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Putting a bit into the polo-box domain of polo-like kinase 1

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Abstract

Polo-like kinase 1 (Plk1) plays key roles in regulating various mitotic processes that are critical for cellular proliferation. A growing body of evidence suggests that Plk1 overexpression is tightly associated with the development of human cancers. Interestingly, various types of cancer cells are shown to be addicted to a high level of Plk1, and the reversal of Plk1 addiction appears to be an effective strategy for selectively killing cancer cells, but not normal cells. Therefore, Plk1 is considered an attractive anticancer drug target. Over the years, a large number of inhibitors that target the catalytic activity of Plk1 have been developed. However, these inhibitors exhibit significant levels of cross-reactivity with related kinases, including Plk2 and Plk3. Consequently, as an alternative approach for developing anti-Plk1 therapeutics, substantial effort is under way to develop inhibitors that target the C-terminal protein–protein interaction domain of Plk1, called the polo-box domain (PBD). In this communication, I will discuss the pros and cons of targeting the PBD in comparison to those of targeting the ATP-binding site within the kinase domain.

Keywords: Polo-like kinase 1; Polo-box domain; Inhibitor; Cancer therapy

Review

Protein phosphorylation by protein kinases represents a fundamental mechanism underlying diverse biochemical and cellular processes that are important for the proliferation of eukaryotic cells (Hanks et al. 1988). Protein kinases are a family of enzymes that catalyze the transfer of the gamma phosphate from adenosine triphosphate (ATP) to a protein substrate and, as a result, induce a change in the conformation and function of the protein substrate. A large body of evidence suggests that deregulating this process can lead to various pathological disorders in humans, including cancers (Lahiry et al. 2010). Therefore, deregulated protein kinases may represent attractive targets for the development of therapeutics against various human disorders. However, unlike initial expectations, targeting protein kinases has proven difficult largely because of the similarities in their primary sequences and conserved structural motifs around the ATP-binding site. Nevertheless, recent advances in our understanding of this family of enzymes have allowed us to overcome these obstacles and develop a sizable number of clinically applicable therapeutic agents. According to the Protein Kinase Inhibitors in Oncology Drug Pipeline Update 2015, small-molecule inhibitors were reported against nearly half of a total of 518 cellular protein kinases. Among them, more than 35 inhibitors are approved by the US Food and Drug Administration for clinical applications, and approximately 500 inhibitors are currently in clinical trials for further development.

Although developing inhibitors that target the catalytic activity of a protein kinase has become a prevailing method, various efforts are under way to develop inhibitors that target a functionally critical protein–protein interaction domain of a kinase. This newly emerging strategy, which is thought to yield a higher level of specificity than conventional ATP analog inhibitors, may lead to the development of a different class of inhibitors that could be used either alone or in combination with available catalytic inhibitors to achieve increased drug efficacy.

Polo-like kinases

Polo-like kinases (collectively known as Plks) belong to the evolutionarily conserved polo subfamily of Ser/Thr protein kinases that play pivotal roles in cell proliferation, differentiation, and adaptive responses (see reviews; Winkles and Alberts 2005; Petronczki

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et al. 2008; Archambault and Glover 2009; Zitouni et al. 2014). In mammalian cells, five Plks (Plk1–5) were identified to date (Fig. 1a), and they exhibit distinct tissue distributions and physiological functions. Except for Plk4, these members contain a signature domain, called the polo-box domain (PBD), which is composed of two motifs with significant homology—polo-box 1 (PB1; residues 405–494 in Plk1) and polo-box 2 (PB2; residues 505–598 in Plk1). Plk4 contains the distantly related cryptic polo box (CPB) and PB3 domains, which interact with its binding targets (Leung et al. 2002; Park et al. 2014).

Among them, Plk1 has drawn a lot of attention because of its tight association with tumorigenesis in human cells. Various studies have shown that Plk1 is highly expressed during the G2 and M phases of the cell cycle (Golsteyn et al. 1995; Lee et al. 1995), and it plays an important role in regulating mitotic entry, centrosome maturation and bipolar spindle assembly, metaphase/anaphase transition, and cytokinesis (Winkles and Alberts 2005; Petronczki et al. 2008; Archambault and Glover 2009; Zitouni et al. 2014) (Fig. 1b). Consistent with the multitude of Plk1 functions, Plk1 has been shown to localize to distinct

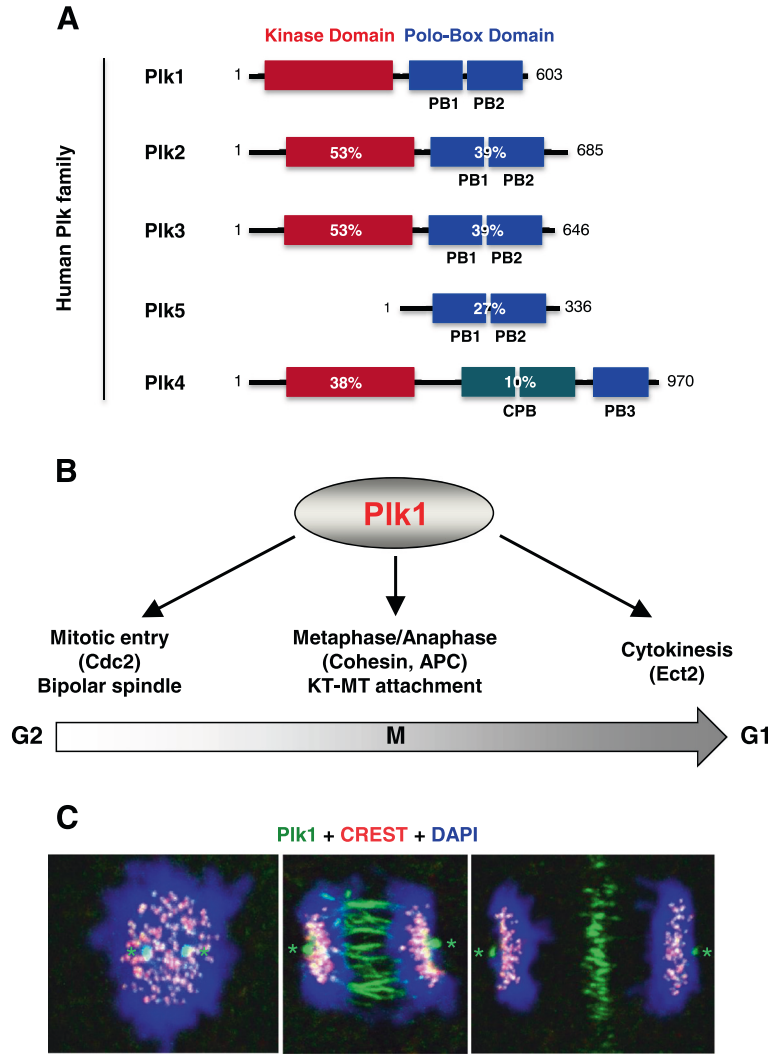

Fig. 1 The human Plk family and the function and subcellular localization of Plk1 during the cell cycle. **a** A schematic diagram illustrating the structures of the human Plk family. Sequence identity (%) with Plk1 is shown. *PB1* polo-box motif 1, *PB2* polo-box motif 2, *CPB* cryptic polo box, *PB3* polo-box motif 3. Numbers, amino acid residue numbers for each Plk. **b** A schematic diagram depicting the mitotic functions of Plk1 from G2/M transition to cytokinesis. **c** Subcellular localization of Plk1 in HeLa cells during the cell cycle. Kinetochore-localized Plk1 signals are colocalized with CREST antigens. *Asterisks* centrosomes. These images were originally published in Journal of Biological Chemistry. Seong YS, et al. A spindle checkpoint arrest and a cytokinesis failure by the dominant-negative polo-box domain of Plk1 in U-2 OS cells. 2002; 277(35):32282-93. © the American Society for Biochemistry and Molecular Biology

subcellular structures, such as centrosomes, kinetochores, and midzones/midbodies, in a temporally and spatially regulated manner (Holtrich et al. 1994; Golsteyn et al. 1995; Lee et al. 1995; Arnaud et al. 1998; Seong et al. 2002) (Fig. 1c). The PBD is largely responsible for directing its catalytic activity of Plk1 to specific subcellular locations (Lee et al. 1998; see review; Park et al. 2010) via its capacity to interact with a phosphorylated Ser/Thr motif, thereby bringing the enzyme in close proximity to its binding targets or substrates localized at these sites (Cheng et al. 2003; Elia et al. 2003; Lowery et al. 2004; Park et al. 2010). As expected, the function of Plk1 PBD is essentially required for proper mitotic progression (Lee et al. 1998, 1999; Seong et al. 2002; Hanisch et al. 2006). As of today, a large number of PBD-binding proteins critically required for various Plk1-dependent mitotic events have been isolated and characterized (Park et al. 2010). Thus, the PBD serves as an essential cis-acting element that mediates various Plk1-dependent biochemical steps and cellular processes at specific subcellular structures.

Distinct from the roles of Plk1 during the late stage of the cell cycle, Plk2 appears to be transiently expressed in G1 and contributes to proper S-phase entry (Simmons et al. 1992; Ma et al. 2003a, b). Other studies showed that Plk2 plays a role in maintaining cell viability after spindle poisoning (Burns et al. 2003). Interestingly, Plk3 is expressed throughout the cell cycle (Chase et al. 1998) and has been implicated in responding to DNA damage and cellular stress (Donohue et al. 1995; Xie et al. 2001a, b, 2002, 2005; Bahassi et al. 2002). Both Plk2 and Plk3 are proposed to function as tumor suppressors (Smith et al. 2006; Yang et al. 2008). On the other hand, Plk4 has been shown to function as a key regulator of centriole biogenesis at the early stage of the cell cycle (Bettencourt-Dias et al. 2005; Habedanck et al. 2005; Duensing et al. 2007; Kleylein-Sohn et al. 2007), suggesting that Plk4-dependent centriole duplication lays a groundwork for Plk1-dependent centrosome maturation and bipolar spindle formation at the time of mitotic entry.

Plk1: a cancer cell-selective anticancer drug target

Consistent with the important role of Plk1 in regulating various mitotic events, Plk1 overexpression is thought to promote neoplastic transformation of human cells (Eckerdt et al. 2005; Strebhardt and Ullrich 2006; Strebhardt 2010). Not surprisingly, Plk1 overexpression appears to be tightly associated with aggressiveness and poor prognosis of various types of human cancers. In addition, recent genome-wide studies have revealed that Plk1 and a number of other mitotically important regulators, such as the anaphase-promoting complex/cyclosomes and the proteasome, are required for the viability of activated *RAS* or inactivated *TP53* mutation-bearing cancer cells, but not for the respective normal cells (Luo et al. 2009a; Sur et al. 2009). These observations suggest that cancer cells are addicted not only to oncogenic *RAS* or the inactivated p53 function, as Bernard Weinstein originally proposed (Weinstein 2002), but also to non-oncogenic Plk1, whose inhibition results in prometaphase accumulation and subsequent death (Luo et al. 2009b) (Fig. 2). These observations suggest that Plk1-dependent biochemical steps and signaling pathways are likely reprogrammed for the survival and proliferation of Plk1-addicted cancer cells. Under these conditions, the reversal of Plk1 addiction may be sufficient for triggering cancer cell-selective mitotic block and apoptotic cell death (Luo et al. 2009b), as has been demonstrated by the reversal of oncogene addictions (McMurray et al. 2008). As an alternative explanation to the oncogene (and also perhaps non-oncogene) addiction, Dean Felsher proposed that oncogene activation may induce a state of cellular amnesia, which allows cells to bypass surveillance mechanisms and, therefore, permits unregulated cell proliferation (Felsher 2008). Whether the altered cellular homeostasis in cancer cells is called addiction or amnesia, studies suggest that the reversal of Plk1 addiction is sufficient for inducing selective cellular senescence or apoptosis in oncogenic *RAS*- or inactivated *TP53*-containing cancer cells (Luo et al. 2009a; Sur et al. 2009) (Fig. 2). Therefore, antagonizing the Plk1 function appears to be

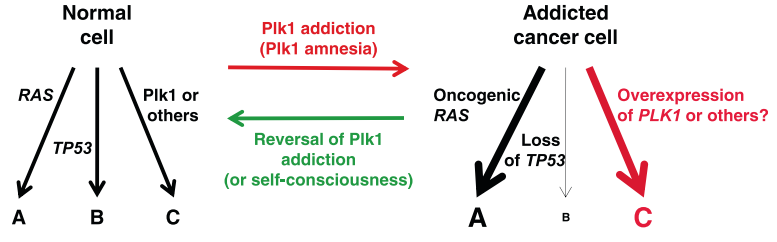

Fig. 2 A schematic diagram illustrating hypothetical biochemical pathways between normal cells and Plk1-addicted cancer cells bearing an oncogenic *RAS* and/or a *TP53* loss-of-function mutation. Reversal of Plk1 addiction (or gaining self-consciousness from Plk1 amnesia) may induce cancer cell–selective mitotic arrest and apoptotic cell death

a particularly appealing strategy for killing oncogenic *RAS*- or inactivated *TP53*-containing cancer cells. Since both Plk2 and Plk3 are required for promoting cell survival (Burns et al. 2003; Xie et al. 2005) and they exhibit properties similar to tumor suppressors (Smith et al. 2006; Yang et al. 2008; Coley et al. 2012), specific inhibition of Plk1, but not Plk2 or Plk3, would be important for selectively killing cancer cells, but not normal cells.

Targeting the kinase domain vs. the PBD of Plk1

Both the kinase domain (KD) and PBD of Plk1 are essentially required for the mitotic functions of Plk1 (Lee et al. 1998; Seong et al. 2002; Hansen et al. 2004), suggesting that they represent two distinct drug targets within one molecule (Fig. 3). The ATP-binding pocket within the KD serves as a well-defined target site that may allow one to achieve the complete annihilation of the kinase's catalytic activity. Consequently, targeting the ATP-binding site has long been a prevailing method for generating kinase inhibitors. Over the years, several ATP analog inhibitors (e.g., BI2536, BI6727, GSK461364A, cyclapolin 1, DAP81, and TAL) have been developed to competitively inhibit Plk1 catalytic activity. BI6727 (also called volasertib) is the most advanced and has been evaluated under phase III clinical trials. However, because of the structural similarities among the catalytic domains of >500 intracellular kinases, these inhibitors largely exhibit a broad inhibitory activity against closely related Plk2 and Plk3 and several other kinases (Steegmaier et al. 2007; Rudolph et al. 2009; Raab et al. 2014), therefore frequently limiting their in vivo applicability because of poor pharmacological safety profiles and dose-limiting toxicity (Lee et al. 2015).

As a new approach to bypass the problems associated with currently available KD inhibitors, a large body of studies has been performed to generate Plk1 PBD antagonists. This new approach is important because targeting protein–protein interactions is considered a highly attractive strategy that provides a potential for developing specific inhibitors against a particular protein. However, finding a targetable protein–protein interaction motif with a distinct binding nature is thought to be difficult because binding surfaces are mostly nondescript. Against the odds, studies with Plk1 PBD revealed that a small and specific phosphopeptide is sufficient for interacting with Plk1 PBD, but not with PBDs from Plk2 or Plk3, with a high affinity (Elia et al. 2003; Yun et al. 2009), suggesting that specific inhibition of Plk1 PBD could be achieved by low-molecular weight, peptide-derived inhibitors, or structurally related compounds. Moreover, recent data suggests that interrogating Plk1 PBD function by expressing a highly specific, suicidal PBD-binding peptide can potently induce mitotic block and apoptosis in tumorigenic or metastatic cancer cells, but not in normal cells (Park et al. 2015). Albeit this promising outlook, small-molecule PBD inhibitors developed to date (Reindl et al. 2008; Watanabe et al. 2009) exhibit only suboptimal Plk1 PBD-binding affinity with an undefined binding mode (Liao et al. 2010). On the other hand, peptide-derived Plk1 PBD inhibitors exhibit superb binding affinity and specificity in vitro. However, they suffer greatly from poor membrane permeability and low bioavailability in cell-based assays (Liu et al. 2011; Qian et al. 2014), thus necessitating further improvement of these inhibitors for in vivo studies.

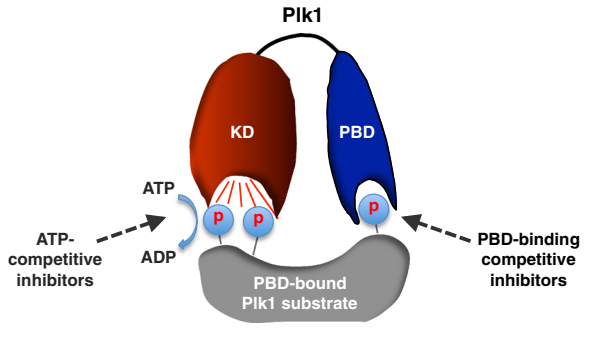

Fig. 3 A schematic diagram illustrating two distinct strategies for targeting Plk1. ATP-competitive analogs have been widely developed to inhibit the catalytic activity of Plk1, while PBD-binding antagonists are being developed to competitively inhibit the function of PBD. *KD* kinase domain, *PBD* polo-box domain. In the diagram, the PBD is shown to interact with a phosphorylated epitope on a substrate, and it tethers the cis-acting N-terminal KD in close proximity to its substrate. *ATP* adenosine triphosphate, *ADP* adenosine diphosphate

Advantages of targeting the PBD of Plk1

Besides being an alternative target for anti-Plk1 drug discovery, it is important to note that inhibiting the PBD is fundamentally different from inhibiting the KD, at both physiological and biochemical levels. Studies showed that inhibiting Plk1 catalytic activity potently induces early mitotic arrest (Sumara et al. 2004; Hanisch et al. 2006; Lenart et al. 2007), whereas inhibiting Plk1 PBD function results in preanaphase arrest (Seong et al. 2002; Hanisch et al. 2006). These findings suggest that this Plk1 kinase activity-dependent process is essentially required from the early stage of mitosis, whereas the PBD-dependent Plk1 function is essential only at a much later stage but prior to the metaphase/anaphase transition. At the biochemical level, the KD binds to ATP as the only ligand, whereas the PBD binds to a large spectrum of binding targets, such as Plk1-binding proteins and physiological substrates (Park et al. 2010). Moreover, the PBD binds to its various targets with different levels of affinity, thus enabling the PBD to mediate diverse Plk1-dependent events in a differentially regulated manner. These fundamental differences in the biochemical properties of the KD versus the PBD predict that ATP analog

inhibitors would annihilate Plk1 catalytic activity in both normal and cancer cells equally, whereas PBD inhibitors could interfere with only a subset of PBD-dependent interactions. Since Plk1-dependent signaling pathways and biochemical steps are predictably rewired in Plk1-addicted cancers, it would be feasible to tailor PBD inhibitors in such a way that they would interfere with PBD-dependent interactions enriched in cancer cells, but not normal cells. Therefore, unlike inhibiting Plk1 KD, antagonizing the PBD function may allow one to uniquely impose an additional layer of selectivity in killing cancer cells, but not normal cells.

Recent advances in developing Plk1 PBD inhibitors

During the past several years, numerous independent efforts have been made to generate both small-molecule- and peptide-based PBD inhibitors (Lee et al. 2015). Small-molecule inhibitors were isolated from in vitro screenings designed to isolate compounds capable of disrupting the Plk1 PBD-dependent interaction with its cognate peptide ligand. These small-molecule inhibitors include a thymoquinone derivative, poloxin (Reindl et al. 2008), a benzylidene-thiazolotriazenedione derivative, poloxipan (Reindl et al. 2009), and a benzotropolone derivative, purpurogallin (Watanabe et al. 2009). However, these inhibitors exhibit only weak inhibitory activity against Plk1 PBD in vitro (Liao et al. 2010), with a substantial level of nonspecific toxicity in cultured cells (Park, J. -E, and K. S. Lee, unpublished data), suggesting that their applicability is likely limited. Optimizing these inhibitors to improve both Plk1 PBD-binding affinity and specificity would be necessary in order to use them in preclinical and clinical studies. The co-crystal structures of Plk1 PBD, in complex with the structurally similar thymoquinone or the oxime fragment of poloxin (called poloxime), have been determined (Yin et al. 2013). The binding mode of thymoquinone or the oxime fragment may potentially serve as a template for anti-PBD drug discovery.

Studies with various PBD-binding proteins led to the identification of a peptide as small as 5-mer PLHSpT (Yun et al. 2009). This peptide was identified from the T78 motif of a kinetochore component called PBIP1 (Kang et al. 2006). PLHSpT falls into the consensus Plk1 PBD-binding target that was previously reported (Elia et al. 2003) and binds to Plk1 PBD with high affinity and specificity (K_d = 450 nM) (Yun et al. 2009). Over the years, substantial progress has been made in developing various PLHSpT derivatives with improved affinity and/or cellular activity (Liu et al. 2011, 2012a, b; Qian et al. 2012, 2014; Murugan et al. 2013a, b; Srinivasrao et al. 2014; Ahn et al. 2015). Among these derivatives, a $C_6H_5(CH_2)_8$ group-conjugated *4j* (Fig. 4a) is considered a prototype, and it exhibits dramatically increased (~500-fold) affinity (K_d = 1–2 nM) with an undiminished level of Plk1-binding specificity (Liu et al. 2011). The unexpected binding mode between the alkylphenyl moiety of *4j* and neighboring hydrophobic residues of Plk1 PBD may provide a new paradigm in the development of Plk1 PBD-binding inhibitors. In addition, the N-terminal Pro motif has been shown to confer Plk1 specificity and ~20-fold affinity to Plk1 PBD, while the C-terminal SpT dipeptide functions as a high-affinity anchor that is crucially required for

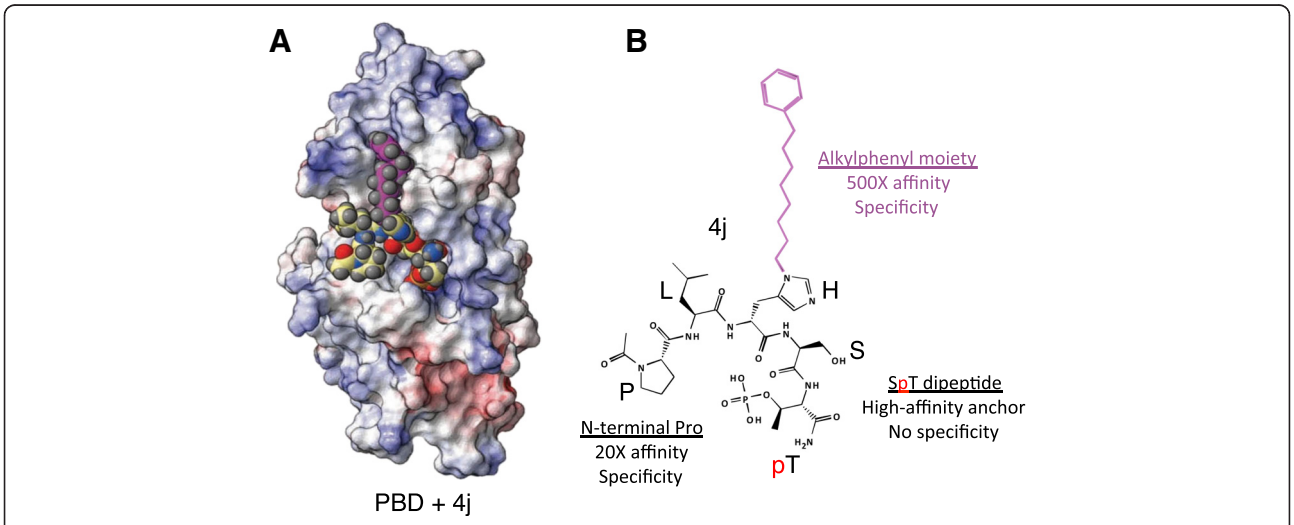

Fig. 4 The mode of binding between Plk1 PBD and its high-affinity ligand, 4j. **a** Electrostatic surface representation of the Plk1 PBD in complex with 4j. Surface colors represent electrostatic potentials with *red* (*negative*), *blue* (*positive*), and *white* (*neutral*). For 4j, oxygen and nitrogen atoms are colored in *red* and *blue*, respectively. The alkylphenyl moiety is indicated in *violet*. The phosphorylated (*red*)-T residue is shown. **b** A schematic diagram showing 4j and its three elements, which are critical for Plk1 PBD binding. PLHSpT is shown in *single letters* except for phosphorylated (*red*)-T. The alkylphenyl moiety of 4j is indicated in *violet*

establishing a stable interaction with the H538 and K540 residues of Plk1 PBD (Yun et al. 2009) (Fig. 4b). Although they exhibit superb binding affinity and specificity, *4j* and its derived inhibitors (Liu et al. 2011, 2012a, b; Qian et al. 2012, 2014) still require high extracellular concentrations (IC_{50} = 80–320 μM) to delocalize Plk1 and induce mitotic block in cultured HeLa cells. This requirement appears to be largely due to these inhibitors' less than acceptable level of membrane permeability and intracellular stability (Liu et al. 2011). Further derivatization and optimization of these inhibitors would be necessary to improve the bioavailability and efficacy of the compounds.

Whether improving peptide-based inhibitors can lead to the development of more clinically applicable PBD antagonists remain to be seen. Nevertheless, the novel binding interactions within the $C_6H_5(CH_2)_8$ group may likely be critical for the future design of PBD-binding antagonists. Based on the earlier success in generating a PLHSpT-based cyclic peptomer (Murugan et al. 2013b), developing *4j*-based cyclic peptides and their derivatives could be important not only to their direct use for in vitro and/or in vivo applications, but also to the design and development of more druggable small-molecule-based inhibitors.

Conclusions

It is now well appreciated that targeting PBD constitutes a fascinating strategy that may lead to the development of a new class of Plk1 inhibitors. Although substantial progress has been made in developing Plk1 PBD inhibitors during the last several years, these inhibitors suffer greatly from low binding affinity, poor specificity, or low membrane permeability. Nevertheless, studies with peptide-based inhibitors revealed that high-affinity and high-specificity binding to Plk1 PBD can be achieved by several structural motifs, such as the SpT high-affinity anchor, the N-terminal Pro moiety, and the recently discovered hydrophobic channel-based interactions. At present, one of the biggest challenges in developing *4j*-based inhibitors is improving membrane permeability by minimizing the anionic change of the phosphorylated Thr residue that is critical for PBD binding. Although developing *4j*-based inhibitors may have its own merits, it may be important to design and develop next-generation small-molecule PBD inhibitors that mimic the structure of *4j* or its derivatives.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JEP, TSK, LM, JKB, and BYK drafted the manuscript and KSL finalized it. All authors read and approved the final manuscript.

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