

Review Article

Targeting Polo-like Kinase 1 for the Treatment of Cancer

Jie Li and Xiaoqi Liu

Purdue University, West Lafayette, IN 47906, USA

*Corresponding author: Jie Li, Purdue University, West Lafayette, IN47906, USA Tel: (+1) 765-588-7903; Email: lijie@purdue.edu

Xiaoqi Liu, Purdue University, West Lafayette, IN47906, USA, Tel: (+1) 765-496-3764; Email: liu8@Purdue.edu

Citation: Li J and Liu X (2016) Targeting Polo-like kinase 1 for the treatment of cancer. Adv Biochem Biotechnol 1: 104. DOI: 10.29011/2574-7258.000004

Received Date: 20 September, 2016; **Accepted Date:** 5 October, 2016; **Published Date:** 13 October, 2016

Polo-Like Kinase Family

Polo-like kinases (Plks) are a family of serine-threonine kinases that regulate multiple intracellular processes, and consist of members Polo-like kinase 1 (Plk1), Plk2, Plk3, Plk4 and Plk5[1,2]

Plk1 levels start to rise significantly at the beginning of S phase, and peak after the G2/M transition[3]. Plk1 plays a critical role in centrosome maturation, kinetochore assembly, and bipolar spindle formation[4]. More importantly, Plk1 plays a key role in ensuring normal mitotic progression. It initiates mitotic entry and mitotic exit by phosphorylating Cyclin B, Cdc25c and by activating the anaphase-promoting complex/cyclosome (APC/C) complex respectively. Briefly, for mitotic entry Plk1 phosphorylates, and thereby regulates, both Cdc25 and Myt1, which are essential for activation of Cdk1/Cyclin B the master regulator of G2/M transition. During the mitotic phase, Plk1 contributes to activation of APC by directly phosphorylating APC and its inhibitor Emi1, which is critical for the onset of anaphase. The impairment of Plk1 function generally interferes with normal onset of anaphase and mitotic exit[5,4]. Inhibition of Plk1 activity by small-molecular inhibitors causes mitotic arrest and induces apoptosis in human cancer cell lines of diverse tissue origin and oncogenomic signature[6]. Plk2 is involved with proper centriole duplication and cell cycle regulation, and whose inhibition could impact chromosomal integrity during mitosis[7]. Plk2 is considered a putative therapeutic target for Parkinson's disease[7]. The expression level of Plk3 remains relatively constant throughout the cell cycle, and its kinase activity peaks in late S/G2. Overexpression of Plk3 in mammalian cells result in growth inhibition as well as apoptosis, and Plk3 deficient mice are known to develop tumors[8]. Furthermore, Plk3 phosphorylation has been observed to stabilize p53 and PTEN which, taken altogether, has resulted in Plk3 being considered a tumor suppressor. Plk4 is essential for centrosome duplication and also has roles in DNA damage response signaling. Plk4 inhibitors have been developed and have shown potent antitumor activity in breast cancer both in vitro and in vivo[9]. Plk5 is a newly identified family member and is expressed mainly in differentiated tissues[10]. Plk5 shows tumor suppressor behavior similar to Plk2 and Plk3.

Structure and Activation of Plk1

Plk1 is the most extensively characterized member of the Plk family[11]. Plk1 can be divided into two parts, the amino-terminal kinase domain and the carboxy-terminal polo-box domain (PBD) [4]. Joining the two domains is a linker region, and a small region known as the polo-box cap (Pc) that probably forms part of the PBD[4,12]. Three key residues are implicated in phosphor peptide binding, Trp414, His538 and Lys540 (numbering for Homo sapiens PLK1), are indicated (Figure 1). Also indicated in the figure are a conserved, activating phosphorylation site in the T-loop (Thr210) and the D-box motif[13] that is required for degradation by the anaphase-promoting complex/cyclosome (APC/C)–Cdh1.

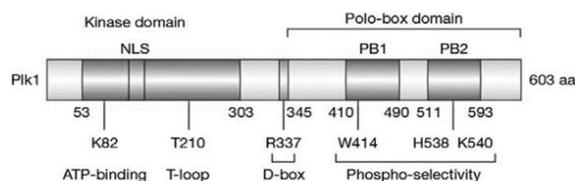


Figure 1: Domain structure of human Plk1[4].

During Plk1 activation, the PBD binds to target proteins following phosphorylation by a priming kinase (not Plk1), resulting in increased polo-like kinase activity. Furthermore, Plk1 activity is stimulated by T-loop phosphorylation (T210) carried out by an upstream activating kinase [4,14]. So far, there are two primary theories on PBD/Plk1 activity order of action[15,16]: 1) Distributive model: When PBD is bound in a phosphorylation dependent manner to ligands, the kinase region of Plk1 is localized to regionally located substrates; 2) Processive model: PBD, after binding to a substrate, allows the Plk1 kinase region to act on that same substrate.

Plk1 and Cancer Treatment

Protein kinases have now become one of the largest classes of anticancer drug targets, and among them Plk1 is one of the most attractive anticancer drug targets because of its strong expression

in cancer tissues, but only weak expression in normal tissue[17]. The specificity of Plk1-dependent biochemical reactions is regulated at two consecutive steps:

1) Polo-Box Domain (PBD)-dependent substrate binding and 2) Kinase Domain (KD)-dependent substrate phosphorylation. Inhibition of either one of these two domains is sufficient for abrogating Plk1 function *in vivo*[18]. The functionally essential KD and PBD of Plk1 represent two distinct drug targets available within one molecule.

However, due to the large number (a total of 518) of protein kinases in mammalian cells, and the high degree of structural conservation among ATP-binding pockets, ATP-competitive Plk1 inhibitors exhibit a significant level of cross-reactivity with other functionally unrelated kinases[19]. For example, BI2536: Although it exhibited impressive anti-tumor efficacy in preclinical studies, BI2536 monotherapy induced only weak clinical efficacy. Coupled with the observed significant adverse effects, monotherapy with BI 2536 was terminated during the Phase II study[20]. To overcome this drug resistance, combinatorial therapy could potentially be a more effective strategy to treat cancers, as it can improve the drug efficacy even at lower doses of each agent, thus maintaining a low toxicity profile. In the following discussion, we will further elaborate on co-targeting Plk1 with other important molecular targets to treat castration-resistant prostate cancer (CRPC) and gemcitabine-resistant pancreatic cancer.

Combined Inhibition of Plk1 and Wnt/B-Catenin Signaling in CRPC

The Wnt/ β -catenin signaling pathway is instrumental in orchestrating proper tissue development in embryos and tissue maintenance in adults[21]. Increasing evidence has indicated that Wnt/ β -catenin signaling is a major pathway associated with developing CRPC[22]. Results from the next generation sequencing studies of CRPC specimens identified components of the Wnt/ β -catenin signaling pathway with significant genomic alterations in CRPC[23]. In low androgen environments, AR and wnt signaling may reinforce each other to elicit specific target genes that promote androgen-independent growth and progression. Given that β -catenin directly contributes to activation of AR signaling, it is essential to define how upstream signaling events regulate the β -catenin pathway so that new approaches to treat AR signaling inhibitor non-responding CRPC can be developed.

In a recent study, we identify Axin2, another member of Axin protein family, as a novel Plk1 substrate[24]. Significantly, our studies revealed that Plk1 phosphorylation of Axin2 is clearly involved in the regulation of β -catenin stability, and consequently PCa growth. Mechanistically, Plk1 phosphorylation of Axin2 facilitates the GSK3 β -dependent phosphorylation of β -catenin

by enhancing the binding between GSK3 β and β -catenin, an essential early step for the subsequent degradation of β -catenin. In cells expressing the Axin2-S311A mutant, the binding between GSK3 β and β -catenin is significantly reduced, leading to inhibited β -catenin phosphorylation and accumulation of β -catenin protein. This novel discovery provides an explanation for how inhibition of Plk1 leads to activation of the Wnt/ β -catenin pathway. It strongly suggests that activation of the Wnt/ β -catenin signaling pathway needs to be carefully considered when Plk1 inhibitors are used in various combination therapies and that targeting inhibition of Plk1 and the Wnt/ β -catenin pathway simultaneously would most likely be an improved and more effective approach for future CRPC therapy (Figure 2).

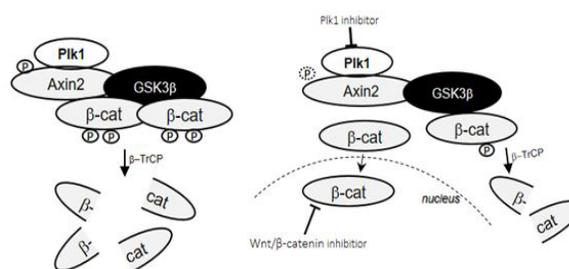


Figure 2: Inhibition of Wnt/ β -catenin signaling significantly potentiates the antineoplastic activity of the Plk1 inhibitor BI2536 in both cultured prostate cancer cells and CRPC xenograft tumors[24].

Inhibition of Plk1 Overcome the Gemcitabine-Resistance in Pancreatic Cancer Pancreatic Ductal AdenoCarcinoma (PDAC) has the highest mortality rate of all cancers, is the fourth leading cause of cancer deaths in the U.S., and is estimated to cost the health care system \$2.4 billion each year[25]. Despite significant research efforts in treating primary and metastatic tumors, only 7% of patients survive 5 years. Gemcitabine is the standard-of-care for chemotherapy in patients with pancreatic adenocarcinoma and it can directly incorporate into DNA or inhibit ribonucleotide reductase to prevent DNA replication and, thus, tumor cell growth[26]. Most pancreatic tumors, however, develop resistance to gemcitabine. Despite its unique molecular properties and its prevalent use in the clinic, a major contributing factor for the dismal prognosis of patients is that they develop gemcitabine resistance where DNA replication and cell survival continue, even in the presence of the drug. Clearly, we need to identify novel targets whose inhibition can enhance therapeutic efficacy in PDAC cases.

Polo-Like Kinase 1 (Plk1), a critical regulator in many cell cycle events, is significantly elevated in human pancreatic cancer[27]. In a recent pancreatic cancer study, we observed that Plk1 is required for the G1/S transition and that inhibition of Plk1 significantly reduces the DNA synthesis rate in human pancreatic cancer cells: an early delay in cell cycle in pancreatic cancer

cells using lower (25 nm) or higher (100 nm) Plk1 inhibitor GSK461364A and an arrest at G2/M phase with higher concentration and at the later time point [28]. Considering that the Plk1 level starts to rise significantly in the G1/S boundary and peaks in mitosis, we would predict that a much higher concentration of Plk1 inhibitor is needed to inhibit its multiple mitotic functions but a relatively low concentration of Plk1 inhibitor is enough to observe the defect during G1/S transition. Furthermore, the combined effect of a specific Plk1 inhibitor GSK461364A with gemcitabine was examined in pancreatic cancer cells. We show that inhibition of Plk1 significantly potentiates the anti-neoplastic activity of gemcitabine in both cultured pancreatic cancer cells and Panc1-derived orthotopic pancreatic cancer xenograft tumors. Overall, this study demonstrates that co-targeting Plk1 can significantly enhance the efficacy of gemcitabine, offering a promising new therapeutic option for the treatment of gemcitabine-resistant human pancreatic cancer.

In summary, inhibition of Plk1 is a very promising strategy for the treatment of various difficult human cancers, and combination of Plk1 inhibitor and other anti-cancer drug (s) represent a novel and more effective approach to manage difficult human cancers.

References

1. Yao LJ, Fan HY, Tong C, Chen DY, Schatten H et al. (2003) Polo-like kinase-1 in porcine oocyte meiotic maturation, fertilization and early embryonic mitosis. *Cell Mol Biol (Noisy-le-grand)* 49: 399-405.
2. Craig SN, Wyatt MD, McInnes C (2014) Current assessment of polo-like kinases as anti-tumor drug targets. *Expert Opin Drug Discov* 9: 773-789.
3. Anger M, Kues WA, Klima J, Mielenz M, Kubelka M et al. (2003) Cell cycle dependent expression of Plk1 in synchronized porcine fetal fibroblasts. *Mol Reprod Dev* 65: 245-253.
4. Barr FA, Sillje HH, Nigg EA (2004) Polo-like kinases and the orchestration of cell division. *Nat Rev Mol Cell Biol* 5: 429-440.
5. Brassac T, Castro A, Lorca T, Le Peuch C, Dorée M et al. (2000) The polo-like kinase Plx1 prevents premature inactivation of the APC(Fizzy)-dependent pathway in the early *Xenopus* cell cycle. *Oncogene* 19: 3782-3790.
6. Steegmaier M, Hoffmann M, Baum A, Lenart P, Petronczki M et al. (2007) BI 2536, a potent and selective inhibitor of polo-like kinase 1, inhibits tumor growth in vivo. *Curr Biol* 17: 316-322.
7. Fitzgerald K, Bergeron M, Willits C, Bowers S, Aubele DL et al. (2013) Pharmacological inhibition of Polo Like Kinase 2 (PLK2) does not cause chromosomal damage or result in the formation of micronuclei. *Toxicol Appl Pharmacol* 269: 1-7.
8. Yang Y, Bai J, Shen R, Brown SAN, Komissarova E et al. (2008) Polo-like kinase 3 functions as a tumor suppressor and is a negative regulator of hypoxia-inducible factor-1 alpha under hypoxic conditions. *Cancer Res* 68: 4077-4085.
9. Sampson PB, Liu Y, Patel NK, Feher M, Forrest B et al. (2015) The discovery of Polo-like kinase 4 inhibitors: design and optimization of spiro[cyclopropane-1,3-[3H]indol]-2-(1'H).ones as orally bioavailable antitumor agents. *J Med Chem* 58: 130-146.
10. De Cárcer G, Escobar B, Higuero AM, García L, Ansón A et al. (2011) Plk5, a polo box domain-only protein with specific roles in neuron differentiation and glioblastoma suppression. *Mol Cell Biol* 31: 1225-1239.
11. Brandwein JM (2015) Targeting polo-like kinase 1 in acute myeloid leukemia. *Ther Adv Hematol* 6: 80-87.
12. Elia AEH, Rellos P, Haire LF, Chao JW, Ivins FJ et al. (2003) The molecular basis for phosphor dependent substrate targeting and regulation of Plks by the Polo-box domain. *Cell* 115: 83-95.
13. Lindon C, Pines J (2004) Ordered proteolysis in anaphase inactivates Plk1 to contribute to proper mitotic exit in human cells. *J Cell Biol* 164: 233-41.
14. Lee KS, Erikson RL (1997) Plk is a functional homolog of *Saccharomyces cerevisiae* Cdc5, and elevated Plk activity induces multiple septation structures. *Mol Cell Biol* 17: 3408-3417.
15. Lowery DM, Lim D, Yaffe MB (2005) Structure and function of Polo-like kinases. *Oncogene* 24: 248-59.
16. van de Weerd BCM, Medema RH (2006) Polo-like kinases: a team in control of the division. *Cell Cycle* 5: 853-864.
17. Zhang J, Li Y, Guo L, Cao R, Zhao P et al. (2009) DH166, a beta-carboline derivative, inhibits the kinase activity of PLK1. *Cancer Biol Ther* 8: 2374-2383.
18. Lee KS, Burke TR, Park J-E, Bang JK, Lee E et al. (2015) Recent Advances and New Strategies in Targeting Plk1 for Anticancer Therapy. *Trends Pharmacol Sci* 36: 858-877.
19. Strebhardt K, Ullrich A (2006) Targeting polo-like kinase 1 for cancer therapy. *Nat Rev Cancer* 6: 321-330.
20. Mross K, Dittrich C, Aulitzky WE, Strumberg D, Schutte J et al. (2012) A randomised phase II trial of the Polo-like kinase inhibitor BI 2536 in chemo-naïve patients with unresectable exocrine adenocarcinoma of the pancreas - a study within the Central European Society Anticancer Drug Research (CESAR) collaborative network. *Br J Cancer* 107: 280-286.
21. Clevers H, Nusse R (2012) Wnt/beta-catenin signaling and disease. *Cell* 149: 1192-1205.
22. Yokoyama NN, Shao S, Hoang BH, Mercola D, Zi X (2014) Wnt signaling in castration-resistant prostate cancer: implications for therapy. *Am J Clin Exp Urol* 2: 27-44.
23. Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, et al. (2012) The mutational landscape of lethal castration-resistant prostate cancer. *Nature* 487: 239-243.
24. Li J, Karki A, Hodges KB, Ahmad N, Zoubeidi A, et al. (2015) Co-targeting Polo-like kinase 1 (Plk1) and the Wnt/beta-catenin signaling pathway in castration-resistant prostate cancer. *Mol Cell Biol* 35: 85-98.
25. Mariotto AB, Yabroff KR, Shao Y, Feuer EJ, Brown ML (2011) Projections of the cost of cancer care in the United States: 2010-2020. *J Natl Cancer Inst* 103: 117-128.

26. Schober M, Javed MA, Beyer G, Le N, Vinci A et al. (2015) New Advances in the Treatment of Metastatic Pancreatic Cancer. *Digestion* 92: 175-184.
27. Song B, Liu XS, Rice SJ, Kuang S, Elzey BD et al. (2013) Plk1 phosphorylation of orc2 and hbo1 contributes to gemcitabine resistance in pancreatic cancer. *Mol Cancer Ther* 12: 58-68.
28. Li J, Wang R, Schweickert PG, Karki A, Yang Y et al. (2016) Plk1 inhibition enhances the efficacy of gemcitabine in human pancreatic cancer. *Cell Cycle* 15: 711-719.