



Polo-like kinase 1 is involved in apoptosis, invasion, and metastasis of pancreatic ductal adenocarcinoma

Yonghuan Mao¹, Ling Xi², Quan Li³, Xinhua Zhang⁴, Chunzhao Yu³

¹Department of General Surgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, China;

²Department of Gerontology, Jiangsu Province Official Hospital, Nanjing, China; ³Department of General Surgery, the Second Affiliated Hospital of Nanjing Medical University, Nanjing, China; ⁴Department of Pediatric Medicine, Jiangsu Province Hospital, Nanjing, China

Contributions: (I) Conception and design: C Yu; (II) Administrative support: C Yu, X Zhang; (III) Provision of study materials or patients: C Yu, Y Mao; (IV) Collection and assembly of data: L Xi, Y Mao; (V) Data analysis and interpretation: Q Li, Y Mao; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Chunzhao Yu. Department of General Surgery, the Second Affiliated Hospital of Nanjing Medical University, 121 Jiangjiayuan, Nanjing 210029, China. Email: slec1989@163.com.

Background: Polo-like kinase 1 (Plk1) is overexpressed in different types of carcinomas, and it is widely pursued as a novel target for treatment of neoplasms. However, the role of Plk1 in invasion and metastasis of pancreatic cancer has not been reported.

Methods: In order to evaluate whether Plk1 can potentially serve as a therapeutic target in pancreatic cancer, we herein explore and discuss the relationship between the inhibition of Plk1 by RNA interference (RNAi) and invasion and metastasis of pancreatic cancer cells. For this, three pancreatic cancer cell lines (AsPC-1, BxPC-3, and PANC-1) were studied. Plk1-specific short hairpin RNAs (shPlk1) were introduced into these cell lines by viral transduction.

Results: We found that Plk1 mRNA and protein levels were significantly reduced in the shPlk1 group. Moreover, knockdown of Plk1 expression by shPlk1 promoted apoptosis, while cell invasion and metastasis potentials were significantly reduced.

Conclusions: Plk1 expression levels were closely correlated with proliferation, invasion, and metastasis of pancreatic cancer, and inhibition of Plk1 expression by RNAi could promote cell apoptosis and suppress metastasis and invasion of pancreatic cancer cells *in vitro*. Furthermore, Plk1 might constitute novel target for treatment of pancreatic cancer, with inhibition of Plk1 potentially providing a new strategy for the prevention of pancreatic cancer invasion and metastasis.

Keywords: Polo-like kinase 1 (Plk1); pancreatic ductal adenocarcinoma; apoptosis; invasion; migration

Submitted Feb 13, 2020. Accepted for publication Sep 30, 2020.

doi: 10.21037/tcr-20-1019

View this article at: <http://dx.doi.org/10.21037/tcr-20-1019>

Introduction

According to statistics from America and Europe, the prognosis of pancreatic cancer (PC) is very poor, ranking it the fourth leading cause of cancer deaths (1). Pancreatectomy is the only effective method to cure this disease, but only 20% of patients are provided with the opportunity to resect the cancer tissue (2). Despite all these efforts, most of the patients are known to relapse (3). Distant metastasis is a major cause of death in cancer

patients, especially with respect to PC. Only few cases do not show resistance to chemo- or radiation therapy (4). Patients with metastatic disease have a median survival of 6 months and a 5-year survival rate of 1%, whereas treatment with surgical resection and other therapeutic approaches is associated with 12–22 months of median survival and a 5-year survival rate of 20–25% (5). Hence, PC poses one of the greatest challenges in cancer research. Nevertheless, our increasing knowledge on the development

of PC will help us find a cure for this debilitating disease (6-12). Nowadays, several signaling pathways were found as targets in PC, for examples, NF- κ B is recognized as a key mediator of inflammation and has been frequently observed to play the crucial role in PC (13). Also, Hippo pathway, a key regulator of organ size, tissue hemostasis and regeneration, was reported to regulate the chemoresistance and prognosis in PC (14).

Polo-like kinase 1 (Plk1) is an important serine/threonine kinase involved in the process of mitosis. Plk1 is highly expressed in most neoplastic tissues compared to healthy tissues (15-18), and its deregulation affects the occurrence and development of tumors (19,20). It is reported that Plk1 could enhance efficacy of Olaparib in castration-resistant prostate cancer (21). Cervical cancer growth was also regulated via c-ABL-Plk1 axis (22). Our previous reports showed that down-regulating Plk1 can activate apoptosis-related pathways, such as caspase-related and Bcl-2 family-mediated pathways, leading to cell death. Furthermore, our findings confirm that suppression of PI3K/Akt and Plk1 combined with gemcitabine could be a potential therapeutic regimen for PC patients, but further efforts are needed (23).

A limited number of studies have demonstrated the possible relationship between the negative regulation of Plk1 and the development of tumors (24-28). Some cancers, like colorectal carcinoma and lung cancer, are caused by K-RAS mutations. Cells with high expression of K-RAS can be selectively killed without hurting normal cells by silencing Plk1 expression. *In vitro* and *in vivo* experiments have highlighted different ways to influence the activity and/or function of Plk1 for induction of tumor cell apoptosis (29,30). A lot of research is committed to using Plk1 as a means for targeted cancer treatment, especially through the development of small molecule inhibitors, which could be used as drugs for the treatment of various cancers (31-35). Knockdown of Plk1 using RNA interference technologies, like small interfering RNAs (siRNAs), led to cell cycle arrest and apoptosis of tumor cells, but had no effect on normal cells (36-38). Of note, 47.7% of invasive PCs are Plk1 positive, highlighting that Plk1 expression is closely related to malignancy of PC in some cases (39). In this study, we report that suppression of Plk1 expression in PC results in inhibition of cell invasion and migration, and induces apoptosis. We present the following article in accordance with the MDAR checklist (available at <http://dx.doi.org/10.21037/tcr-20-1019>).

Methods

Construction of recombinant adenovirus (rAd)-Plk1-shRNAs (rAd-shPlk1)

We selected different regions of the Plk1 transcript according to its gene sequence, and synthesized four shRNAs using the pYr-1.1 vector (hU6/EGFP/Neo) (Changsha Yingrun Biotechnology Co. Ltd, China) (Tables 1,2). After gene sequence analysis, pYr-1.1-Plk1-shRNA2 and pYr-1.1-Plk1-shRNA4 (Figure 1A) were introduced into the adenoviral vector pAd/PL-DEST (Changsha Yingrun Biotechnology Co. Ltd, China) through homologous recombination *in vitro*. HEK 293 cells were used for packing in to rAd (Figure 1B). By restructuring, both Plk1-sh2 and -sh4 could work effectively, and Plk1-sh4 proved superior to Plk1-sh2 (Figure 1C). Plk1-sh4 was finally selected for the following study, and was set as rAd-shPlk1, while rAd-EGFP (empty vector) served as experimental control.

Cell culture, adenovirus transduction, and RNAi

We selected the PC cell lines AsPC-1, BxPC-3, and PANC-1as research objects. The cells were cultured at 37 °C and 5% CO₂ in RPMI 1640 supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 U/mL). They were divided into three groups: control group (untransduced cells), rAd-EGFP group (transduced with the empty vector control), and rAd-shPlk1 group (transduced with rAd-shPlk1). The cells were seeded on 6-well plates and cultured to 60% confluence prior to adenoviral transduction. For this, they were incubated with adenoviruses carrying vectors for expression of either EGFP or shPlk1 at a multiplicity of infection of approximately 10⁶. Cells were collected for quantitative real-time PCR (qRT-PCR) and western blotting at 12 and 24 hours after transduction.

Detection of changes in Plk1 mRNA levels by qRT-PCR

TRIzol reagent was used for isolation of total RNA, and cDNA was synthesized according to the manufacturer's instructions (Promega, Madison, WI, US). Afterwards, we employed the same amount of cDNA for each of the samples for analysis by qRT-PCR. Specific forward (5'-GCTGGGCAACCTTTTCCTG-3') and reverse (5'-GCAGTGGGATCTGTCTGAAGC-3')

Table 1 Information on the shRNA4 of the Plk1 transcript

Number	shRNA sequences (5'-3')
PLK1-shRNA1	CGAGCTGCTTAATGACGAGTTCTCGAGAACTCGTCATTAAGCAGCTCGTTTTT
PLK1-shRNA2	CCCGAGGTGCTGAGCAAGAACTCGAGTTTCTTCTCAGCACCTCGGGTTTTT
PLK1-shRNA3	GCTCATCTTGTGCCCACTGATCTCGAGATCAGTGGGCACAAGATGAGCTTTTTT
PLK1-shRNA4	GCAGCGTGCAGATCAACTTCTCTCGAGAGAAGTTGATCTGCACGCTGCTTTTTT

Table 2 Information on the targeting different regions of the Plk1 transcript

Number	Target sequences (5'-3')	Target region
PLK1-shRNA1	CGAGCTGCTTAATGACGAGTT	CDS
PLK1-shRNA2	CCCGAGGTGCTGAGCAAGAAA	CDS
PLK1-shRNA3	GCTCATCTTGTGCCCACTGAT	CDS
PLK1-shRNA4	GCAGCGTGCAGATCAACTTCT	CDS

primers were used to detect Plk1 gene expression in cells of the different groups. GAPDH served as internal standard, using the following primers: 5'-ATCCCATCACCATCTTCCAGG-3' (forward) and 5'-CCATCACGCCACAGTTTCC-3' (reverse). Plk1 and GAPDH gene amplifications were carried out for 30 and 25 cycles, respectively.

Analysis of changes in Plk1 protein levels by western blotting

Cells were washed three times with phosphate buffered saline (PBS, 4 °C), and cellular protein was subsequently collected by lysis in 80 µL of RIPA buffer at 4 °C for 20 minutes. Removal of cell debris was performed by centrifugation at 12,000×g and 4 °C for 15 minutes, and the supernatant was collected for subsequent analyses. Afterwards, the Bio-Rad protein assay was used to determine the protein concentrations, and the same amount of protein (40 µg) per group was separated by electrophoresis, and subsequently transferred to polyvinylidene fluoride membranes (Amersham, USA). The membranes were blocked by incubation in 5% non-fat milk in Trisbuffered saline for 1 hour, followed by incubation with primary antibodies overnight at 4 °C. Anti-Plk1 monoclonal antibody (Cell Signaling Technology, Inc., USA) was used at 1 µg/mL. The membranes were then incubated for 1 h with horseradish peroxidase-conjugated goat anti-rabbit antibody (Santa Cruz Biotechnology, Inc.,

USA) at a 1:30,000 dilution. Electrochemiluminescence was detected on a ChemiDoc XRS+ Gel Imaging System. Next, in order to ensure that the same amount of protein had been loaded, the membranes were washed three times with Trisbuffered saline and re-incubated with an antibody directed against GAPDH.

Apoptosis analysis by flow cytometry

We collected the cells transduced with adenovirus at different time points, after which they were washed twice with PBS (4 °C), and re-dissolved in 1× binding buffer (BD Pharmingen, USA) to obtain a concentration of 1×10^6 cells/mL. Afterwards, 100 µL of the suspension (1×10^5 cells) were transferred to a 5 mL tube. After addition of 5 µL of APC AnnexinV (BD Pharmingen, USA) and 7-AAD (BD Pharmingen, USA) each, 400 µL of 1× binding buffer were added to each tube, after which the cells were incubated at 25 °C in the dark for 15 minutes. Cells were subsequently analyzed by flow cytometry (BD FACSCalibur equipped with CellQuest Pro) within 1 hour.

Transwell methods to detect cell migration and invasion

Sub-culturing of the cells in the upper chamber was performed until reaching 90% confluence. Next, 5×10^6 cells were suspended in 1 mL of serum-free culture medium. Half of the cell suspension (500 µL) was cultured in the upper chamber of the 24-well transwell chamber, and 800 µL

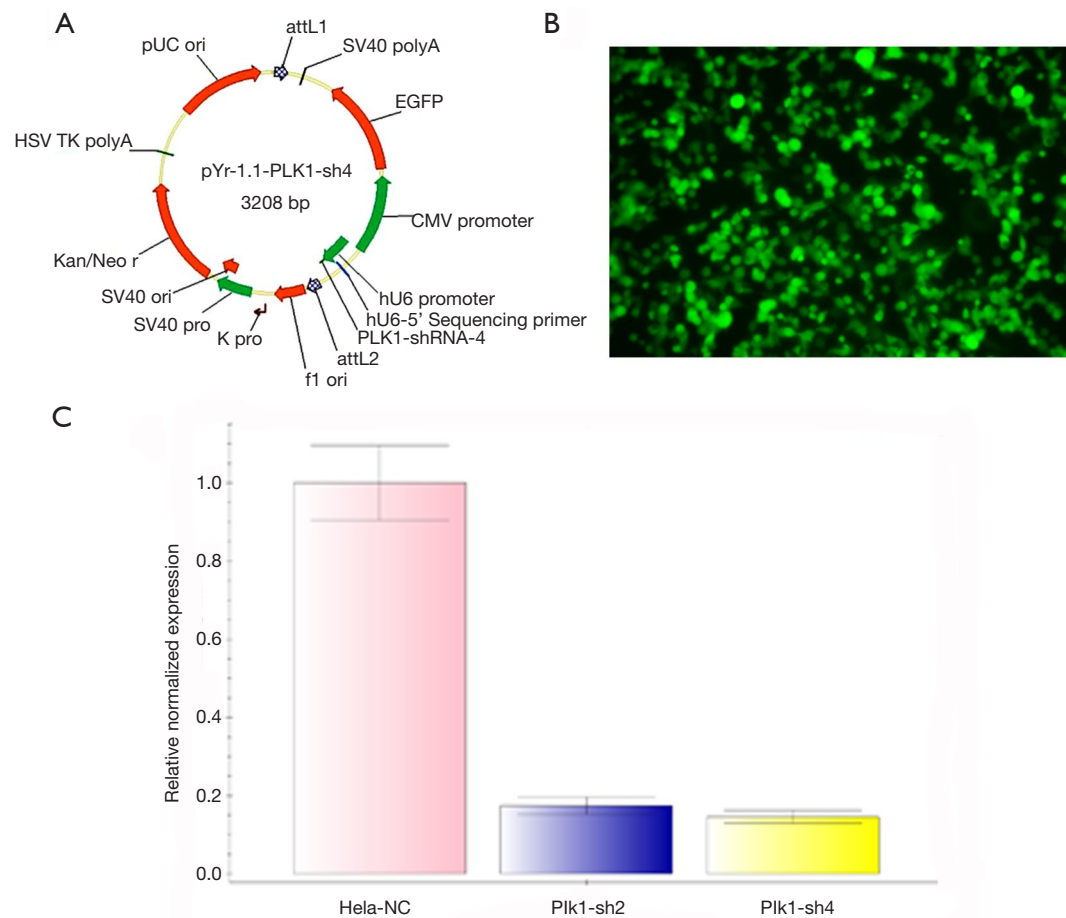


Figure 1 Construction of rAd-shPlk1. (A) Structure of pYr-1.1-Plk1-shRNA4. (B) Accumulation of rAd in HEK 293 cells. (C) Verification and validation of rAd-shPlk1 using HeLa cells through qRT-PCR.

of culture medium mixed with 2% fetal bovine serum were added to the lower chamber. The Transwell cultures were cultured at 37 °C and 5% CO₂ in an incubator for 12 hours to detect cell migration. Different from migration assays, matrix (Corning Matrigel) mixed with serum-free RPMI 1640 medium at a 1:5 ratio was added to the upper chamber, and the cells were then added and incubated at 37 °C and 5% CO₂ for 24 hours before being used to detect cell invasion. The remaining liquid was removed before use. The cells were sub-cultured in the invasion chamber until reaching 80% confluence. As for the cell migration assay, 2.5×10⁵ cells in 500 μL were cultured within one invasion chamber on a 24-well plate, and 800 μL culture medium mixed with 5% fetal bovine serum were added to the lower chamber. Subsequently, the cells were cultured under the aforementioned conditions for 24 hours to detect cell invasion. Following this, the cells on the upper

surface of the membrane were cleared, and the cells on the underside of the membrane were stained with 0.1% crystal violet for 15 minutes. The samples were observed and imaged on a microscope at 10× to 20× total magnification, depending on the cell density. Crystal violet was rinsed off using 33% acetic acid, and the plate readout was performed on an ultraviolet spectrophotometer. Cell numbers were converted to OD values (except for AsPC-1 cells, which were counted at 100× magnification).

Statistical analysis

All of the experiments were repeated three to four times, and the data are presented as mean ± SD. Statistical analysis was performed using SPSS 21.0 software. Comparisons between two groups were conducted using Welch's *t*-test. ANOVA was used for more than two groups, followed

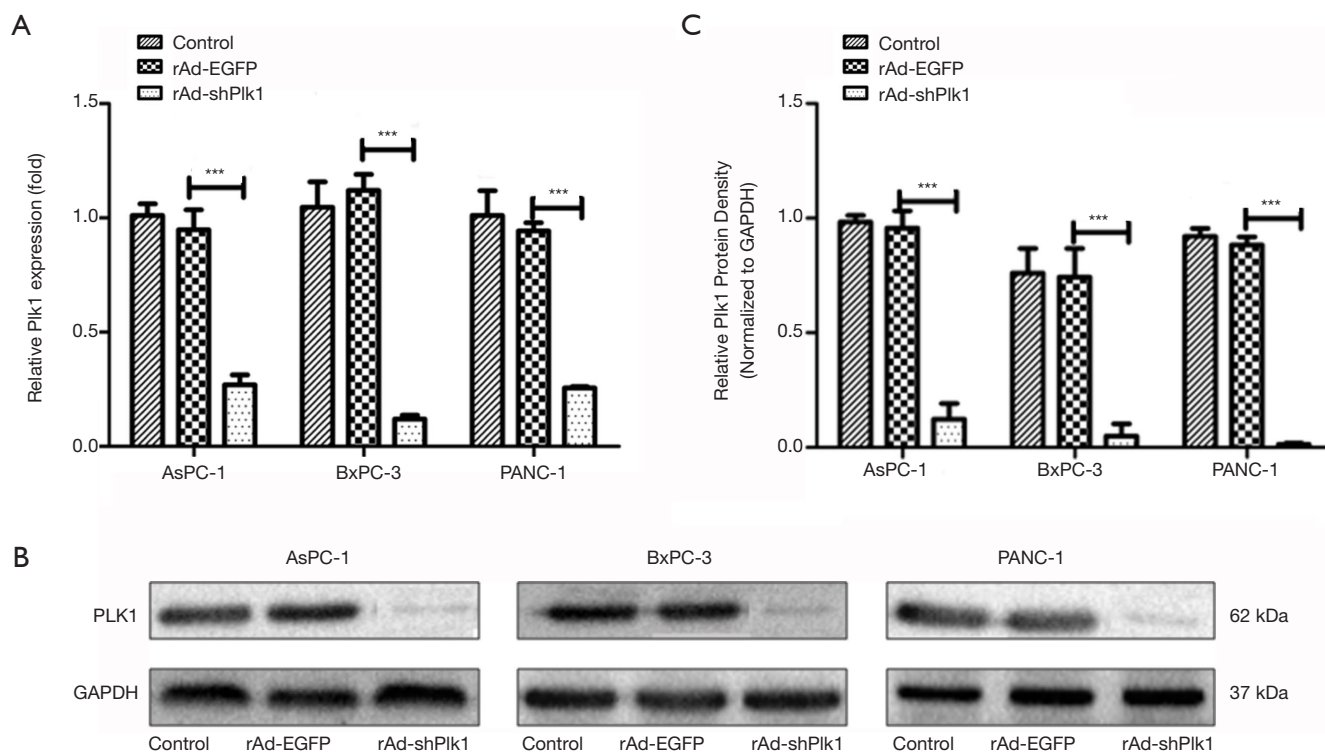


Figure 2 Plk1 expression was inhibited in the rAd-shPlk1 group in pancreatic cancer cells. (A) Quantified relative expression levels of Plk1 in AsPC-1, BxPC-3, and PANC-1 cells, with GAPDH serving as reference. (B) Western blotting (WB) assays for detection of Plk1 in AsPC-1, BxPC-3, and PANC-1 cells. (C) Relative Plk1 protein density normalized to GAPDH according to the WB results. rAd-EGFP vs. rAd-shPlk1 groups, *** $P < 0.001$.

by Bonferroni test among different groups. Differences were considered statistically significant at $P < 0.05$. P values indicating statistically significant differences were set as * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Results

Transduction with shPlk1 down-regulates Plk1 mRNA and protein levels in human PC cell lines

To detect the effect of shPlk1 on mRNA and protein levels of Plk1 in PC cell lines, we used qRT-PCR and western blotting, respectively. Results obtained by qRT-PCR indicated that the Plk1 mRNA levels were significantly different in the three groups, while total RNA levels were equal. More specifically, expression in the rAd-shPlk1 group was markedly lower than in the rAd-EGFP group ($P = 0.0006$) (Figure 2A). Western blotting results indicated that the total amount of Plk1 protein in the rAd-shPlk1 group was lower than in the rAd-EGFP group ($P = 0.0009$)

(Figure 2B,C). According to the above results, we found that Plk1 was down-regulated by shPlk1 with regard to both mRNA and protein levels in all PC cell lines tested.

Knockdown of Plk1 by shPlk1 induces apoptosis in PC cells

To test the effect of shPlk1-mediated down-regulation of Plk1 in pancreatic tumor cell lines, we used flow cytometry for analyzing apoptosis in the three groups. The results of this experiment are shown in Figure 3A,B,C. Cytograms of APC Annexin V binding (abscissa) versus 7-AAD uptake (ordinate) show three distinct populations: (I) viable cells (low APC and low 7-AAD signals) in gate LL; (II) early apoptotic cells (high APC and low 7-AAD signals) in gate LR, and (III) cells that have lost membrane integrity as a result of very late apoptosis (high APC and high 7-AAD signals) in gate UR. According to these data, we can conclude that within 24 hours, there is no significant difference in the three groups with regard to their apoptosis rates ($P = 0.058$); however, after 48 hours, the apoptosis rate

in the rAd-shPlk1 group was higher than in the other two groups ($P=0.007$). In line with this, the viable cell number in the rAd-shPlk1 group was significantly lower than in the rAd-EGFP group at all time points analyzed ($P=0.003$) (Figure 3D,E,F). Therefore, we inferred that shPlk1 could induce apoptosis of PC cells.

Down-regulation of Plk1 can inhibit PC cell invasion and migration

In order to detect whether down-regulation of Plk1 could affect PC cell invasion and migration, we transduced PC cells with rAd-shPlk1, and then evaluated cell migration and invasion capacities through transwell assays. Thereby, we showed that the number of cells that had migrated through the artificial basement membrane or/and Matrigel matrix from the rAd-shPlk1 group was significantly reduced compared the other two groups ($P=0.004$) (Figure 4).

Discussion

As mentioned above, the high mortality rate of PC patient urgently requires us to find the factors associated with cell proliferation, invasion, metastasis, and apoptosis, as well as the molecular mechanisms underlying these processes. Plk1, an indispensable factor in the process of cell proliferation, is highly expressed in a great proportion of PC cases, and in many other tumors (40). Lots of researches showed that metastasis in cancer patients and overall survival rates are associated with high Plk1 expression (41-43). Our previous study indicated that forced Plk1 overexpression promoted cell proliferation and an increase in the percentage of cells in the G1/S phase, but a reduction in the G2/M phase cell population. We also found that siRNA-mediated knockdown of Plk1 caused cell cycle arrest in G2/M and proliferation inhibition (42). This research was devised to investigate the effect of Plk1 in apoptosis, invasion, and migration of human PC cell lines.

There are several strategies available, like anti-sense oligonucleotides (ASO), siRNAs, and small molecules, which can be employed to deplete or inhibit expression of Plk1 (43-46). However, in our present study, an shRNA against Plk1 was our method of choice on account of the same interference efficiency, but with a longer interference time compared to the siRNA-based strategy.

This was our first time to construct an adenoviral vector for introduction of shPlk1. In order to exclude that apoptosis induced by shPlk1 had an impact on invasion

and migration, we examined PC cell apoptosis at different time points, and found that there was no significant difference in apoptosis induction among the three different conditions tested at 24 hours. Together, our data suggest that suppression of Plk1 expression caused an inhibition of invasion and migration capacities of PC cells. This finding powerfully supports the idea that Plk1 is a significant, decisive factor for invasion and metastasis of PC cells. Although our study showed that down-regulation of Plk1 exerts this effect on invasion and migration in PC cells, lower levels of Plk1 are not necessarily beneficial. The treatment effect of Plk1 in pancreatic cancer was still unknown. We need further study *in vivo* and *in vitro* to verify the effect of Plk1 on pancreatic cancer. It was reported that down-regulating the expression of Plk1 could still lead to aneuploidy and tumorigenesis (47). Therefore, it is important to maintain endogenous levels of Plk1 for mitosis to proceed normally, and attention should be paid to side effects, such as tumorigenesis, should Plk1 inhibitors be used in clinical trials.

At present, it is believed that Plk1 leads to epithelial-mesenchymal transition and affects the invasion and metastasis of tumor tissues (48). Cai *et al.* found that Plk1 regulates both metastasis and epithelial-mesenchymal transition of gastric cancer cells through regulation of the AKT pathway, and Wu *et al.* showed Plk1 to be involved directly in the phosphorylation of CRAF, with subsequent stimulation of the MEK1/2-ERK1/2-Fra1-ZEB1/2 signaling pathway, thus leading to epithelial-mesenchymal transition, which in turn regulates invasion and metastasis of metastatic prostate cancer (49,50). During this study, the exact molecular mechanisms underlying the effects of Plk1 on invasion and metastasis of PC were not investigated, and this will be addressed in future studies.

Conclusions

In conclusion, our study shows that the expression of Plk1 is closely related to invasion and metastasis of PC, and that shRNA-dependent down-regulation of Plk1 can lead to inhibition of PC cell migration and invasion *in vitro*. Further studies using animal models will pave the way for future clinical trials for the development and implementation of Plk1 inhibitors or RNAi-mediated Plk1 gene silencing strategies to complement current therapeutic approaches for PC. Certainly, further efforts to investigate the mechanisms behind PC development and progression induced by Plk1 are warranted.

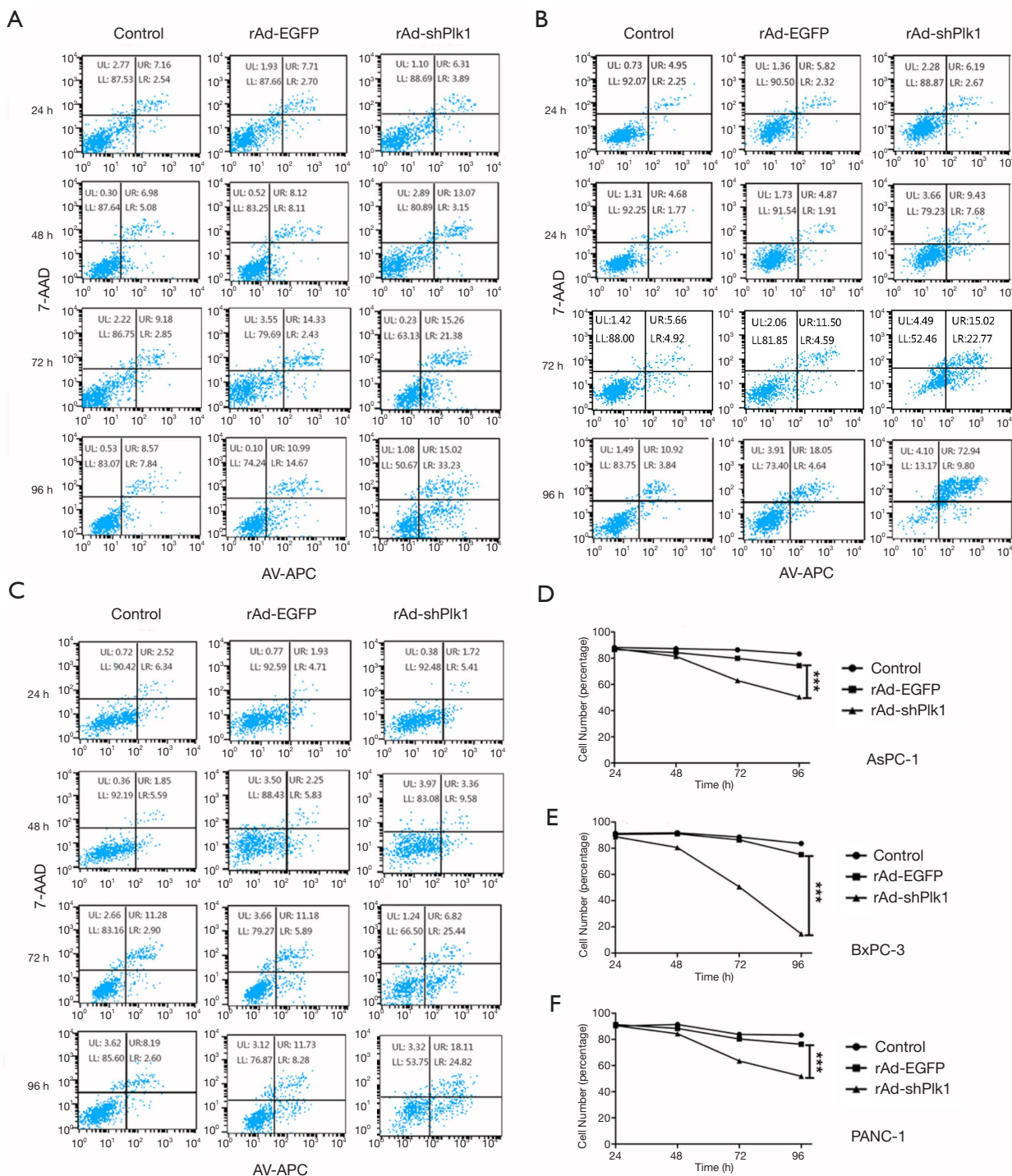


Figure 3 Analysis of apoptosis in control, rAd-EGFP, and rAd-shPlk1 groups at 24, 48, 72, and 96 hours through flow cytometry. AsPC-1 (A), BxPC-3 (B), and PANC-1 (C) were transduced with vectors carrying rAd-shPlk1, rAd-EGFP (vector control), and control constructs. Apoptosis induction in the three cell lines was determined at 24, 48, 72, and 96 hours after the treatment, respectively. (D,E,F) There was no significant difference among the three separate treatments after 24 hours. Compared to control and rAd-EGFP groups, the number of viable cells of the rAd-shPlk1 group was significantly decreased after 48 hours (***, $P < 0.001$).

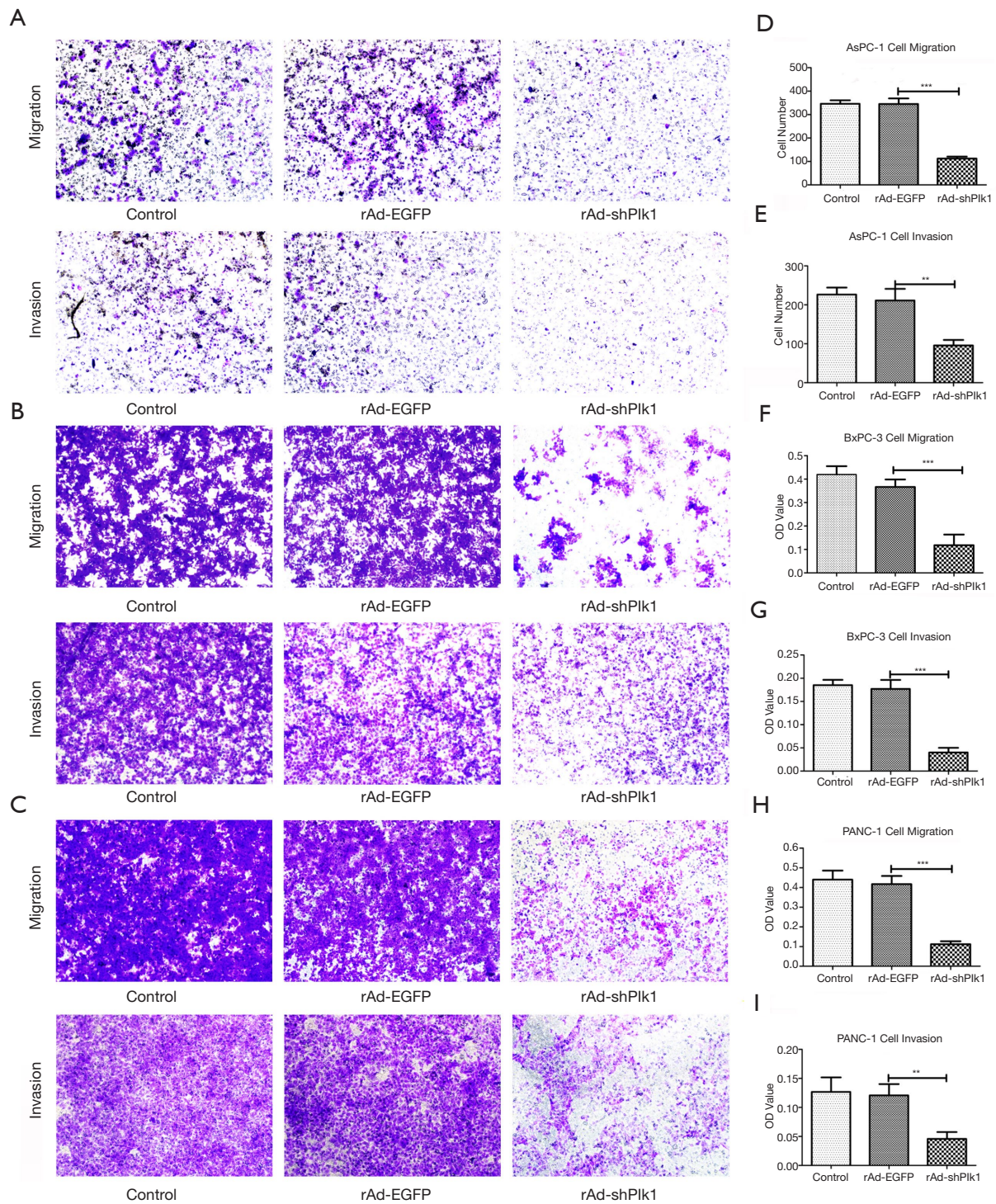


Figure 4 Effect of rAd-shPlk1 on pancreatic cancer cell migration and invasion. Images of migrating and invading AsPC-1 (A), BxPC-3 (B), and PANC-1 (C) cell lines taken on a microscope at a magnification of 100× (crystal violet staining). (D,E,H) Compared to the rAd-EGFP group, the migration ability of the rAd-shPlk1 group was markedly decreased (**, $P < 0.01$; ***, $P < 0.001$). (E,G,I) Comparison of the invasion capacities of the three groups, highlighting a significant reduction in the invasion capacity of the rAd-shPlk1 group (**, $P < 0.01$; ***, $P < 0.001$).

Acknowledgments

Funding: This work was supported in part by grants from the National Natural Science Foundation of China (No. 30972910, No. 81172269) and Natural Science Foundation of Jiangsu Province, China (No. 050104313).

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at <http://dx.doi.org/10.21037/tcr-20-1019>

Data Sharing Statement: Available at <http://dx.doi.org/10.21037/tcr-20-1019>

Peer Review File: Available at <http://dx.doi.org/10.21037/tcr-20-1019>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr-20-1019>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: <https://creativecommons.org/licenses/by-nc-nd/4.0/>.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin* 2019;69:7-34.
2. Butturini G, Stocken DD, Wentz MN, et al. Influence of resection margins and treatment on survival in patients with pancreatic cancer: meta-analysis of randomized controlled trials. *Arch Surg* 2008;143:75-83; discussion 83.
3. Long J, Zhang Y, Yu X, et al. Overcoming drug resistance in pancreatic cancer. *Expert Opin Ther Targets* 2011;15:817-28.
4. Stathis A, Moore MJ. Advanced pancreatic carcinoma: current treatment and future challenges. *Nat Rev Clin Oncol* 2010;7:163-72.
5. Neoptolemos JP, Stocken DD, Bassi C, et al. Adjuvant chemotherapy with fluorouracil plus folinic acid vs gemcitabine following pancreatic cancer resection: a randomized controlled trial. *JAMA* 2010;304:1073-81.
6. Van Cutsem E, Vervenne WL, Bennouna J, et al. Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. *J Clin Oncol* 2009;27:2231-7.
7. Van Laethem JL, Verslype C, Iovanna JL, et al. New strategies and designs in pancreatic cancer research: consensus guidelines report from a European expert panel. *Ann Oncol* 2012;23:570-6.
8. Matthaei H, Schulick RD, Hruban RH, et al. Cystic precursors to invasive pancreatic cancer. *Nat Rev Gastroenterol Hepatol* 2011;8:141-50.
9. Yachida S, Jones S, Bozic I, et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature* 2010;467:1114-7.
10. Campbell PJ, Yachida S, Mudie LJ, et al. The patterns and dynamics of genomic instability in metastatic pancreatic cancer. *Nature* 2010;467:1109-13.
11. Garrido-Laguna I, Tan AC, Uson M, et al. Integrated preclinical and clinical development of mTOR inhibitors in pancreatic cancer. *Br J Cancer* 2010;103:649-55.
12. Iacobuzio-Donahue CA. Genetic evolution of pancreatic cancer: lessons learnt from the pancreatic cancer genome sequencing project. *Gut* 2012;61:1085-94.
13. Pramanik KC, Makena MR, Bhowmick K, et al. Advancement of NF-kappaB Signaling Pathway: A Novel Target in Pancreatic Cancer. *Int J Mol Sci* 2018;19:3890.
14. Ansari D, Ohlsson H, Althini C, et al. The Hippo Signaling Pathway in Pancreatic Cancer. *Anticancer Res* 2019;39:3317-21.
15. Weichert W, Denkert C, Schmidt M, et al. Polo-like kinase isoform expression is a prognostic factor in ovarian carcinoma. *Br J Cancer* 2004;90:815-21.
16. Weichert W, Schmidt M, Gekeler V, et al. Polo-like kinase 1 is overexpressed in prostate cancer and linked to higher tumor grades. *Prostate* 2004;60:240-5.
17. Takahashi T, Sano B, Nagata T, et al. Polo-like kinase 1 (PLK1) is overexpressed in primary colorectal cancers. *Cancer Sci* 2003;94:148-52.
18. Ito Y, Miyoshi E, Sasaki N, et al. Polo-like kinase 1

- overexpression is an early event in the progression of papillary carcinoma. *Br J Cancer* 2004;90:414-8.
19. Ando K, Ozaki T, Yamamoto H, et al. Polo-like kinase 1 (Plk1) inhibits p53 function by physical interaction and phosphorylation. *J Biol Chem* 2004;279:25549-61.
 20. Strebhardt K, Ullrich A. Targeting polo-like kinase 1 for cancer therapy. *Nat Rev Cancer* 2006;6:321-30.
 21. Li J, Wang R, Kong Y, et al. Targeting Plk1 to Enhance Efficacy of Olaparib in Castration-Resistant Prostate Cancer. *Mol Cancer Ther* 2017;16:469-79.
 22. Yang X, Chen G, Li W, et al. Cervical Cancer Growth Is Regulated by a c-ABL-PLK1 Signaling Axis. *Cancer Res* 2017;77:1142-54.
 23. Mao Y, Xi L, Li Q, et al. Combination of PI3K/Akt Pathway Inhibition and Plk1 Depletion Can Enhance Chemosensitivity to Gemcitabine in Pancreatic Carcinoma. *Transl Oncol* 2018;11:852-63.
 24. Schmit TL, Ledesma MC, Ahmad N. Modulating polo-like kinase 1 as a means for cancer chemoprevention. *Pharm Res* 2010;27:989-98.
 25. Benoit DS, Henry SM, Shubin AD, et al. pH-responsive polymeric sirna carriers sensitize multidrug resistant ovarian cancer cells to doxorubicin via knockdown of polo-like kinase 1. *Mol Pharm* 2010;7:442-55.
 26. Chun G, Bae D, Nickens K, et al. Polo-like kinase 1 enhances survival and mutagenesis after genotoxic stress in normal cells through cell cycle checkpoint bypass. *Carcinogenesis* 2010;31:785-93.
 27. Peter B, Gleixner KV, Cerny-Reiterer S, et al. Polo-like kinase-1 as a novel target in neoplastic mast cells: demonstration of growth-inhibitory effects of small interfering RNA and the Polo-like kinase-1 targeting drug BI 2536. *Haematologica* 2011;96:672-80.
 28. Kawata E, Ashihara E, Maekawa T. RNA interference against polo-like kinase-1 in advanced non-small cell lung cancers. *J Clin Bioinforma* 2011;1:6.
 29. Valianou M, Cox AM, Pichette B, et al. Pharmacological inhibition of Polo-like kinase 1 (PLK1) by BI-2536 decreases the viability and survival of hamartin and tuberlin deficient cells via induction of apoptosis and attenuation of autophagy. *Cell Cycle* 2015;14:399-407.
 30. Weiss L, Efferth T. Polo-like kinase 1 as target for cancer therapy. *Exp Hematol Oncol* 2012;1:38.
 31. Mok WC, Wasser S, Tan T, et al. Polo-like kinase 1, a new therapeutic target in hepatocellular carcinoma. *World J Gastroenterol* 2012;18:3527-36.
 32. Berg T. Small-molecule inhibitors of protein-protein interactions. *Curr Opin Drug Discov Devel* 2008;11:666-74.
 33. McInnes C, Mezna M, Fischer PM. Progress in the discovery of polo-like kinase inhibitors. *Curr Top Med Chem* 2005;5:181-97.
 34. Schmit TL, Ahmad N. Regulation of mitosis via mitotic kinases: new opportunities for cancer management. *Mol Cancer Ther* 2007;6:1920-31.
 35. Schoffski P. Polo-like kinase (PLK) inhibitors in preclinical and early clinical development in oncology. *Oncologist* 2009;14:559-70.
 36. Spankuch-Schmitt B, Bereiter-Hahn J, Kaufmann M, et al. Effect of RNA silencing of polo-like kinase-1 (PLK1) on apoptosis and spindle formation in human cancer cells. *J Natl Cancer Inst* 2002;94:1863-77.
 37. Jalili A, Moser A, Pashenkov M, et al. Polo-like kinase 1 is a potential therapeutic target in human melanoma. *J Invest Dermatol* 2011;131:1886-95.
 38. Nihal M, Stutz N, Schmit T, et al. Polo-like kinase 1 (Plk1) is expressed by cutaneous T-cell lymphomas (CTCLs), and its downregulation promotes cell cycle arrest and apoptosis. *Cell Cycle* 2011;10:1303-11.
 39. Weichert W, Schmidt M, Jacob J, et al. Overexpression of Polo-like kinase 1 is a common and early event in pancreatic cancer. *Pancreatology* 2005;5:259-65.
 40. Gray PJ Jr, Bearss DJ, Han H, et al. Identification of human polo-like kinase 1 as a potential therapeutic target in pancreatic cancer. *Mol Cancer Ther* 2004;3:641-6.
 41. Takai N, Hamanaka R, Yoshimatsu J, et al. Polo-like kinases (Plks) and cancer. *Oncogene* 2005;24:287-91.
 42. Kneisel L, Strebhardt K, Bernd A, et al. Expression of polo-like kinase (PLK1) in thin melanomas: a novel marker of metastatic disease. *J Cutan Pathol* 2002;29:354-8.
 43. Qian Y, Hua E, Bisht K, et al. Inhibition of Polo-like kinase 1 prevents the growth of metastatic breast cancer cells in the brain. *Clin Exp Metastasis* 2011;28:899-908.
 44. Yu C, Zhang X, Sun G, et al. RNA interference-mediated silencing of the polo-like kinase 1 gene enhances chemosensitivity to gemcitabine in pancreatic adenocarcinoma cells. *J Cell Mol Med* 2008;12:2334-49.
 45. Jimeno A, Rubio-Viqueira B, Rajeshkumar NV, et al. A fine-needle aspirate-based vulnerability assay identifies polo-like kinase 1 as a mediator of gemcitabine resistance in pancreatic cancer. *Mol Cancer Ther* 2010;9:311-8.
 46. Yao YD, Sun TM, Huang SY, et al. Targeted delivery of PLK1-siRNA by ScFv suppresses Her2+ breast cancer growth and metastasis. *Sci Transl Med* 2012;4:130ra48.
 47. Lu LY, Yu X. The balance of Polo-like kinase 1 in tumorigenesis. *Cell Div* 2009;4:4.
 48. Fu Z, Wen D. The Emerging Role of Polo-Like Kinase

- 1 in Epithelial-Mesenchymal Transition and Tumor Metastasis. *Cancers (Basel)* 2017;9:131.
49. Cai XP, Chen LD, Song HB, et al. PLK1 promotes epithelial-mesenchymal transition and metastasis of gastric carcinoma cells. *Am J Transl Res* 2016;8:4172-83.
50. Wu J, Ivanov AI, Fisher PB, et al. Polo-like kinase 1 induces epithelial-to-mesenchymal transition and promotes epithelial cell motility by activating CRAF/ERK signaling. *Elife* 2016;5:e10734.

Cite this article as: Mao Y, Xi L, Li Q, Zhang X, Yu C. Polo-like kinase 1 is involved in apoptosis, invasion, and metastasis of pancreatic ductal adenocarcinoma. *Transl Cancer Res* 2020;9(11):6672-6682. doi: 10.21037/tcr-20-1019