has become a potential therapeutic target in cancer due to its role in controlling centriole duplication and its deregulation in multiple tumors. In addition, PLK4 is a low-abundance enzyme expressed only in normal proliferating tissues but is aberrantly expressed in cancer, which makes it a selective drug target.

CFI-400945 was the first orally available potent PLK4 inhibitor; this agent selectively inhibits PLK4 with an IC_{50} value of 2.8 ± 1.4 nM. The small molecule inhibitor was identified via a drug discovery program, and shows selective inhibition of PLK4 rather than other PLK family members (Mason et al. 2014). In U2OS osteosarcoma cells and MDA-MB-468 and MDA-MB-231 breast cancer cell lines, CFI-400945 treatment showed similar effects on centriole number as RNAi-mediated-reduction of PLK4 levels, leading to centriole duplication defects and cell death. Interestingly, CFI-400945 displayed bimodal effects on centriole number, with higher doses of the compound suppressing centrosome duplication and lower doses driving an increase in centriole number. Researchers attributed this effect to the self-regulation pattern of PLK4. At low concentrations, partial inhibition of PLK4 was not sufficient for its own degradation but was sufficient for substrate phosphorylation, causing an increase in PLK4 levels and centriole number. In contrast, higher concentrations completely blocked PLK4 activity and thereby inhibited centriole duplication (Mason et al. 2014).

The conclusion that CFI-400945 elicits antineoplastic effects has been extended to other solid tumors. In four of six human pancreatic cancer xenograft models, CFI-400945 treatment reduced tumor growth and prolonged survival (Lohse et al. 2017). Kawakami et al. showed that CFI-400945 treatment blocked cell proliferation and induced polyploidy, apoptosis and mitotic aberrations in lung tumor cells and inhibited tumor growth in a murine lung cancer xenograft model (Kawakami et al. 2018a). Considering its dose-dependent inhibitory effects in a diverse panel of human cancer cell lines and patient-derived xenograft tumors, CFI-400945 has entered Phase I clinical trials for solid tumors (ClinicalTrials.gov identifier: NCT01954316) (Holland and Cleveland 2014). As of 2018, four CFI-400945 trials were cited in the ClinicalTrials.gov register. All four clinical trials are currently recruiting patients and the most recent one (ClinicalTrials.gov identifier: NCT03187288) is aimed at evaluating the safety and tolerability of CFI-400945 in patients with relapsed or refractory acute myeloid leukemia (AML) or MDS. Tables 1 and 2 summarize current preclinical studies and clinical trials, respectively.

Although it exhibits considerable antiproliferative actions in cancer, whether CFI-400945 exerts its cellular effects through PLK4 inhibition remains under debate since this inhibitor was shown to have activity against aurora kinase B (AURKB) Kawakami et al. (2018a, b; Mason et al. 2014). It is noteworthy that IC50 of CFI-400945 is 2.8 nM against PLK4 and 98 nM against Aurora kinase B, a 35-fold differential. Changes in centriole number caused by CFI-400945 was at a low dose of 10 nM, indicating that this effect was unlikely caused by Aurora B kinase inhibition Kawakami et al.(2018a). In addition, the antitumor effects of CFI-400945 treatment are consistent with PLK4 inhibition Kawakami et al. (2018a, b). Moreover, whether cytokinesis failure by CFI-400945 is due to off-target inhibition of Aurora kinase B is worth discussing. CFI-400945 treatment on cancer cells led to significant reduction of PLK4 midbody-positive cancer cells and further formation of

Table 1 PLK4 inhibitors

Inhibitor	Structure	Target	IC50	Mainly studied cancer cells	Effects	References
CFI-400945		PLK4, but has activity against AURKB, TRKA, TRKB, Tie2/TEK	IC50=2.8±1.4 nM	Osteosarcoma cells; breast cancer cells; lung cancer cells	Centriole duplication defects; multipolar spindles formation; cytokinesis failure; Apoptosis; cell arrest and cell death	(Kawakami et al. 2018a; Mason et al. 2014)
Centrinone/ Centrinone-B		PLK4	IC50=2.71nM (Centrinone); IC50=8.69nM (Centrinone-B)	Cervical carcinoma cells; colon carcinoma cells	Reversible centriole loss; cell arrest; cell proliferation inhibition	(Suri et al. 2019; Wong et al. 2015)
YLT-11		PLK4	IC50=22nM	Breast cancer cells	Cell proliferation inhibition; centriole duplication defects; mitosis defects; aneuploidy; subsequent cell death induction	(Lei et al. 2018)

AURKB aurora kinase B, *TRKA* neurotrophic tyrosine kinase, receptor, type 1, *TRKB* neurotrophic tyrosine kinase, receptor, type 2, *Tie2/TEK* TEK tyrosine kinase, endothelial

The two-dimensional structures of chemical compounds were taken from database PubChem, http://pubchem.ncbi.nlm.nih.gov

Table 2 Clinical trials of PLK4 inhibitors

Trial ID	Inhibitor	Status	Phase	Disease	Treatment regimens	Number of patients/age/sexes	Results
NCT03187288	CFI-400945	Recruiting	I	AML, MDS, relapsed cancer, refractory cancer	Orally, 64, 72, 96, 128, 176 or 224 mg/day, every day until intolerable side effects or disease progression	48/18 +/all	Pending
NCT01954316	CFI-400945	Recruiting	I	Advanced cancer	Orally, 3, 6, 11, 16, 24, and 32 mg/day	48/18 +/all	Pending
NCT03624543	CFI-400945	Recruiting	II	Breast cancer	64 mg orally day 1–7 every 14 days (cycle 1) and 64 mg orally daily for 28 days (cycle 2)	72/18 +/female	Pending
NCT03385655	CFI-400945	Recruiting	II	Prostate cancer	64 mg orally day 1–7 every 14 days	500/18 +/male	Pending

AML acute myeloid leukemia, *MDS* myelodysplastic syndromes

polyploidy, indicating that the effects of CFI-400945 on cytokinesis were likely attributed to PLK4 inhibition (Press et al. 2019).

Centrinone and centrinone B were developed as highly selective PLK4 inhibitors and block centriole duplication and deplete centrosomes by preventing centriole assembly. In Hela cells, centrinone treatment led to p53-mediated cell cycle arrest in G1 phase. In human melanoma cell lines except p53 mutant cell lines, centrinone B treatment resulted in a significant decrease in cell viability and an increase in apoptosis (Denu et al. 2018). However, in some cancer cell lines, centrinone treatment led to cell proliferation independent of centrosome loss, indicating an intrinsic "set point" for centrosome number. Thus, the authors suggested that centrosome depletion was insufficient for cancer therapy and should be combined with other targeted drugs (Wong et al. 2015).

PLK4 inhibition has been investigated in combination with other drugs. Kawakami and colleagues found that a combination of cyclin-dependent kinase 2 (CDK2) antagonists and CFI-400945 showed synergistic effects against lung cancer cells (combination index < 1) (Kawakami et al. 2018a). Both of these drugs triggered multipolar mitosis in cancer cells. CDK2 antagonism inhibited centrosome clustering, while low concentrations of CFI-400945 generated supernumerary centrosomes. The distinct mechanisms of these two drugs makes their combination feasible for cancer treatment Kawakami et al. (2018a). Additionally, PLK4 inhibitors increased the susceptibility of cancer cells to DNA-damaging agents in rhabdoid tumors and pediatric medulloblastoma, suggesting the feasibility of combination with cytotoxic agents Sredni et al. (2017a). Recently, Zhang et al. evaluated the effects of PLK4 on temozolomide (TMZ) sensitivity in glioblastoma (Zhang et al. 2019). The combination of PLK4 depletion and TMZ enhanced the antitumor effect and decreased the IC50 of TMZ. Furthermore, a combination of CFI-400945 and TMZ reduced the tumor volume and improved survival in tumor-bearing mice (Zhang et al. 2019).

Further studies are required to investigate potential combination therapies with PLK4 inhibition. To promote CIN in cancer cells, drugs targeting microtubule dynamics can be added. To prevent the unfavorable immune response caused by CFI-400945-mediated DNA response, combination with immune checkpoint inhibitors can also be attempted Kawakami et al. (2018a). Additionally, with the discovery of STAT3 involvement in centrosome clustering, researchers discovered that breast cancer cells with CA exhibited increased sensitivity to STAT3 inhibition, suggesting an approach for combination therapy with CFI-400945 and STAT3 inhibitors in cancer (Morris et al. 2017).

Conclusions and future perspectives

The effects of centrosome abnormalities on human cancers have recently become very important in recent years (Anderhub et al. 2012; Denu et al. 2016; Levine et al. 2017). PLK4 is a regulator of centrosome duplication. Overexpression of PLK4 induces CA and may contribute to carcinogenesis, indicating that PLK4 is a therapeutic target in cancer. To date, genotoxins, a class of DNA damage-inducing drugs, have been widely used to treat tumors. Increasing evidence implies that PLK4 may function as a DNA-damage sensitizer, improving the efficacy of chemotherapy. Thus, the role of PLK4 inhibition should be further explored as a mitosis-targeting strategy in combination with other anticancer agents.

In this review, we discussed the regulation and expression of PLK4. However, our understanding of aberrant PLK4 modifications in the development of cancer is far from complete. Additional studies are required to identify additional

upstream and downstream regulatory elements, with an aim to identify novel mechanisms for their involvement in the maintenance of genome stability and novel targeted treatment strategies.

Funding This study was funded by National Natural Science Foundation (No. 81270598, No. 81473486, and No. 81770210); Key Research and Development Program of Shandong Province (No. 2018CXGC1213); Technology Development Projects of Shandong Province (No. 2017GSF18189); Taishan Scholar Foundation of Shandong Province; Shandong Provincial Engineering Research Center of Lymphoma; Key Laboratory for Kidney Regeneration of Shandong Province.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Statement of human and animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Human participants were not involved in the study.

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