Polo-like Kinase 1 Facilitates Loss of Pten Tumor Suppressor-induced Prostate Cancer Formation[S]

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Background: Loss of Pten is thought to mediate the majority of prostate cancers.
Results: Polo-like kinase 1 confers the tumorigenic competence of Pten-deleted prostate cancer cells.
Conclusion: Polo-like kinase 1 facilitates loss of Pten-induced prostate cancer formation.
Significance: These results provide a mechanism explaining how cancer cells overcome oncogenesis-associated cellular stress.

Loss of the tumor suppressor Pten (phosphatase and tensin homolog deleted on chromosome 10) is thought to mediate the majority of prostate cancers, but the molecular mechanism remains elusive. In this study, we demonstrate that Pten-depleted cells suffer from mitotic stress and that nuclear function of Pten, but not its phosphatase activity, is required to reverse this stress phenotype. Further, depletion of Pten results in elevated expression of Polo-like kinase 1 (Plk1), a critical regulator of the cell cycle. We show that overexpression of Plk1 correlates with genetic inactivation of Pten during prostate neoplasia formation. Significantly, we find that elevated Plk1 is critical for Pten-depleted cells to adapt to mitotic stress for survival and that reintroduction of wild-type Pten into Pten-null prostate cancer cells reduces the survival dependence on Plk1. We further show that Plk1 confers the tumorigenic competence of Pten-deleted prostate cancer cells in a mouse xenograft model. These findings identify a role of Plk1 in facilitating loss of Pten-induced prostate cancer formation, which suggests that Plk1 might be a promising target for prostate cancer patients with inactivating Pten mutations.

In principle, cancer arises from an accumulation of genetic mutations. Cancer cells are characterized by a common set of properties including sustenance of proliferative signaling, evasion of growth suppressors, resistance of cell death, gain of replicative immortality, induction of angiogenesis, and activation of invasion and metastasis (1). In addition, the stress phenotypes associated with cancers are proposed as new hallmarks, such as DNA damage/replication stress, proteotoxic stress, mitotic stress, metabolic stress, and oxidative stress (2). However, how cancer cells adapt to these stresses is largely unknown.

The tumor suppressor Pten[2] (phosphatase and tensin homolog deleted on chromosome 10) is a lipid and protein phosphatase, which is well known to balance the PI3K/Akt pathway by dephosphorylation of phosphoinositide 3,4,5-trisphosphate, an activator of Akt (3). Genetic inactivation of Pten is common in prostate cancer, and the activation of the PI3K/Akt pathway resulting from loss of Pten is thought to be a major driving factor for this neoplasm (4–6). However, emerging evidences suggest that Pten functions beyond suppression of the PI3K/Akt pathway (7) and that other molecular factors also contribute to loss of Pten-induced prostate cancer formation (8, 9).

Polo-like kinase 1 (Plk1) plays pivotal roles in multiple aspects of mitosis, such as mitotic entry, centrosome maturation, sister chromatid segregation, and cytokinesis (10). Recent studies suggest that Plk1 acts as a coordinator between cell cycle progression and checkpoint pathways (11) and that Plk1 is essential for reinitiation of mitosis by silencing the G_2 checkpoint (12, 13). High levels of Plk1 expression correlate with cellular proliferation and poor prognosis of patients with various cancers (10, 14), including prostate cancer (15). Plk1 has been reported to mediate the chemoresistance of DNA damage-based chemotherapy via checkpoint adaptation (16). Moreover, overexpression of Plk1 in NIH 3T3 cells leads to malignant transformation (17).

The present study reports that Plk1 facilitates loss of Pten-induced prostate cancer formation. We show that depletion of Pten leads to elevated expression of Plk1. Overexpressed Plk1 adapts Pten-depleted cells to the mitotic stress. Significantly, Plk1 confers the tumorigenic competence of Pten-deleted prostate cancer cells, suggesting a strategy for prostate cancer therapy.

EXPERIMENTAL PROCEDURES

Mice—Prostate-specific Pten deletion mice (Pten^{fl/fl}; probasin-Cre) were generated as described previously (18).

[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Experimental Procedures and Figs. S1–S7.

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[2] The abbreviations used are: Pten, phosphatase and tensin homolog deleted on chromosome 10; Plk1, Polo-like kinase 1; DMSO, dimethyl sulfoxide; APC, anaphase-promoting complex/cyclosome; Cre, causes recombination.

Mouse Xenograft Model—DU145 control or Pten-depleted cells (5×10^6 cells per mouse) were mixed with an equal volume of Matrigel (Collaborative Biomedical Products) and inoculated into the flank of athymic nude mice (Harlan Laboratories). 1 week later, animals were randomized into treatment and control groups of five mice each. BI 2536 was dissolved in 0.1 N HCl, diluted with 0.9% NaCl, and injected into the tail vein twice weekly for 3 weeks. Tumor volumes, estimated using the formula: $V = L \times W^2/2$ (V, mm³; L, mm; W, mm), were measured every other day with digital calipers.

Statistical Analysis—A two-tailed, unpaired Student's *t* test was used to assess the difference between the effects of treatment on two cell lines. One-way analysis of variance was used to determine statistically significant differences from the mean in the xenograft study.

Cell Culture and Transfection, Construction of Vectors, RNAi, Fluorescence in Situ Hybridization, Chromosome Spreading, Immunofluorescence Staining, Western Blotting, Immunohistochemistry, Cell Viability Assay, and Soft Agar Quantitative Assay—Detailed information for these procedures is described in the supplemental Experimental Procedures.

RESULTS

Pten-depleted Cells Undergo Mitotic Stress—To understand loss of Pten-induced transformation, we used DU145 cells, a prostate cancer cell line with wild-type (WT) Pten, to generate Pten-depleted cells by RNAi (supplemental Fig. S1). Significantly higher percentages of chromosome misalignment and lagging chromosomes in Pten-depleted cells were observed when compared with DU145 control cells (Fig. 1*A*). As a consequence of the higher chromosomal instability, Pten-depleted cells show a higher percentage of aneuploidy in interphase and a higher percentage of cells with an abnormal number of chromosomes in metaphase (Fig. 1, *B* and *C*). These data suggest that Pten is essential for accurate chromosome distribution during mitosis, and thus its depletion might result in mitotic stress. To test this hypothesis, we treated DU145 control and Pten-depleted cells with mitotic poisons and compared the responses. FACS analysis showed that more G_2/M populations accumulated upon treatment by either nocodazole (inhibiting microtubule polymerization) or taxol (inhibiting microtubule depolymerization) in Pten-depleted cells than in Pten WT cells (Fig. 1, *D, left panel*, and *E, left panel*). We further compared mitotic indices in these two isogenic cell lines. In agreement with the FACS profiles, Pten-depleted cells showed a higher mitotic index than Pten WT cells (Fig. 1, *D, right panel*, and *E, right panel*), confirming the mitotic stress associated with Pten-depleted cells.

Mitotic Stress of Pten-depleted Cell Depends on Pten Nuclear Function but Not Its Phosphatase Activity—To dissect the mechanism for Pten loss-induced mitotic stress, we reintroduced Pten WT, Pten-K13E,K289E (nucleus-excluded mutant), or Pten-C124S (phosphatase-inactive mutant) into PC-3 cells (Pten-null) and examined their effects on this stress phenotype. When GFP-Pten-WT was introduced into PC-3 cells (supplemental Fig. S6*A*), we found that PC-3 cells expressing GFP-Pten-WT became less sensitive to mitotic perturbation than control PC-3 cells (supplemental Fig. S2, *A* and *B*),

confirming that mitotic stress is caused by Pten depletion. Reintroduction of Pten-C124S but not Pten-K13E,K289E into PC-3 cells reversed the stress phenotype (supplemental Fig. S2, *C–F*), suggesting that this mitotic stress depends on Pten nuclear function but not its phosphatase activity. However, how these cells adapt to the stress is unknown.

Loss of Pten Results in Plk1 Overexpression—Plk1, a critical mitotic player, is essential for checkpoint recovery and adaptation (12, 19). Moreover, Plk1 is overexpressed in prostate cancer and linked to higher tumor grade (15). To investigate the mechanism of mitotic stress adaptation after loss of Pten, we first compared the Plk1 expression levels in prostate tissue from Pten$^{+/+}$, Pten$^{+/-}$, or Pten$^{-/-}$ mice, which mimic human prostate cancer progression. As shown in Fig. 2*A*, increasing Plk1 expression in prostate epithelial cells is associated with a gradual loss of Pten and is accompanied by prostate luminal cell over-proliferation. These data suggest a possible role of high Plk1 expression in facilitating prostate tumor formation induced by loss of Pten.

Loss of Pten leads to the activation of phosphoinositide 3,4,5-trisphosphate downstream targets including Akt kinase (6). We reasoned that a positive correlation between Plk1 elevation and Akt activation is expected if Plk1 plays a critical role in Pten loss-induced prostate cancer progression. Accordingly, we analyzed the levels of Plk1 and the states of Akt in a human prostate cancer tissue array that included samples from normal prostate, prostate hyperplasia, and prostate malignant tumor. As indicated in Fig. 2*B*, among 20 prostate hyperplasia tissue samples examined, nine cases exhibited high Plk1 levels, seven cases with activation of Akt (detected by phospho-Ser-473 Akt staining), and in six of these cases, Plk1 overexpression correlated with activation of Akt. Furthermore, among 50 prostate malignant tumor samples, 24 cases exhibited Plk1 overexpression, 20 cases with activation of Akt, and in 16 of these cases, Plk1 overexpression correlated with activation of Akt. Pten protein level was also examined in this human prostate cancer array. We observed that two among 20 hyperplasia samples and six among 50 malignant samples show Pten-negative staining, and these samples are correlated with Plk1 overexpression (Fig. 2*B*, *panels j* and *k*). 18 among 20 hyperplasia samples and 44 among 50 malignant samples show Pten-positive staining (Fig. 2*B*, *panels h* and *i*), which is consistent with previous studies that complete loss of Pten is not a high frequent event for human prostate cancer (20, 21). Overall, we observed a positive correlation between Plk1 elevation and inactivation of Pten during human prostate cancer progression. In addition, we noticed that in these human prostate hyperplasia samples, Plk1 expression in stromal cells is higher than in epithelial cells, which is different from Plk1 overexpression in Pten$^{-/+}$ mouse prostate epithelial cells. The reason for this difference is that knockout of Pten is limited in mouse prostate epithelial cells by probasin-driven Cre recombination. In addition, it was reported that inactivation of Pten in stromal fibroblasts accelerated the initiation and progression of epithelial tumors (22). Whether the elevated Plk1 in human prostate stroma is involved in this process needs further experimentation.
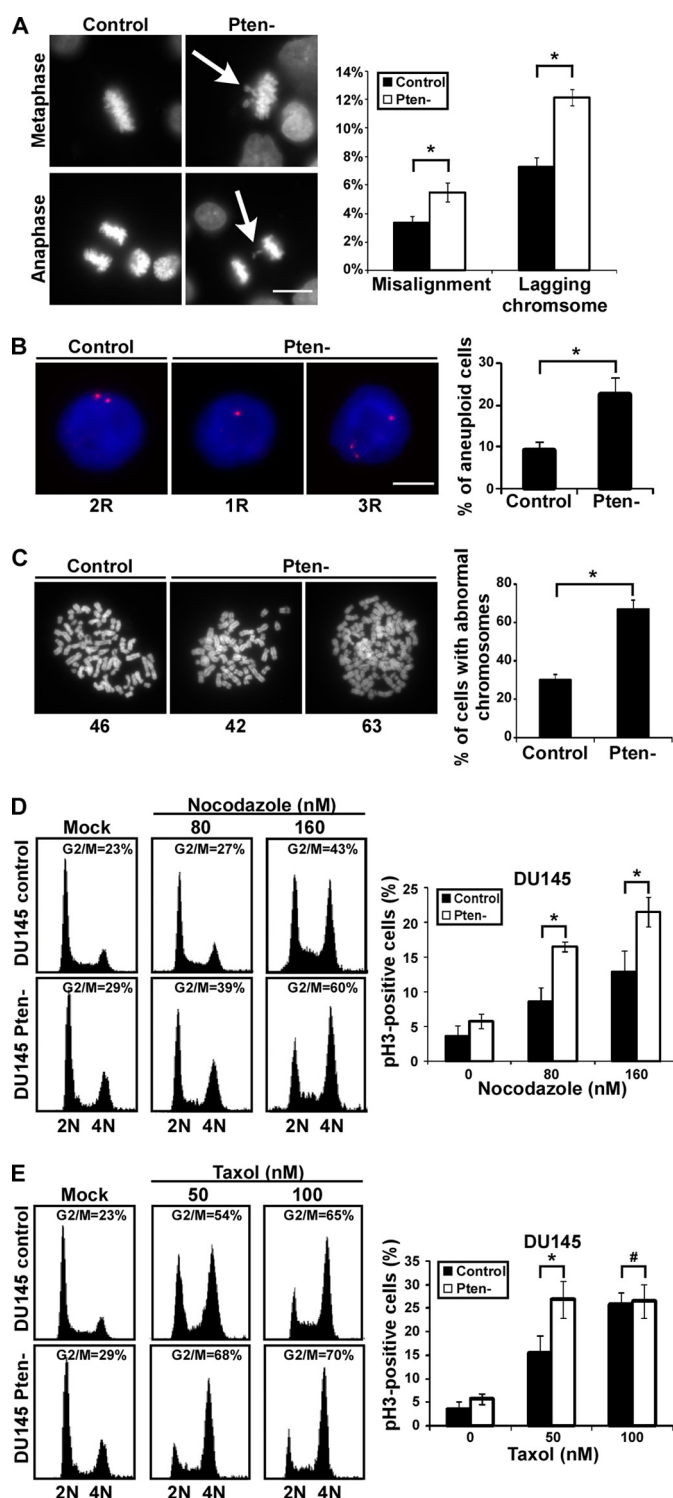
FIGURE 1. **Pten-depleted cells undergo mitotic stress.** *A–C*, Pten depletion leads to chromosomal instability. *A*, DU145 control and Pten-depleted cells were synchronized by a double thymidine block (16 h of thymidine block, 8 h of release, and a second thymidine block for 16 h) to arrest cells at the G_1/S boundary. Cells were released into growth medium containing 24 μM deoxycytidine and 50 μM monastrol for 8 h to enrich at prometaphase. Cells were then released into normal growth medium for 20 min to enrich at metaphase and 40 min to enrich at anaphase, and the percentages of cells with misaligned chromosomes in metaphase and lagging chromosomes in anaphase were counted (*, $p < 0.05$; *scale bar* on *left* indicates 10 μm). *B*, interphase fluorescence *in situ* hybridization analysis was performed with a probe against chromosome 2 (*red*) for DU145 control and Pten-depleted cells. The percentages of aneuploidy were calculated from at least 100 cells each (*, $p < 0.05$; *scale bar* on *left* indicates 5 μm). *C*, chromosome spreading was

Plk1 Confers the Tumorigenic Competence of Pten-depleted Prostate Cancer Cells—To evaluate the significance of Plk1 in Pten-depleted prostate cancer cells, we inhibited Plk1 activity by using BI 2536, a specific Plk1 inhibitor under clinical trial (23–25). FACS analysis and mitotic index measurements suggested that Pten-depleted cells responded to inhibition of Plk1 more rapidly than Pten WT cells (Fig. 3*A*). Significantly, Plk1 inhibition produced a more severe apoptosis response in Pten-depleted cells than in Pten WT cells, as indicated by a higher level of cleaved poly(ADP-ribose) polymerase, a marker for apoptosis (Fig. 3*B*, *left panel*). Plk1 protein levels increased in both cell lines upon treatment with 20 mM BI 2536, which is due to enrichment of mitotic cells by inhibition of Plk1. Interestingly, Plk1 protein level was elevated after Pten depletion even without BI 2536 treatment (Fig. 3*B*, *left panel*, compare *lane 1* and *lane 4*), which is consistent with the observation in the prostate tissue array study (Fig. 2*B*). Inhibition of Plk1 preferentially decreased the viability of Pten-depleted cells, but not Pten WT cells (Fig. 3*B*, *right panel*). Moreover, a similar result was observed in two additional Pten-depleted DU145 cell lines generated by Pten RNAi targeting different sites (supplemental Fig. S3). To rule out the possibility of off-target effect of BI 2536, we also inhibited Plk1 by RNAi. We found that knockdown of Plk1 had a more pronounced effect on Pten-depleted cells than Pten WT cells because Plk1 knockdown led to a higher mitotic index and a lower cell survival rate in the Pten-depleted cells (supplemental Fig. S4). These data suggest that Plk1 is critical for Pten-depleted prostate cancer cells to adapt to mitotic stress for survival.

To extend these observations to a more relevant process for prostate cancer progression, we generated a Pten-depleted line of RWPE-1 (supplemental Fig. S5), a non-transformed prostate cell line. Plk1 expression is elevated after Pten depletion (supplemental Fig. S5), and Pten-depleted RWPE-1 cells were hypersensitive to inhibition of Plk1 (Fig. 3, *C* and *D*), confirming the role of Plk1 in counteracting mitotic stress induced by loss of Pten.

Consistent with recent study by Song *et al.* (26), we also observed that restoration of Pten in Pten-null cells attenuates the dependence on Plk1 (supplemental Fig. S6, *A–C*), and this restoration depends on Pten nuclear function but not its phosphatase activity (supplemental Fig. S6, *D and E*). To gain more insight for the role of Plk1 during prostate cancer progression, we asked whether Plk1 functions similarly in androgen-dependent prostate cancer cells that represent an early stage of prostate cancer formation. Toward this end, we reintroduced Pten into LNCap cells, which contain androgen receptors and respond to androgen treatment. As shown in supplemental Fig. S7, reintroduction of GFP-Pten in LNCap cells also attenuates the sensitivity to Plk1 inhibition, suggesting a role of Plk1 in adapting to mitotic stress in androgen-dependent prostate cancer cells.

performed on DU145 control and Pten-depleted cells. The percentages of cells with abnormal chromosome numbers were calculated from at least 50 cells each (*, $p < 0.05$). *D* and *E*, Pten-depleted cells are sensitive to mitotic perturbation. DU145 control and Pten-depleted cells were treated with nocodazole (80 or 160 nM) (*D*), taxol (50 or 100 nM) (*E*), or DMSO as control and subjected to FACS analysis after a 12-h treatment (*left panel*) or to phospho-histone H3 staining to measure the mitotic index after a 10-h treatment (*right panel*) (*, $p < 0.05$, #, not significant). *Error bars* in *A–E* indicate S.E.
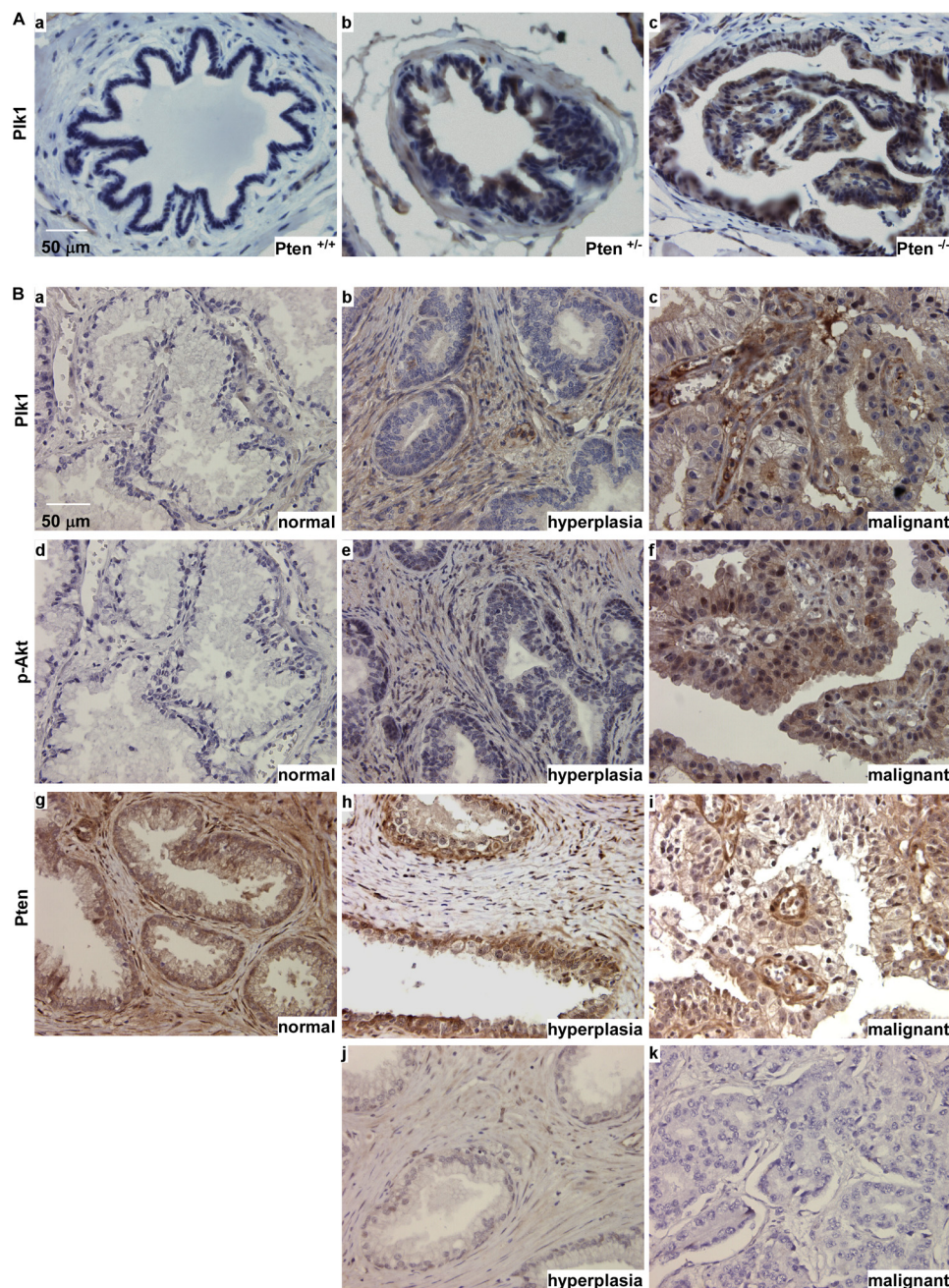
FIGURE 2. **Loss of Pten results in Plk1 overexpression.** *A*, diaminobenzidine staining of Plk1 in prostate tissue of wild-type ($^{+/+}$), heterozygous depletion ($+/-$), or homozygous depletion ($-/-$) Pten mice at the age of 3 months. Pten was depleted in a prostate-specific manner. *B*, diaminobenzidine staining of Plk1, phospho-Ser-473 Akt (*p-Akt*), and Pten in a human prostate cancer tissue array including 10 normal tissues, 20 hyperplasia tissues, and 50 malignant tumors. Representative images show normal prostate tissue (*panels a, d,* and *g*), prostate hyperplasia tissue (*panels b, e, h,* and *j*), and prostate malignant tumor (*panels c, f, i,* and *k*).

To further evaluate the significance of Plk1 on tumorigenic properties of Pten-depleted prostate cancer cells, we performed a soft agar assay to compare the inhibitory effect of Plk1 inhibition on anchorage-independent growth of Pten-depleted cells and Pten WT cells. As shown in Fig. 3*E*, Plk1 inhibition had a more pronounced effect in reducing colony numbers in Pten-depleted cells than in Pten WT cells, suggesting that Pten-depleted cells rely more on Plk1 than Pten WT cells for anchorage-independent growth.

Given that results based on cell-based assay do not necessarily reflect the consequence *in vivo*, we used Pten-depleted and Pten WT DU145 cells to compare the dependence of Plk1 on tumor formation in a mouse xenograft model. We found that Pten-depleted DU145 cells without BI 2536 treatment showed the most aggressive tumor growth (Fig. 3*F*), indicating that loss of Pten up-regulates the tumorigenic competence. A 3-week treatment of BI 2536 (25 mg/kg) completely suppressed the growth of tumors derived from Pten-depleted DU145 cells but had no significant inhibitory effect on the growth of tumors derived from WT DU145 cells (Fig. 3*F*). These *in vivo* data suggest that Plk1 confers the tumorigenic competence of Pten-depleted prostate cancer cells.
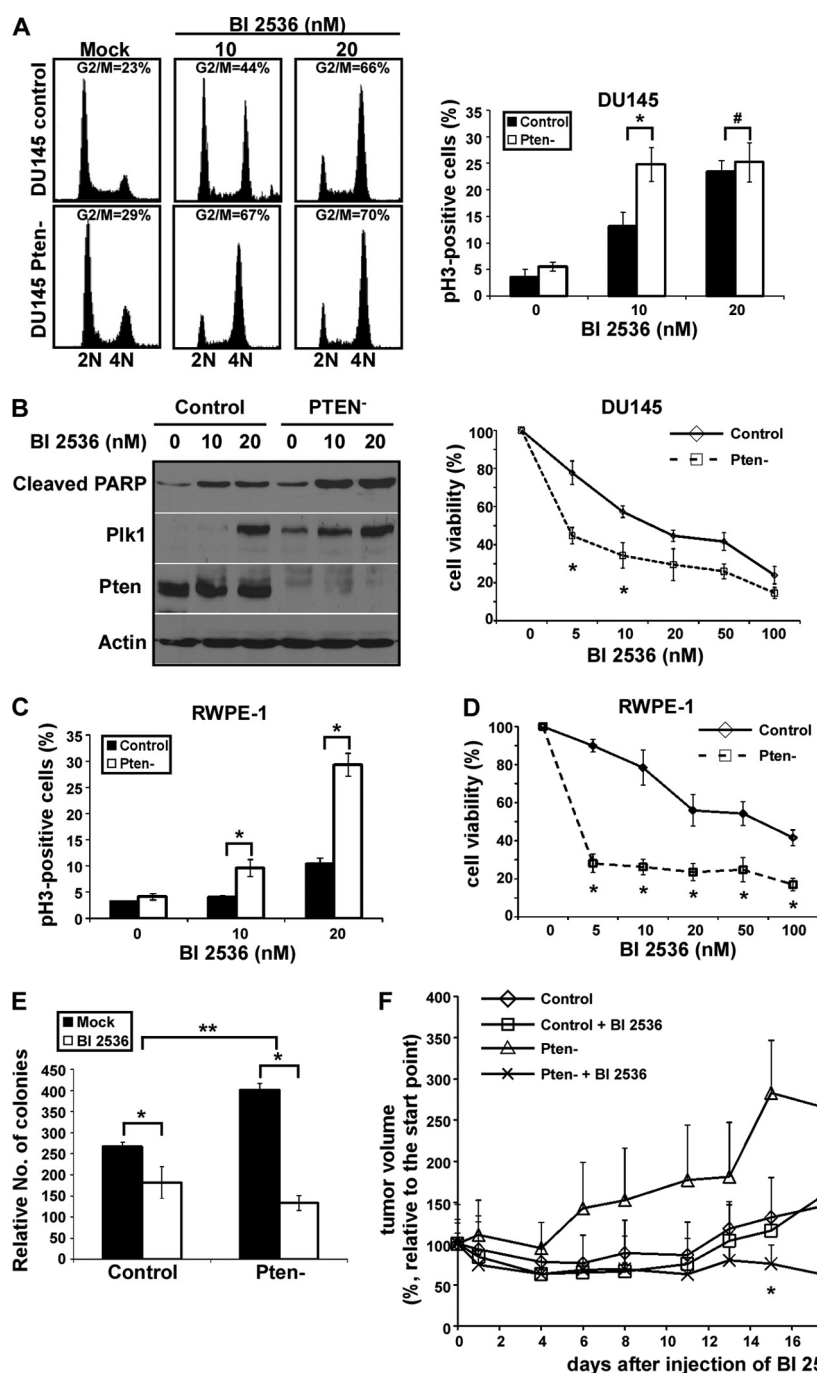
FIGURE 3. **Plk1 confers the tumor formation competence of Pten-depleted prostate cancer cells.** *A,* DU145 control and Pten-depleted cells were treated with BI 2536 (10 or 20 nM) or DMSO and subjected to FACS analysis after a 12-h treatment (*left panel*) or phospho-histone H3 staining to measure the mitotic index after a 10-h treatment (*right panel*). (*, $p < 0.05$, #, not significant) *B,* DU145 control and Pten-depleted cells were treated with BI 2536 (10 or 20 nM) or DMSO for 16 h and analyzed by cleaved poly(ADP-ribose) polymerase (*PARP*) Western blotting to examine apoptosis (*left panel*). DU145 control or Pten-depleted cells (5,000) were grown in 96-well plates, treated with BI 2536 (5, 10, 20, 50, 100 nM) for 3 days, and subjected to cell viability assay (*right panel*). (*, $p < 0.05$). *C,* RWPE-1 control and Pten-depleted cells were treated with BI 2536 (10 or 20 nM) or DMSO for 10 h, and subjected to phospho-histone H3 staining to measure the mitotic index (*, $p < 0.05$). *D,* RWPE-1 control and Pten-depleted cells (5,000) were grown on 96-well plates, treated with increasing concentrations of BI 2536 for 3 days, and harvested for cell viability assay (*, $p < 0.05$). *E,* DU145 control and Pten-depleted cells (2,000) were grown on soft agar and treated with BI 2536 (10 nM) for 7 days, and the relative colony number was determined by CellTiter-Glo luminescent cell viability assay (*, $p < 0.05$, **, $p < 0.01$). *F,* DU145 control or Pten-depleted cells (5×10^6) were inoculated into nude mice. 1 week after inoculation, the mice were treated with BI 2536 (25 mg/kg) twice weekly for 3 weeks, and the size of the tumors in each group was plotted against the number of days after the first day of BI-2536 injection (*, $p < 0.01$). *Error bars* in *A–F* indicate S.E.

DISCUSSION

Most phenotypes associated with loss of Pten can be explained by the activation of PI3K/Akt pathway (4–6), but emerging evidences indicate that Pten functions beyond sup-

pression of the PI3K/Akt pathway (7–9). Pten is reported to maintain centromere integrity by direct interaction with CENP-C, a key centromeric protein (27). Consistent with the function of Pten during mitosis, we demonstrated that Pten-

depleted prostate cancer cells suffer with mitotic stress and that this stress phenotype is dependent on Pten nuclear function but not its phosphatase activity. We found that Plk1 expression is elevated in these cells and that Plk1 is required to adapt to the mitotic stress for cell survival. Significantly, we further showed that Plk1 plays a critical role in maintaining the tumorigenic competence of Pten-depleted prostate cancer cells. These observations identify a role of Plk1 in facilitating loss of Pten-induced prostate cancer formation and provide a mechanism explaining how cancer cells overcome oncogenesis-associated cellular stress.

Inhibition of Plk1 preferentially suppresses tumor growth of Pten-depleted cells in a mouse xenograft model (Fig. 3*F*), suggesting that Plk1 might be a promising target for prostate cancer patients with genetic inactivation of Pten. A major concern for applying a Plk1 inhibitor such as BI 2536 for cancer treatment is the potential toxicity to normal tissues within its effective concentration. Our data indicate that Pten status plays an important role in determining the sensitivity of cells for inhibition of Plk1 because we found that 5 nM BI 2536 treatment efficiently inhibited the growth of prostate cancer cells without Pten (Fig. 3*B*, *right panel*) but did not affect normal prostate cells (Fig. 3*D*), suggesting that inhibition of Plk1 can specifically kill Pten-null cancer cells within a reasonable therapeutic window.

During the preparation of this manuscript, Song *et al.* (26) reported that nuclear Pten regulates the E3 ubiquitin ligase APC-Cdh1 (anaphase-promoting complex/cyclosome), and exclusion of Pten from the nucleus impairs its ubiquitination activity. Plk1 degradation is triggered by APC-Cdh1 in the late mitosis, so this study provides a mechanistic explanation for how loss of Pten results in overexpression of Plk1. However, why Pten-depleted cells are hypersensitive to Plk1 inhibition is not clear. We demonstrate that Pten depletion leads to mitotic stress and that Plk1 plays an essential role in adapting to this mitotic stress. Consistent with the study by Song *et al.* (26), we found that mitotic stress induced by loss of Pten depends on Pten nuclear function but not its phosphatase activity.

REFERENCES

1. Hanahan, D., and Weinberg, R. A. (2000) *Cell* **100,** 57–70
2. Luo, J., Solimini, N. L., and Elledge, S. J. (2009) *Cell* **136,** 823–837
3. Maehama, T., and Dixon, J. E. (1998) *J. Biol. Chem.* **273,** 13375–13378
4. Stambolic, V., Suzuki, A., de la Pompa, J. L., Brothers, G. M., Mirtsos, C., Sasaki, T., Ruland, J., Penninger, J. M., Siderovski, D. P., and Mak, T. W. (1998) *Cell* **95,** 29–39
5. Sun, H., Lesche, R., Li, D. M., Liliental, J., Zhang, H., Gao, J., Gavrilova, N., Mueller, B., Liu, X., and Wu, H. (1999) *Proc. Natl. Acad. Sci. U.S.A.* **96,** 6199–6204
6. Majumder, P. K., and Sellers, W. R. (2005) *Oncogene* **24,** 7465–7474
7. Vivanco, I., Palaskas, N., Tran, C., Finn, S. P., Getz, G., Kennedy, N. J., Jiao, J., Rose, J., Xie, W., Loda, M., Golub, T., Mellinghoff, I. K., Davis, R. J., Wu, H., and Sawyers, C. L. (2007) *Cancer Cell* **11,** 555–569
8. Majumder, P. K., Yeh, J. J., George, D. J., Febbo, P. G., Kum, J., Xue, Q., Bikoff, R., Ma, H., Kantoff, P. W., Golub, T. R., Loda, M., and Sellers, W. R. (2003) *Proc. Natl. Acad. Sci. U.S.A.* **100,** 7841–7846
9. Blanco-Aparicio, C., Renner, O., Leal, J. F., and Carnero, A. (2007) *Carcinogenesis* **28,** 1379–1386
10. Strebhardt, K. (2010) *Nat. Rev. Drug Disc.* **9,** 643–660
11. Takaki, T., Trenz, K., Costanzo, V., and Petronczki, M. (2008) *Curr. Opin. Cell Biol.* **20,** 650–660
12. van Vugt, M. A., Brás, A., and Medema, R. H. (2004) *Mol. Cell* **15,** 799–811
13. Liu, X. S., Li, H., Song, B., and Liu, X. (2010) *EMBO Rep.* **11,** 626–632
14. Eckerdt, F., Yuan, J., and Strebhardt, K. (2005) *Oncogene* **24,** 267–276
15. Weichert, W., Schmidt, M., Gekeler, V., Denkert, C., Stephan, C., Jung, K., Loening, S., Dietel, M., and Kristiansen, G. (2004) *Prostate* **60,** 240–245
16. Jimeno, A., Rubio-Viqueira, B., Rajeshkumar, N. V., Chan, A., Solomon, A., and Hidalgo, M. (2010) *Mol. Cancer Ther.* **9,** 311–318
17. Smith, M. R., Wilson, M. L., Hamanaka, R., Chase, D., Kung, H., Longo, D. L., and Ferris, D. K. (1997) *Biochem. Biophys. Res. Commun.* **234,** 397–405
18. Haverkamp, J. M., Charbonneau, B., Crist, S. A., Meyerholz, D. K., Cohen, M. B., Snyder, P. W., Svensson, R. U., Henry, M. D., Wang, H. H., and Ratliff, T. L. (2011) *Prostate* **71,** 1139–1150
19. Syljuåsen, R. G., Jensen, S., Bartek, J., and Lukas, J. (2006) *Cancer Res.* **66,** 10253–10257
20. Ali, I. U., Schriml, L. M., and Dean, M. (1999) *J. Natl. Cancer Inst.* **91,** 1922–1932
21. Salmena, L., Carracedo, A., and Pandolfi, P. P. (2008) *Cell* **133,** 403–414
22. Trimboli, A. J., Cantemir-Stone, C. Z., Li, F., Wallace, J. A., Merchant, A., Creasap, N., Thompson, J. C., Caserta, E., Wang, H., Chong, J. L., Naidu, S., Wei, G., Sharma, S. M., Stephens, J. A., Fernandez, S. A., Gurcan, M. N., Weinstein, M. B., Barsky, S. H., Yee, L., Rosol, T. J., Stromberg, P. C., Robinson, M. L., Pepin, F., Hallett, M., Park, M., Ostrowski, M. C., and Leone, G. (2009) *Nature* **461,** 1084–1091
23. Schöffski, P., Blay, J. Y., De Greve, J., Brain, E., Machiels, J. P., Soria, J. C., Sleijfer, S., Wolter, P., Ray-Coquard, I., Fontaine, C., Munzert, G., Fritsch, H., Hanft, G., Aerts, C., Rapion, J., Allgeier, A., Bogaerts, J., and Lacombe, D. (2010) *Eur. J. Cancer* **46,** 2206–2215
24. Steegmaier, M., Hoffmann, M., Baum, A., Lénárt, P., Petronczki, M., Krssák, M., Gürtler, U., Garin-Chesa, P., Lieb, S., Quant, J., Grauert, M., Adolf, G. R., Kraut, N., Peters, J. M., and Rettig, W. J. (2007) *Curr. Biol.* **17,** 316–322
25. Hofheinz, R. D., Al-Batran, S. E., Hochhaus, A., Jäger, E., Reichardt, V. L., Fritsch, H., Trommeshauser, D., and Munzert, G. (2010) *Clin. Cancer Res.* **16,** 4666–4674
26. Song, M. S., Carracedo, A., Salmena, L., Song, S. J., Egia, A., Malumbres, M., and Pandolfi, P. P. (2011) *Cell* **144,** 187–199
27. Shen, W. H., Balajee, A. S., Wang, J., Wu, H., Eng, C., Pandolfi, P. P., and Yin, Y. (2007) *Cell* **128,** 157–170