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Polo-like kinase 1, on the rise from cell cycle regulation to prostate cancer development

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ABSTRACT

Polo-like kinase 1 (Plk1), a well-characterized member of serine/threonine kinases Plk family, has been shown to play pivotal roles in mitosis and cytokinesis in eukaryotic cells. Recent studies suggest that Plk1 not only controls the process of mitosis and cytokinesis, but also, going beyond those previously described functions, plays critical roles in DNA replication and Pten null prostate cancer initiation. In this review, we briefly summarize the functions of Plk1 in mitosis and cytokinesis, and then mainly focus on newly discovered functions of Plk1 in DNA replication and in Pten-null prostate cancer initiation. Furthermore, we briefly introduce the architectures of human and mouse prostate glands and the possible roles of Plk1 in human prostate cancer development. And finally, the newly chemotherapeutic development of small-molecule Plk1 inhibitors to target Plk1 in cancer treatment and their translational studies are also briefly reviewed.

KEYWORDS   polo-like kinase 1, cell cycle regulation, Orc2, DNA replication, early embryonic development, prostate cancer, Pten, cancer initiation and progression, translational studies

INTRODUCTION

Polo gene, encoding an important enzyme, was first identified in fruit fly (*D. melanogaster*) in two decades ago (Llamazares et al., 1991). It was shown that polo gene, whose mutations led to abnormal mitosis, plays a critical role in mitosis of *Drosophila* larval neuroblasts. Subsequently, the homolog of polo gene was identified and cloned from murine (*M. musculus*) hematopoietic progenitor cells and shown to encode a protein kinase that is highly conserved with fruit fly's polo

gene and named polo-like kinase gene, designated as Plk gene (Clay et al., 1993). Since the discovery of polo gene, a wealth of functional information on Plks has been collected from various organisms. Plks belong to a family of serine/threonine kinases, which are highly conserved among eukaryotes from yeast to mammals. Both budding yeast (*S. cerevisiae*) and fission yeast (*S. pombe*) have only one homolog of Plks, designated as Cdc5 and Plo1, respectively. Both of them are known to play important roles in cell cycle regulation, including mitotic entry, mitotic exit, and cytokinesis (Bahassi, 2011). Three known Plks (Plx1–3) were identified in *Xenopus laevis* (Santamaria et al., 2007) and five Plks (Plk1–5) were found in mammals (Glover et al., 1998; Barr et al., 2004; Andrysik et al., 2010). Recent advances in basic functional studies of Plks in various organisms have shown that these Plks have multiple functions in tight regulation of the cell cycle progression. Mutations in these genes lead to abnormal cell cycle in these organisms. All Plks in mammalian cells have a similar structure, with a canonical serine/threonine kinase domain at the N terminus, followed by a regulatory domain containing two polo-box domains (PBD) at the C terminus (Fig. 1A). Although similar architecture was shown among Plk members, the human Plk1, 2, 3, 4, and 5 genes have distinct chromosomal localization and expression profiles, and show unique biological functions from extensive studies in the past two decades (Hudson et al., 2001; Ma et al., 2003; Barr et al., 2004).

Plk1, a well-characterized member of Plk family, localizes to various subcellular structures, such as centrosomes, spindle poles, kinetochores, midzones and midbodies by PBD binding (Elia et al., 2003a) to its substrates. Plk1 mediates many mitotic events, including entry into mitosis, centrosome maturation, assembly of the bipolar spindle, sister chromatid splitting, activation of the Anaphase-Promoting Complex/Cyclosome (APC/C), and exit from mitosis with the initiation of cytokinesis (Fig.1B). Many excellent reviews have exten-
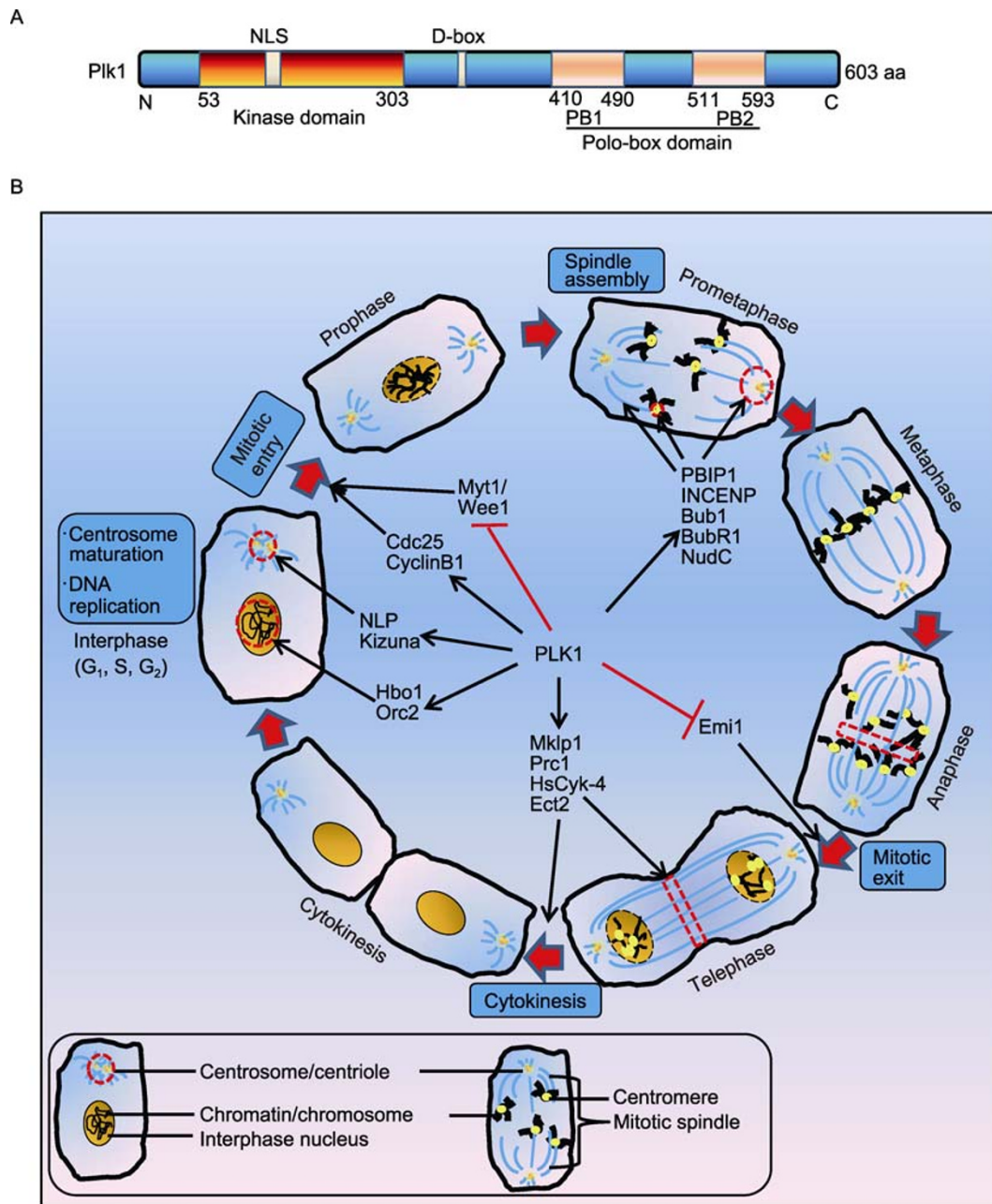
A

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Figure 1.   The schematic structure of Plk1 and its roles in cell cycle regulation. (A) Domain structure of human Plk1. The length of amino acid (aa) is shown on the right. The positions of kinase domain and polo-box domain PB1 and PB2 are depicted, and the numbers designate the start and the end positions of the domains. (B) A schematic diagram of the mammalian cell cycle, emphasizing the functions of Plk1 in multiple stages of cell cycle regulation. Functions of Plk1 and its substrates are listed in blue boxes. Localization of Plk1 to subcellular structures is indicated by red dashed objects. Related subcellular structures of cells are indicated in inset box

sively addressed these aspects (Glover et al., 1998; Barr et al., 2004; van de Weerdt and Medema, 2006; Barr and Gruneberg, 2007; Petronczki et al., 2008; Takaki et al., 2008; Archambault and Glover, 2009; Strebhardt, 2010). In addition, increasing evidences show that the functions of Plk1 go be-

yond its roles in mitotic regulation. First, recent publications on new Plk1 substrates reveal that Plk1 not only functions in mitotic regulation but also plays pivotal roles in other cell cycle phases, and even some novel functions go beyond the cell cycle regulation. For example, diverse functions of sub-

strates of Plk1 indicate that Plk1 mediates the functions involving microtubule dynamics (CLIP-170 and p150^{glued}) (Yang et al., 2009a; Li et al., 2010a, 2010b), DNA replication (Hbo1 and Orc2) (Fig. 2) (Wu and Liu, 2008; Song et al., 2011), chromosome dynamics (Topo II and Trf1) (Li et al., 2008; Wu et al., 2008), p53 regulation (Topors and GTSE1) (Yang et al., 2009b; Liu et al., 2010a), and recovery from the G$_2$ DNA damage checkpoint (van Vugt et al., 2010). Second, a recent publication of our lab links Plk1 to the tumor suppressor Pten, revealing an important role of Plk1 in cancer initiation and progression (Liu et al., 2011). The mitotic functions of Plk1 have been thoroughly discussed in recent reviews (Strebhardt and Ullrich, 2006; van de Weerdt and Medema, 2006; Petronczki et al., 2008; Lee et al., 2008; Takaki et al., 2008; Liu et al., 2010b; Park et al., 2010; Strebhardt, 2010; Bahassi, 2011). In the current review, we will briefly introduce the functions of Plk1 in mitosis and go beyond mitosis to highlight the recent surprising findings of Plk1 functions in DNA replication and prostate cancer development.

ROLES OF PLK1 IN CELL CYCLE REGULATION

Polo-box domain dependent binding of Plk1 to target its substrates

The most striking feature of Plk1 is that it orderly changes its localization to various subcellular structures during mitotic progression, both spatially and temporally (Fig. 1B; Table 1) (Strebhardt, 2010). During cell cycle progression, Plk1 binds to the pre-replicative complex in interphase (Fig. 2) (Song et al., 2011), sublocalizes to centrosomes in prophase, becomes enriched at kinetochores in pro-metaphase and metaphase, then is recruited to the central spindles in anaphase, and finally redistributes to the midbodies in telephase and cytokinesis (Fig. 1B). How does Plk1 precisely and properly translocalize to these subcellular structures to mediate the specific events of cell cycle? The C-terminal PBD of Plk1 was first found to function as a phosphor-peptide binding domain in 2003 (Elia et al., 2003a). This discovery, as an answer to the above question, suggests a mechanism of Plk1

Table 1 Substrates of polo-like kinase 1 in cell cycle regulation

| Substrates	Phosphorylation sites	Functions	References
S phase			
Hbo1	Ser57	Histone acetyltransferase. Required for pre-RC formation and DNA replication licensing	Wu and Liu, 2008
Orc2	Ser188	Component of ORC. Facilitating DNA replication under stressful conditions	Song et al., 2011
G$_2$/M phase			
NLP	Ser237, 1463	Ninein-like protein for centrosome maturation	Casenghi et al., 2003
Kizuna	Thr379	Centrosome architecture maintenance	Oshimori et al., 2006
Cdc25	Ser198	Phosphatase. Activation of Cdk1/Cyclin B1 complex for promoting G$_2$/M phase transition	Toyoshima-Morimoto et al., 2002
Cyclin B1	Ser133,147	Activation of Cdk1/Cyclin B1 complex on centrosome for promoting G$_2$/M phase transition	Toyoshima-Morimoto et al., 2001
Myt1	Ser426, Thr495	Cdk1 inhibitory kinase, regulating Cdk1 activity	Nakajima et al., 2003
Wee1	Ser53	Cdk1 inhibitory kinase, regulating Cdk1 activity	Watanabe et al., 2004
M phase			
PBIP1	Thr78	PBD interacting protein. Recruitment of Plk1 to kinetochores/centrosomes	Kang et al., 2006
INCENP	ND[*]	The inner centromere protein. Recruitment of Plk1 to kinetochores/centrosomes	Goto et al., 2006
Bub1	ND	Mitotic checkpoint kinase. Targeting Plk1 at kinetochores	Qi et al., 2006
BubR1	Ser676	Spindle checkpoint component. Recruitment of Plk1 to kinetochores/centrosomes	Elowe et al., 2007
NudC	Ser274, 326	Recruitment of Plk1 to kinetochores	Zhou et al., 2003
Emi1	Ser145, 149	APC/C complex inhibitor, regulating APC/C complex activity	Hansen et al., 2004; Moshe et al., 2004
Cytokinesis			
Mklp1	Ser904, 905	Motitic kinesin-like motor protein. Component of centraspindlin. Recruiting Plk1 to midzone in cytokinesis regulation	Liu et al., 2004
Prc1	Thr602	Recruitment of Plk1 to spindle midzone in cytokinesis regulation	Neef et al., 2007
HsCyk-4	Ser149, 157, 164, 170, 214, 260	Component of centraspindlin, recruitment of Ect2 to spindle midzone and then activation of Ect2	Wolfe et al., 2009; Burkard et al., 2009
Ect2	Thr412	Rho guanine-nucleotide exchange factor (RhoGEF), for RhoA activation in cytokinesis regulation	Niiya et al., 2006

* Not determined
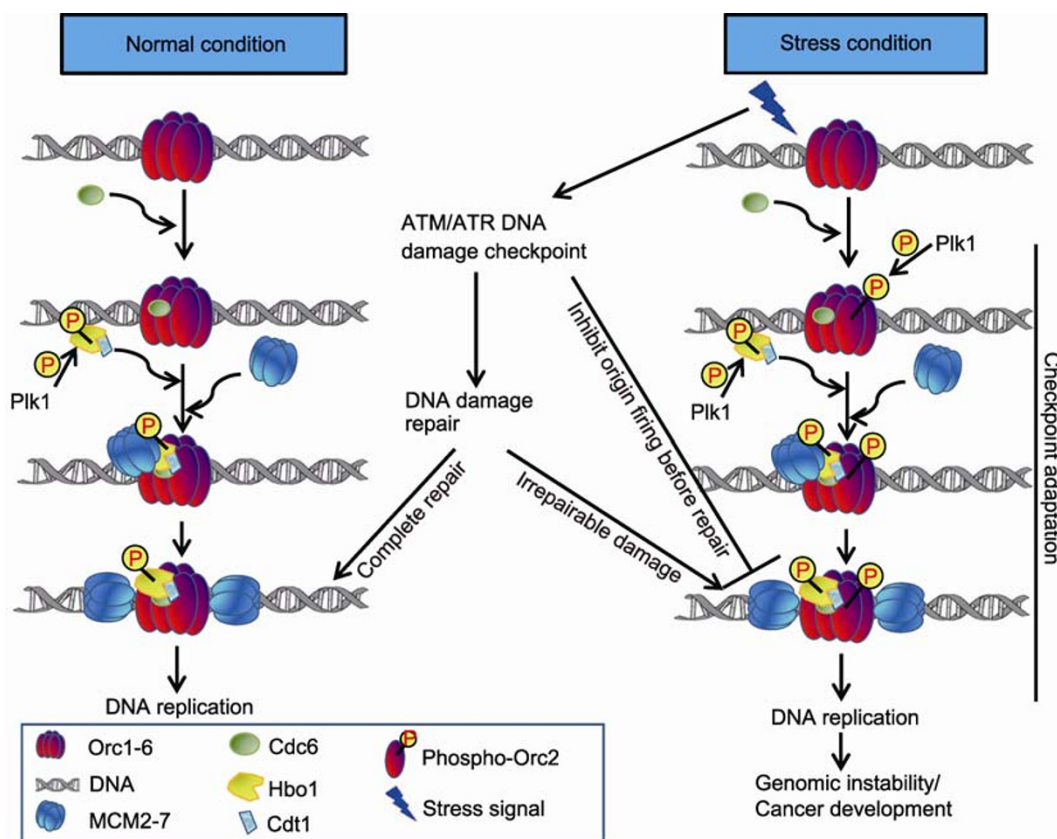
Figure 2. The model of the mechanism of Plk1 in maintaining the stability of pre-replicative complex for facilitating DNA replication under stressful conditions. In normal condition, Plk1 phosphorylated Hbo1 is required pre-RC formation and DNA replication licensing (left panel). Under stressful conditions, stress signal causes DNA damage and results in ATM/ATR-mediated checkpoint signaling pathway activation and cell cycle arrest for DNA repair (middle panel). Meanwhile, Plk1 recruits to pre-RC and phosphorylates Orc2 to maintain the stability of pre-RC for facilitating DNA replication and therefore reentering the cell cycle with complete repair of damaged DNA (checkpoint recovery) or with irrepairable damaged DNA (checkpoint adaptation). Checkpoint adaptation might result in genomic instability and be the cause of cancer development.

targeting subcellular structures by which Plk1 can dock, via its PBD, to specifically phosphorylated targets (Petronczki et al., 2008). Supporting this model, the subsequent crystallization studies of human PBD of Plk1 showed how the phosphor-peptides bind to an interface that is formed between the two polo boxes (Cheng et al., 2003; Elia et al., 2003b). In addition, Plk1 binds to target proteins that have previously been marked as docking sites through a 'priming' phosphorylation (Elia et al., 2003a) by Cdc2 or other proline-directed kinases, or by self-priming phosphorylation *in vivo* (Park et al., 2010). The 'priming' phosphorylation to generate docking sites for Plk1 and the different subcellular distributions of Plk1 docking partners confer very important elements of temporal and spatial control over the activity of Plk1, respectively (Barr et al., 2004). Thus, PBD-dependent binding of Plk1 targeting its substrates confers Plk1 the ability to orderly regulate various cell cycle events in a temporal and spatial manner.

Plk1, a key regulator in mitosis and cytokinesis

The roles of Plk1 in mitosis and cytokinesis have been well-established. In prophase, Plk1 is essential for centrosome functions and the assembly of bipolar spindles (Fig. 1B). One of these functions is the recruitment of γ-tubulin ring complexes (γ-TuRC), which can nucleate new microtubules, to centrosomes during prophase (Lane and Nigg, 1996). This process is referred to as centrosome maturation. Ninein-like protein (NLP), a component of the centrosome and a substrate of Plk1, recruits γ-TuRC and stimulates microtubule nucleation during interphase (Casenghi et al., 2003). Phosphorylation of NLP by Plk1 at both Ser237 and Ser1463 sites leads to its dissociation from centrosome during mitosis and might facilitate the recruitment of γ-TuRC and switch the centrosome from interphase state to the mitotic state (Table 1) (Casenghi et al., 2003, 2005). Another substrate of Plk1, Kizuna, is essential for the mitotic centrosome architecture maintenance (Fig. 1B; Table 1) (Oshimori et al., 2006). De-

pletion of Kizuna or inhibition of its phosphorylation by Plk1 leads to the fragmentation and dissociation of pericentriolar material from centrioles in pro-metaphase, and results in multipolar spindles.

During mitotic entry, Plk1 controls the activity of Cdk1/Cyclin B1 complex and promotes the G_2/M phase transition (Fig. 1B). Cdk1/Cyclin B1, the key driver for the mitotic progression, is controlled by sophisticated regulatory network to prevent its being prematurely activated before the completion of DNA replication to maintain the genome integrity. The Cdk1/Cyclin B1 complex is kept inactive by inhibitory phosphorylation of Cdk1 at Thr14 and Tyr15 by Wee1 and Myt1 kinases, respectively, although with the accumulation of Cyclin B1 during G_2 phase. At the onset of mitosis, Plk1 activates Cdk1/Cyclin B1 by both activating phosphorylation of positive regulator Cdc25 and inhibitory phosphorylation of negative regulators, Wee1 and Myt1 (Fig. 1B; Table 1) (Kumagai and Dunphy, 1996; Inoue and Sagata, 2005). In addition, Cyclin B1 is also a physiological substrate of Plk1. Early reports suggested that Cyclin B1 was phosphorylated by Plk1 at Ser133 and Ser147 sites, which located within cytoplasmic retention signal (CRS) and nuclear-export signal (NES), respectively, for facilitating the translocation of Cyclin B1 to the nucleus during prophase, therein, activating the activity of Cdk1/Cyclin B1 complex and promoting G_2/M phase transition in vertebrate cells (Table 1) (Toyoshima-Morimoto et al., 2001; Yuan et al., 2002). However, a subsequent study found that the preferential phosphorylation of Plk1 at Ser133 site of Cyclin B1 occurred and is activated on centrosomes, but did not cause the nucleocytoplasmic distribution of Cyclin B1 during prophase (Jackman et al., 2003). Although the discrepancy exists among these reports, the notion of Plk1 phosphorylated activation of Cdc25 and Cyclin B1 and phosphorylated inhibition of Myt1/Wee1 to activate the activity of Cdk1/Cyclin B1 for promoting mitotic entry cooperatively is evident.

In metaphase, Plk1 is recruited by several proteins, including the polo-box interacting protein (PBIP1), the inner centromere protein (INCENP), the spindle checkpoint component BubR1, and the mitotic checkpoint kinase (Bub1) to kinetochores/centromeres to perform its functions at these sites (Fig. 1B; Table 1) (Goto et al., 2006; Kang et al., 2006; Qi et al., 2006; Elowe et al., 2007). In early mitosis, PBIP1 is targeted by Plk1 phosphorylation for degradation (Kang et al., 2006). So the other partners might contribute to localization of Plk1 at kinetochores during mitosis (Petronczki et al., 2008). For example, Bub1 is recognized by PBD of Plk1 once Bub1 has been 'primed' by Cdk1 (Qi, et al., 2006), INCENP and MCAK also shared the similar mechanism to target Plk1 at kinetochores (Goto et al., 2006; Rosasco-Nitcher et al., 2008). In addition, NudC is another partner for recruitment of Plk1 to kinetochores (Nishino et al., 2006). It has been shown that NudC bound to Plk1 and was phosphorylated by Plk1 at Ser274 and Ser326 sites (Table 1) (Zhou et al., 2003).

Plk1-phosphoryated NudC recruits Plk1 to the outer plate of the kinetochores. Kinetochore-targeted Plk1 stimulates the kinetochore recruitment of CENP-E, which promotes stable end-on microtubule–kinetochore interactions and proper congression of chromosomes to the metaphase plate. Depletion of NudC reduced end-on microtubule attachments at kinetochores and resulted in the defects in chromosome congression at the metaphase plate (Nishino et al., 2006). Finally, the observation that the level of Plk1 is particularly high on the kinetochores of those chromosomes that have not yet been attached to both poles of the mitotic spindle and are not under tension (Ahonen et al., 2005; Lénárt et al., 2007) suggests that Plk1 might play critical roles in promoting kinetochore attachment or in spindle checkpoint regulation (Petronczki et al., 2008).

In late mitosis, Plk1 mediates the activity of APC/C, an E3 ubiquitin ligase, which is responsible for timely degradation of various mitotic regulators, to regulate chromosome segregation, exit from mitosis, and a stable subsequent G_1 phase (Fig. 1B) (Wäsch and Engelbert, 2005). The APC/C complex is first activated by the protein Cdc20, targeting proteins containing a destruction box (D box), like Securin. Cdc20 is then degraded and replaced by Cdh1 when the mitotic exit is initiated by APC/C^{Cdc20}, thus, allowing the degradation of a wider spectrum of substrates (Eckerdt and Strebhardt, 2006). Emi1 is a major APC/C inhibitor in mitotic cells. At late stage of mitosis, Emi1 is phosphorylated by priming kinase, Cdk1/Cyclin B1, and then phosphorylated by Plk1, resulting in increased binding of Emi1 to the Skp1-Cullin-F-box$^{\beta\text{-TRCP}}$ (SCF$^{\beta\text{-TRCP}}$) ubiquitin ligase complex (Table 1) (Eckerdt and Strebhardt, 2006; Martin and Strebhardt, 2006). This leads to the destruction of Emi1 and release of APC/C from inhibition, thus promoting the mitotic exit. Moreover, once there are unfavorable signals originated from kinetochores, Plk1 activates spindle checkpoint signaling pathway to prevent the pre-matured activation of APC/C, which promotes mitosis exit, by indirectly targeting Cdc20 to inhibit APC/C activity (Eckerdt and Strebhardt, 2006). Plk1 phosphorylates 3F3/2 epitope that is recognized by 3F3/2 antibody at kinetochores which are not under tension to generate tension-sensing 3F3/2 phosphoepitope (Ahonen et al., 2005; Wong and Fang, 2005). During this process, Plk1 recruits checkpoint protein Mad2 to kinetochores. Also, unattached kinetochores control spindle checkpoint by activating the activity of the checkpoint kinase, BubR1. Activation of BubR1 recruits heterodimer Mad1-Mad2 and activates Mad2. Activated Mad2 and/or BubR1 interact with Cdc20 and inhibit Cdc20 to prevent the activation of APC/C activity.

In cytokinesis, Plk1 controls cleavage furrow formation in mammalian cells (Fig. 1B; Table 1). Cytokinesis has to be tightly regulated in both time and space so that the cytoplasm is only divided after chromosome segregation has occurred and only in between the two sets of segregated chromatids (Barr and Gruneberg, 2007). Mitotic spindle plays important

roles in determining the cleavage plane. Bundled arrays of overlapping antiparallel microtubules form a structure between the two sets of segregated sister chromatids referred to as spindle midzone. The key component of the midzone is called centralspindlin, composed of two molecules of the kinesin Mklp1 and two molecules of HsCyk-4 (RhoGAP) (Pavicic-Kaltenbrunner et al., 2007). At the anaphase, HsCyk-4 binds to Rho guanine-nucleotide exchange factor (RhoGEF) Ect2 and thereby recruits Ect2 to the midzone (Somers and Saint, 2003; Yüce et al., 2005; Zhao and Fang, 2005; Nishimura and Yonemura, 2006). This interaction is thought to enhance the activity of Ect2 and further activate the RhoA, whose activation at equatorial cortex controls the assembly and ingression of the contractile ring by inducing the activity of myosin II and the polymerization of actin (Petronczki et al., 2008). In this process, Plk1 phosphorylation events play critical roles in regulating the activity of cytokinesis regulators. A microtubule binding protein, Prc1, acting as a Plk1 docking site, recruits Plk1 to spindle midzone by Plk1 'self-priming' phosphorylation of Prc1 at Thr602 (Table 1) (Neef et al., 2007). Plk1 is recruited to the spindle midzone and therein phosphorylates HsCyk-4 at Ser149, 157, 164, 170, 214, and 260 sites (Table 1), consequently generating a recognition site for BRCT domain of Ect2, which contains a phosphor-peptide binding motif (Manke et al., 2003). Binding of Ect2 to HsCyk-4 results in recruitment and activation of Ect2, thereby activating RhoA and initiating the cytokinesis.

NOVEL FUNCTION OF PLK1 IN DNA REPLICATION

Prior to mitotic entry of cell cycle, DNA must be strictly replicated to maintain the genome integrity of cells. During DNA replication, the replication fork is strictly monitored by a regulation network to ensure the completion of entire genome replication. Defects of the replication fork assembly and monitoring proteins lead to genomic instability, thus resulting in carcinogenesis or a series of diseases known as the 'chromosome instability syndrome (Masai et al., 2010).' In this section, we couldn't cover every aspects of DNA replication due to space limitation, and will mainly focus on the novel function of Plk1, which involves in the assembly and regulation of pre-replicative complex (pre-RC) for facilitating DNA replication under stressful conditions (Fig. 2; Table 1) (Wu and Liu, 2008; Song et al., 2011).

The initiation of DNA replication in eukaryotic cells is a precisely regulated event. From late M phase to early G_1 phase, an ordered assembly of pre-RC at replicative origins is required for initiation of DNA replication (Fig. 2) (Bell and Dutta, 2002; Blow and Dutta, 2005). The first complex recruited to origins is Origin Recognition Complex (ORC), which contains six subunits (Orc1–6). The ORC, which was first discovered in budding yeast (Bell and Stillman, 1992; Bell and Dutta, 2002), is an ATP-regulated DNA binding protein complex. In mammalian cells, Orc2 to 5 form a stable core

complex, while Orc1 and Orc6 are weakly interacted with the core complex (Giordano-Coltart et al., 2005). The ATP-bound ORC binds to origin DNA, followed by subsequent recruitment of Cdc6, licensing factor Cdt1, and Hbo1 (Fig. 2). Mcm2-7 helicase, which is essential for DNA unwinding prior to DNA replication (Liang et al., 1995), is then recruited to the ORC complex. Therein ATP is hydrolyzed and induces the dissociation of Cdc6 and Cdt1 from the ORC, resulting in the stable binding of the MCM with origin DNA.

Going beyond the well-characterized Plk1 functions in mitosis and cytokinesis, recent studies unveil a novel role of Plk1 in S phase. It has been reported that depletion of Plk1 by RNAi resulted in the disruption of pre-RC formation and DNA synthesis reduction in the first cell cycle. This indicates that Plk1 is indeed required for DNA replication in S phase (Yim and Erikson, 2009). Another study showed that Plk1 physically interacted with pre-RC proteins and thereby they were co-localized on centrosomes (Tsvetkov and Stern, 2005; Stuermer et al., 2007), further suggesting a possible role of Plk1 in S phase. To search for direct evidence that Plk1-associated kinase activity might regulate DNA replication, our lab identified two members of DNA replication machinery as Plk1 substrates recently. Aforementioned Hbo1 (histone acetyltransferase binding to the origin recognition complex 1), a binding partner of the Orc1 protein (Iizuka and Stillman, 1999), was one of such protein (Wu and Liu, 2008). Hbo1 binds to Mcm2, functioning as a coactivator of Cdt1 for facilitating the loading of MCM complex (Fig. 2). Plk1 directly interacts with Hbo1 in mitosis and phosphorylates Hbo1 at Ser57 *in vitro* and *in vivo* (Table 1). During mitosis, Hbo1 is first phosphorylated at Thr85/88 by Cdk1 to generate a docking site for subsequent recruitment of Plk1. Overexpression of the Plk1 unphosphorylatable mutant (Hbo1-S57A) leads to a G_1/S phase arrest, MCM loading inhibition, and DNA replication reduction. Similarly, Hbo1 depletion results in decreased DNA replication and a failure of MCM complex binding to chromatin. Significantly, both phenotypes can be partially rescued by the ectopic expression of WT Hbo1 but not Hbo1-S57A, suggesting that Plk1 phosphorylation of Hbo1 is indeed required for pre-RC formation and DNA replication licensing (Wu and Liu, 2008). This is the first piece of direct evidence to support a notion that Plk1 acts as a link to coordinate mitotic events with DNA replication.

Orc2 (origin recognition complex 2), another novel Plk1 substrate recently described, also has a well-documented role in DNA replication (Table 1) (Song et al., 2011). Plk1 was shown to interact with Orc2 in S phase and phosphorylates Orc2 at Ser188 *in vitro* and *in vivo*. Strikingly, Orc2-Ser188 phosphorylation is significantly enhanced when DNA replication is under various stresses, including UV, hydroxyurea, gemicitabine, or aphidicolin treatments. Overexpression of Plk1 unphosphorylatable mutant Orc2-S188A leads to defects in DNA synthesis under stress, suggesting that this phosphorylation event is critical to maintain DNA replication

under stressful conditions. In line with this observation, mechanistic dissection of the role of Plk1 phosphorylation of Orc2 in DNA replication under stress reveals that Orc2 phosphorylated at Ser188 associates with DNA replication origins and the Orc2-S188A mutant fails to maintain the functional pre-RC under replication stress (Fig. 2). Consequently, expression of the Orc2-S188A mutant results in intra-S phase checkpoint activation and delay of S-phase progression. This study suggests a novel role of Plk1 in facilitating DNA replication and maintaining genomic integrity under stressful conditions (Song et al., 2011).

This intriguing finding is reminiscent of us the functions of Plk1 in DNA checkpoint adaption discovered in yeast (*S. cerevasiae*). In response to DNA damage-induced cell cycle arrest in G_2, cells will undergo three possible scenarios. First, cells reenter cell cycle after a complete repair of the damaged DNA (checkpoint recovery). Second, apoptosis allows the elimination of cells with unrepaired DNA. Third, cells continue to the cell cycle with damaged DNA (checkpoint adaptation), which is potentially deleterious for cell survival (Bahassi, 2011). In interphase, when DNA damage occurs under stressful conditions, cell cycle is arrested and ATM/ATR-mediated checkpoint signaling pathway is activated, therefore providing time for DNA repair prior to the onset of mitosis (Fig. 2). After DNA repair is complete, Plk1-associated kinase activity mediates the checkpoint recovery for the progression of ongoing cell cycle. However, a mechanism for 'adaptation' to the G_2/M DNA damage checkpoint and overriding the checkpoint that permits cells with irreparable damage to reenter the cell cycle was found in yeast (*S. cerevasiae*) (Shirayama et al., 1998). This mechanism may be beneficial for unicellular organisms because it provides opportunities for cells to repair the damaged chromosomes in the next cell cycle, thus, enhancing their chances for survival (Galgoczy and Toczyski, 2001; Lee et al., 2001). This checkpoint adaption was later shown to be mainly mediated by yeast Plk, Cdc5 (Toczyski et al., 1997). In *Xenopus*, a similar scenario was observed. Nuclei containing egg extracts with replication inhibitor aphidicolin can adapt to replication stress and enter into mitosis with unreplicated DNA (Yoo et al., 2004). How does Plk1 regulate the inactivation of DNA damage checkpoint and reenter mitosis under stressful conditions? Plx, a *Xenopus* homolog of Plk1, was shown to inactivate Chk1 by regulating Claspin (Yoo et al., 2004). Claspin is an adaptor protein that is essential for Chk1 activation (Kumagai and Dunphy, 2003). Under genotoxic stress, Claspin is highly phosphorylated in an ATR-dependent manner, and it appears to be necessary for activation of Chk1 by ATR (Kumagai and Dunphy, 2003). However, ATR phosphorylation of Claspin at Thr906 creates a docking site for Plx binding, and the recruited Plx further phosphorylates Claspin at Ser934. Plk1 phosphorylation of Claspin leads to dissociation of Claspin from chromatin, resulting in inactivation of Chk1 and mitotic reentry.

Although checkpoint adaptation in *X. laevis* and *S. cerevisiae* is well-established, whether the similar mechanism exists in mammalian system is still controversial. Adaptation would appear to be unfavorable for mammalian systems because of the obvious mutator effect and the enhanced risk of cancer (Bahassi, 2011). However, Syljuåsen et al. (2006) showed that, following ionizing radiation-induced G_2 checkpoint arrest, human osteosarcoma cells can enter mitosis with γ-H2AX foci, a marker for unpaired DNA DSBs (double-strand breaks). Plk1 and Chk1 seem to control this process, at least partially, via independent signal pathways. Inhibition of Chk1 promotes exit from G_2 checkpoint, whereas overexpression of Chk1 or depletion of Plk1 delays this process. These results suggest that human cells are able to exit the checkpoint arrest and divide before damage has been fully repaired. In line with this notion, under DNA replication stressful conditions, the enhancement of Plk1 phosphorylation of Orc2 was shown to maintain the pre-RC and promote DNA replication at S phase in human cells, albeit intra-S phase checkpoint activation in the Orc2-S188A mutant-expressing cells would delay the progression of cell cycle. Although it is still not known whether the mammalian cells can actually enter mitosis and finish cell division with unrepaired DNA damage that was introduced in S phase, nevertheless, this finding provides additional evidence to support possible existence of checkpoint adaptation mechanism in human cells. Still, it is somewhat surprising that this process occurs in higher eukaryotes, as cell division with damaged DNA may be detrimental for genomic stability and enhances the risk of cancer development (Syljuåsen et al., 2006). It will be very intriguing to speculate that checkpoint adaptation might occur only in cancer cells, but not in normal cells. Further investigation needs to be done to provide more convincing evidence to support this model in mammalian cells.

PLK1 IS ESSENTIAL FOR EARLY EMBRYONIC DEVELOPMENT

Although much work has focused on Plk1 in cell cycle regulation, little is known about the physiological functions of Plk1 *in vivo*. In a recent study, Lu and colleagues generated knockout mice to investigate the functions of Plk1 *in vivo*. Plk1 homozygous null mice were embryonic lethality, and early Plk1$^{-/-}$ embryos failed to survive after the eight-cell stage. However, Plk1$^{+/-}$ mice are born and fertile, with no obvious effects except a slight decrease in Plk1 levels by comparison with those in Plk1 WT mice. Surprisingly, the increased incidence of tumors in Plk1 heterozygous mice is highly significant by comparing with Plk1 WT mice (Plk1$^{+/-}$ 27.5% vs Plk1 WT 9%). This data suggests that Plk1 is important for early embryonic development and may function as a haploinsufficient tumor suppressor (Lu et al., 2008). Moreover, Plk1 also was shown to be very critical for mitosis in the embryonic development of zebrafish. In the early embryonic

development of zebrafish, the expression of Plk1 was ubiquitous by the bud stage, but became restricted to the proliferating tissues, such as brain, eyes, spinal cord, somites, fin folds, and tail bud, by 24 h post fertilization. Depletion of Plk1 resulted in mitotic arrest and finally death by 6 days post-fertilization (Jeong et al., 2010). Taken together, these studies suggest that Plk1 is essential for early embryonic development and normal level of Plk1 is critical for maintaining chromosomal stability. Deregulation of Plk1 in embryonic development leads to embryonic lethality or increased risk of tumorigenesis in development.

DEREGULATION OF PLK1 LEADS TO ONCOGENIC TRANSFORMATION

In normal cells, the expression level and activity of Plk1 is strictly controlled by its upstream regulators and proteasomal degradation, respectively, for orderly regulating cell cycle events. The expression and activity of Plk1 is low throughout G_0, G_1, and S phase, begins to rise in G_2 phase and peaks during M phase (Lake and Jelinek, 1993; Golsteyn et al., 1994, 1995; Lee et al., 1995). Plk1 is then degraded by the APC/C starting in late mitosis and continuing throughout G_1. In many cancer cell lines, Plk1 can be easily detected in G_1 phase with apparent nuclear localization (Li et al., 2008). Deregulated activity of Plk1 results in abnormalities in centrosome duplication, maturation and/or microtubule dynamics, therefore causing increased centrosome size and/or centrosome number, both of which show a positive and significant correlation with aneuploidy and chromosomal instability (Takai et al., 2005). Therefore, Plk1 has been accepted as a marker for cell proliferation and associates with oncogenic transformation. A well-known study shows overexpression of murine Plk1 in NIH3T3 cells led to a transformed phenotype. The transformed cells formed colonies and were capable of growth in soft agar and formation of tumors in nude mice (Smith et al., 1997). Furthermore, it has been reported that elevated levels of Plk1 were found in non-small-cell lung cancer (Wolf et al., 1997), head/neck squamous cell carcinomas (HNSCC) (Knecht et al., 1999), esophageal carcinoma (Tokumitsu et al., 1999), oropharyngeal carcinomas (Knecht et al., 2000), melanomas (Strebhardt et al., 2000), breast cancer (Wolf et al., 2000), endometrial carcinomas (Takai et al., 2001b), colorectal cancer (Macmillan et al., 2001; Takahashi et al., 2003), ovarian cancer (Takai et al., 2001a; Weichert et al., 2004a), pancreatic cancer (Gray et al., 2004), prostate carcinomas (Weichert et al., 2004b) and in papillary carcinomas (Ito et al., 2004). These studies suggest that deregulation of Plk1 highly correlates with carcinogenesis of various tumors in human.

Possible roles of Plk1 in prostate cancer development

Prostate cancer is the most common cancer in Western men

and the leading cause of cancer deaths in the United States. Although we still could not fully understand the mechanisms of prostate cancer initiation and progression, recent advances in high throughput genetic technologies together with various animal model studies and human tissues of prostate cancer allow a comprehensive analysis and functional validation of the molecular changes, therefore identifying critical genetic and epigenetic changes that promote prostate cancer initiation and progression, and developing novel therapeutic approaches that can help to combat this disease (Knudsen and Vasioukhin, 2010).

Architecture of prostate gland

Human prostate gland, which surrounds the urethra, is resided at the base of the bladder. It is a walnut-sized organ. Adult prostate gland lacks discernible lobular structures. McNeal defined the human prostate as a zonal structure, including central zone, peripheral zone, transition zone, and anterior fibromuscular zone (Fig. 3A) (McNeal, 1969, 1981, 1988; Timms, 2008). It is worthy to note that the outmost peripheral zone occupies the most volume of the prostate and is the carrier of the majority of malignant prostate carcinomas, whereas the benign prostatic hyperplasia (BPH), a common non-malignant tumor, arises from the transition zone (Fig. 3A and 3B) (Shen and Abate-Shen, 2010). In contrast to human prostate, mouse prostate gland is composed of several distinct lobular structures, including ventral, lateral, dorsal, and anterior lobes (Fig. 3A). The dorsolateral lobe of mouse prostate gland is most similar to the peripheral zone of those in human by comparison of the gene expression profiling of human and mouse prostate gland (Berquin et al., 2005).

Both human and mouse prostate glands consist of two compartments: epithelial and stromal (Fig. 3B). And these two compartments are separated by basement membrane, which is the packed structure of collagen fibers containing various extracellular proteins secreted from both epithelial and stromal cells (Bonkhoff et al., 1991, 1992). The epithelial layers contain three differentiated cell types: luminal, basal, and neuroendocrine (Fig. 3B) (Foster et al., 2002; van Leenders and Schalken, 2003; Hudson, 2004; Shappell et al., 2004; Peehl, 2005). The polarized columnar epithelial cells form a continuous layer, luminal layer, which is responsible for the prostatic secretions. Luminal cells express high levels of androgen receptor (AR), cytokeratin 8/18, CD57, and Nkx3.1. In contrast, basal epithelial cells, which separate the luminal layer from stroma (Brawer et al., 1985; Nagle et al., 1987; Liu et al., 1997; van Leenders and Schalken, 2003), express cytokeratins 5/14, P63, CD44, GSTP1, with low or undetectable levels of AR (Knudsen and Vasioukhin, 2010; Shen and Abate-Shen, 2010). Neuroendocrine cells, which are morphologically indistinguishable from basal cells, express endocrine markers, such as Chromgranin A and synaptophysin, but do not express AR (Shen and Abate-Shen,
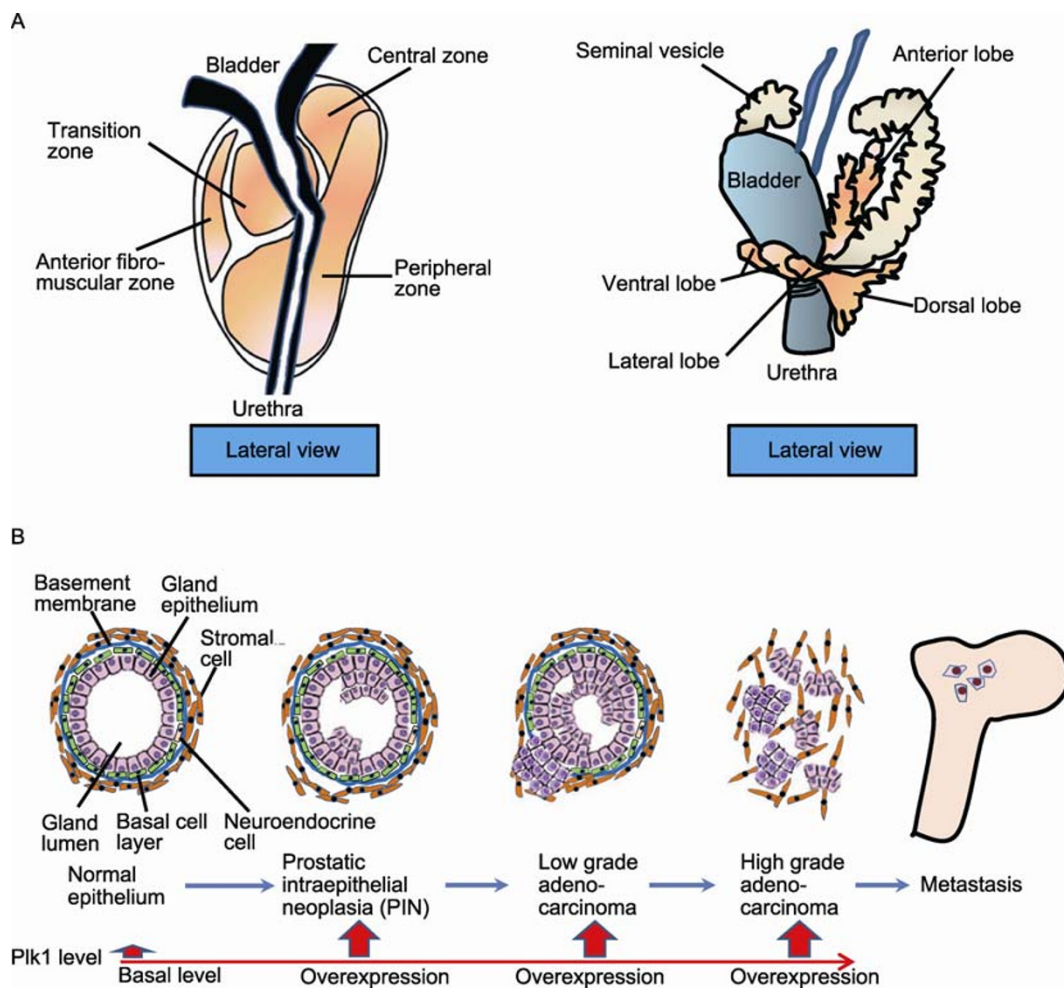
Figure 3. The comparison of the architectures of human and mouse prostate glands (A) and model of histological changes during prostate cancer initiation and progression (B). It has been shown that the overexpression of Plk1 in each stage of prostate carcinoma of tissue specimens by comparing with normal prostate tissues (Weichert et al., 2004b) (bottom panel).

2010). Cell type specific expression is often used to analyze the prostate gland and to detect glandular pathology (Shen and Abate-Shen, 2010). The stromal cell compartment of prostate gland contains several different cell types, including smooth muscle cells, fibroblasts, and endothelial cells with smooth muscle cells as the most abundant cell type (Bartsch et al., 1979).

Overexpression of Plk1 in prostate cancer development

Advanced age is the most highly risk factor for prostate cancer initiation. It has been reported that men who are younger than 40 have one in 10,000 chance of developing prostate cancer and that the risk increases to one in seven by the age of 60 (American Cancer Society, 2009). However, prostate cancer is not simply a by-product of aging, since incidence varies considerably among different populations (Shen and Abate-Shen, 2010). This phenomenon more likely reflects the

interplay of environmental, physiological, and molecular influences with normal consequences of aging that presumably exacerbate the effects of these influences (Shen and Abate-Shen, 2010). The development of human prostate cancer includes four stages, which develops from prostatic intraepithelial neoplasia (PIN) to high grade adenocarcinoma, and then metastasis (Fig. 3B). Several important factors which have been implicated in prostate cancer initiation and progression, including inflammation, oxidative stress and DNA damage, telomere shortening, senescence, genomic alterations, genetic factors, and epigenetic alterations, together with molecular mechanisms of prostate carcinogenesis, have been subjected to extensive reviews (Knudsen and Vasioukhin, 2010; Shen and Abate-Shen, 2010). We here pay more attention to our lab's recent finding on the role of Plk1 in prostate cancer initiation.

It has been shown that Plk1 associates with prostate cancer initiation and progression. In the study by Weichert

and his colleagues, 78 tissue specimens of human prostate carcinoma, adjacent normal prostate tissue, and benign hyperplasia, were used to investigate the expression of Plk1. It was shown that 52.6% of prostate cancer specimens had strong expression of Plk1 and that expression of Plk1 positively correlated with Gleason grade (Fig. 3B) (Weichert et al., 2004b). In contrast, no significant Plk1 expression was detected in normal prostate epithelium and stroma as well as in the benign prostate hyperplasia specimens. This study demonstrated that Plk1 is overexpressed in prostate cancer and is linked to higher tumor grades (Weichert et al., 2004b). Moreover, knockdown of Plk1 by RNAi resulted in a significant decrease in cell viability, induction of apoptosis, defects in important mitotic processes (failure of cytokinesis, and the defects in centrosome integrity and maturation), and G_2/M phase arrest in human prostate cancer cell lines representing different stages of disease progression (Reagan-Shaw and Ahmad, 2005b). Although the link between Plk1 and prostate cancer was well-established in these studies, the mechanism underlying the role of Plk1 in prostate cancer initiation and progression remains elusive. In a recent publication, Liu and colleagues unveiled the mechanism of Plk1 in facilitating loss of Pten-induced prostate cancer formation. In this report, prostate cancer cell lines, prostate-specific Pten-deletion mouse model, xenograft mouse model were used to investigate the role of Plk1 in loss of Pten-induced prostate cancer. Pten knockout study showed that depletion of Pten results in an elevated level of Plk1 in $Pten^{-/-}$ mouse prostate tissues and that the elevation of Plk1 is critical for Pten-depleted cells to adapt to mitotic stress, which is conferred by Pten depletion, for survival. Moreover, overexpression of Plk1 correlates with genetic inactivation of Pten during human prostate neoplasia formation (Liu et al., 2011). Prostate cancer is associated with genetic alterations involving the PI3K and AR pathways, both of which mediate survival signals in prostate cancer (Carver et al., 2011). About 40% of primary and 70% of metastatic prostate cancers have genetic alterations in the PI3K pathway, mostly through loss of Pten (El Sheikh et al., 2008; Reid et al., 2010; Taylor et al., 2010). Studies based on conditional prostate-specific Pten deletion and Pten silenced cell lines have established that loss of Pten promotes resistance to castration (Gao et al., 2006; Jiao et al., 2007), suggesting that loss of Pten might be one of the causes of the development of castration resistance prostate cancer (CRPC). Furthermore, it is most likely that Plk1 mediates the Pten loss after castration, therefore resulting in the development of CRPC. It will be very intriguing to test whether Plk1 directly targets Pten and causes its inactivation. Nevertheless, this finding sheds light on further dissection of the mechanism of Plk1 in prostate cancer initiation and progression.

Targeting Plk1 in prostate cancer treatment

It is well-characterized that Plk1 is a key player in cell prolif-

eration and that overexpression of Plk1 contributes to oncogenic transformation (Guan et al., 2005). A study of overexpression of Plk1 in NIH3T3 cells which confers the transformed phenotype (Eckerdt et al., 2005) suggested that Plk1 overexpression is a cause but not a result of oncogenic transformation (Ahmad, 2004; Spänkuch et al., 2004; Eckerdt et al., 2005; Reagan-Shaw and Ahmad, 2005a). So inhibition of Plk1 might become a promising new chemotherapeutic approach for prostate cancer treatment (Weichert et al., 2004b). Depletion of Plk1 by siRNA resulted in induction of apoptosis of human cancer cells (Liu and Erikson, 2003). In a further detailed study, knockdown of Plk1 in three prostate cancer cell lines, LNCaP, DU145, and PC-3, by siRNA approach also resulted in a significant decrease in growth and viability with concurrent induction of apoptosis (Reagan-Shaw and Ahmad, 2005b). Therefore, Plk1 has been proposed as a promising drug target for prostate cancer treatment (Reagan-Shaw and Ahmad, 2005a). Indeed, numerous small-molecule Plk inhibitors have been developed to target ATP-binding pocket and PBD domain of Plk1, respectively. However, because of the high level of sequence identity of kinase domain of Plk1 with those of Plk2, Plk3, and Plk4, ATP-competitive inhibitors of Plk1, such as PHA-680626 and BI2536, scarcely discriminate between Plk1 and Plk3 (Kothe et al., 2007a, 2007b; Steegmaier et al., 2007). Therefore, the development of specific ATP-competitive Plk1 inhibitors is still challenging (Johnson et al., 2007). Targeting the PBD of Plk1 is another strategy for developing Plk1 inhibitors. Recently, small molecule inhibitors for impairing the interaction of Plk1 PBD with its substrates were identified with a high-throughput screening assay based on fluorescence polarization (Reindl et al., 2008a, 2008b). Some specific PBD inhibitors, such as poloxin (Hanisch et al., 2006), poloxipan (Reindl et al., 2009), and purpurogallin (Watanabe et al., 2009), were identified and their antitumor activity were under investigation. In addition, several other Plk1 inhibitors, including BI2536, BI6727, ON01910.Na, HMN-214, GW843682X, GSK461364, NMS-P937, have been developed to evaluate their potential in cancer treatments clinically (Strebhardt, 2010).

CLINICAL-TRANSLATIONAL TRIALS OF PLK INHIBITORS

The aforementioned Plk1 inhibitors have been investigated in clinical trials. The results from phase I or II studies of four Plk1 inhibitors, BI2536, ON01910.Na, HMN-214, and GSK461364, have been reported recently (Garland et al., 2006; Jimeno et al., 2008; Mross et al., 2008; Pandha et al., 2008; Sehnoy et al., 2008; Vose et al., 2008; Gandhi et al., 2009; Olmos et al., 2009; Sebastian et al., 2009).

BI2536, a highly selective ATP-competitive Plk1 inhibitor, causes perturbation of the spindle assembly, resulting in mitotic arrest and apoptosis. BI2536 has been studied in patients with solid tumors or hematologic malignancies. In

phase I trials, various dose-ranging schedules were used to assess the maximum-tolerated dose (MTD), overall safety, pharmacokinetics (PK), and efficacy of i.v. BI2536. The MTD of 175 mg or 200 mg was established for the patients with relapsed or refractory non-Hodgkin's lymphoma (NHL), or advanced solid tumors, respectively, when administered every 21 days. Nausea, fatigue, and anorexia were of moderate to mild intensity (Mross et al., 2008; Vose et al., 2008). Phase II studies of BI2536 in patients with non-small lung cancer and hormone refractory prostate cancer showed modest activity. Mild fatigue and nausea were also observed (Pandha et al., 2008; Sebastian et al., 2009).

ON01910.Na has been shown to inhibit the growth of cancer cells and lead to the apoptosis in multiple cancer cell lines *in vitro* and *in vivo* (Gumireddy et al., 2005). The treatment of the cells with ON01910.Na resulted in abnormalities of spindle and centrosomes and cell cycle arrest at G_2/M phase. In phase I trials, the MTD of 3120 mg of ON01910.Na was established when administered in 4-week cycles (2-h infusion, twice weekly for 3 weeks). The common adverse events, including fatigue, pain, nausea, vomiting, and abdominal pain, were observed (Jimeno et al., 2008).

HMN-214 is an oral stilbene derivative and is a prodrug of the active agent HMN-176. HMN-176 interferes with the subcellular localization of Plk1, resulting in G_2/M arrest and destruction of the spindle polar bodies and DNA fragmentation. Two phase I studies in patients with advanced solid tumors were performed to assess the MTD and the pharmacokinetics of HMN-214. An MTD of 8 mg/m^2/d was established when administered orally, daily for 21 days of a 28-day cycle. Severe myalgia and/or bone pain syndrome and hyperglycemia with dose-limiting toxicity (DLTs) at a dose of 9.9 mg/m^2/d were observed in this dosing schedule. In 5-day dosing schedule, the DLTs, neutropenic sepsis and prolonged neutropenia were observed in the heavily pretreated patients, while DLTs were febrile neutropenia, electrolyte disturbances, neuropathy, and myalgia in lightly pretreated patients (Patnaik et al., 2003; von Hoff et al., 2004; Garland et al., 2006).

GSK461364 is a selective i.v. thiophene amide ATP-competitive inhibitor of Plk1. High concentration (> 300 nmol/L) of GSK461364 caused G_2 phase arrest and low concentration (10–300 nmol/L) caused M phase arrest. In phase I studies, an MTD of 225 mg was established when administered weekly for 3 weeks in a 4-week cycle, while the MTD was 75 mg when administered twice weekly for 3 weeks in 4-week cycle. The observed DLTs were neutropenia, thrombocytopenia, and bone marrow suppression (Olmos et al., 2009).

FURTHER PERSPECTIVES—PLK1 RISES FROM CELL CYCLE REGULATION TO CANCER RESEARCH

There is no doubt that great advances have been made in elucidating the roles of Plk1 in cell cycle regulation over the past 20 years. These advances have greatly improved our understanding of how Plk1 works in mediating many cell cycle events, including DNA replication, centrosome maturation, bipolar spindle formation, cytokinesis, cell cycle checkpoint activation and inactivation by phosphorylation of different specific substrates. Although many studies have linked Plk1 with oncogenic transformation and carcinogenesis, and come out with a wealth of phenotypic information to support the correlation of Plk1 elevation with various cancer initiation and progression, little is known about the 'in-depth' mechanism of Plk1 in carcinogenesis, or at least, in prostate carcinogenesis. In future studies, going beyond the classical cell cycle-related Plk1 functions, identifying new Plk1 partners, and linking Plk1 with oncogenic signaling pathways would be an attractive direction for the next wave of Plk1 research.

ABBREVIATIONS

AR, androgen receptor; CRS, cytoplasmic retention signal; INCENP, the inner centromere protein; γ-TuRC, γ-tubulin ring complexes; MTD, maximum-tolerated dose; NES, nuclear- export signal; NLP, ninein-like protein; ORC, Origin Recognition Complex; PBD, polo-box domains; PBIP1, polo-box interacting protein; Plk1, polo-like kinase 1

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