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Deficiency of PTEN leads to aberrant chromosome segregation through downregulation of MAD2

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Abstract. Proper spindle formation and accurate chromosome segregation are essential for ensuring mitotic fidelity. Phosphatase and tensin homolog (PTEN) is a multifunctional protein, which is able to maintain the stability of the genome and chromosomes. The present study described an essential role of PTEN in regulating chromosome segregation to prevent gross genomic instability via regulation of mitotic arrest deficient 2 (MAD2). PTEN knockdown induced cell cycle arrest and abnormal chromosome segregation, which manifested as the formation of anaphase bridges, lagging chromosomes and premature chromatid separation. In addition, MAD2 was identified as a potential target of PTEN. Furthermore, the present study revealed that PTEN knockdown resulted in MAD2 degradation via the ubiquitin-proteasomal pathway, while restoration of MAD2 expression partially ameliorated the mitotic defects induced by PTEN loss. The results from the present study proposed a novel mechanism by which PTEN maintains chromosome stability.

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Introduction

Phosphatase and tensin homolog (PTEN) is a multifunctional gene, which encodes a dual-specific phosphatase that dephosphorylates phosphatidylinositol (3,4,5)-triphosphate (PIP3) to phosphatidylinositol 4,5-bisohosphate and inhibits the phosphorylation of Akt (1). Mutations in the PTEN gene are common in human cancer (2-5). The protein expression levels of PTEN notably affect a variety of cellular processes, including cell death, proliferation, migration and metabolism (6,7). In addition, previous studies have indicated that PTEN serves fundamental functions in maintaining chromosomal stability (8-12). Mutations in the PTEN gene usually lead to aneuploidy (8,13,14); however, how PTEN controls the stability of chromosomes remains unknown.

Mitotic arrest deficient 2 (MAD2) protein is essential for the function of the spindle assembly checkpoint (SAC). At the beginning of mitosis, MAD2 maintains the activity of the SAC complex until all kinetochores are correctly connected to spindle microtubules (15). MAD2, and other proteins (cell division cycle 20, budding uninhibited by benzimidazole-related 1, BUB3 mitotic checkpoint protein and MAD3), constitute the mitotic checkpoint complex, which may inhibit the activity of Anaphase promoting complex/cyclosome (APC/C) to ensure cells remain in metaphase (16,17). Once the chromosomes have properly attached to the spindle, the APC/C complex facilitates the degradation of securin and cyclin B1, promoting cells to enter anaphase (15,18,19). Accumulating evidence has suggested that human tumors with chromosome instability exhibit abnormal MAD2 expression. Investigations using $MAD2^{+/-}$ mice revealed an increased frequency of aneuploidy (20-22); however the mechanism underlying the regulation of MAD2 remains unknown.

The present study demonstrated that depletion of PTEN induced cell cycle arrest and inhibited SAC, which led to aberrant chromosome segregation and abnormal chromosome number, suggesting that PTEN is important for chromosome stability. In addition, it was determined that PTEN deficiency decreased the stability of MAD2, which resulted in increased protein degradation via the ubiquitin-proteasome pathway, whereas the recovery of MAD2 expression partially rescued aberrant chromosome segregation in PTEN-knockdown cells. The results of the present study indicated that MAD2 function may be mediated by a mechanism in which PTEN regulates chromosome segregation.

Materials and methods

Cell culture, short hairpin RNA (shRNA), plasmids and chemicals. HeLa cells were obtained from The Cell Bank of Type Culture Collection of Chinese Academy of Sciences. Mouse embryonic fibroblasts (MEFs) were a gift from Dr Yuxin Yin of the Department of Pathology, Peking University. The cells were grown in Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (Sijiqing; Zhejiang Tianhang Biotechnology Co., Ltd.), and 1X penicillin-streptomycin and cultured in 5% CO_2 incubator at 37˚C.

PTEN-targeted shRNA (5'-GACAAAGCCAACCGA TACTTT-3') and control shRNA (5'-TTCTCCGAACGTGTC ACGT-3'), and MAD2 overexpression and control vectors were obtained from Shanghai GenePharma Co., Ltd. The ubiquitin expression vector was purchased from OriGene Technologies, Inc. In total, 25 μl shRNA containing lentivirus ($1x10^8$ transducing U/ml) was used for transfection. Plasmids were transfected into cells using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturers' protocol. The cells were cultured for a further 48 h before they were collected for subsequent experiments. MG132 and nocodazole were purchased from Sigma-Aldrich; Merck KGaA, and cycloheximide (CHX) was obtained from VWR International, LLC.

Western blot analysis and ubiquitination assay. Total HeLa cell extracts were prepared using a radioimmunoprecipitation assay buffer (Beyotime Institute of Biotechnology); proteins were quantified with a BCA kit (Beyotime Institute of Biotechnology). A total of 30 μg protein were separated using 10% SDS-PAGE and then transferred to a polyvinylidene difluoride membrane (EMD Millipore). The membrane was blocked with 5% skimmed milk in TBS + 0.5% Tween-20 (TBS-T) for 2 h at room temperature, and then incubated with primary antibodies at 4˚C overnight. Following 3 washes with TBS-T, the membrane was incubated with a horseradish peroxidase (HRP)-labeled secondary antibody for 2 h at room temperature. Subsequently, the membrane was washed 3 times and the bands were detected using an Enhanced Chemiluminescent substrate (Pierce; Thermo Fisher Scientific, Inc.) and analyzed using ImageJ (version 1.48; National Institute of Health). The following primary antibodies were used: Rabbit anti-human PTEN (1:2,000; Cell Signaling Technology, Inc.; cat. no. 9188L), rabbit anti-human MAD2 (1:1,000) and cyclin B1 (1:4,000; Santa Cruz Biotechnology, Inc.; cat. nos. sc-28261 and sc-752, respectively), and mouse anti-GAPDH (1:20,000; ProteinTech Group, Inc.; cat. no. 60004-1-Ig) were used. HRP-labelled goat anti-rabbit IgG (1:10,000) and rabbit anti-mouse IgG (1:10,000) were obtained from Santa Cruz Biotechnology, Inc. (cat. nos. sc-2004 and sc-2005, respectively). GAPDH was used as an internal control.

For the ubiquitination assay, HeLa cells were incubated with 20 μM MG132 for 4 h and collected 48 h after transfection of the ubiquitin expression plasmid; cells were then lysed in Triton lysis buffer (50 mM Tris-HCl pH 7.4, 0.25 M NaCl, 0.1% Triton X-100, 50 mM NaF). Cell lysates were pre-cleared using Protein A/G Agarose (cat. no., sc-2003; Santa Cruz Biotechnology, Inc.) and then incubated with anti-MAD2 antibody overnight at 4˚C; Protein A/G Agarose was added 2 h prior to centrifugation (1,000 x g for 3 min at 4˚C) to obtain cell lysates. Triton lysis buffer was used to wash the immunocomplexes 5 times. Finally, the samples were eluted by resuspending in loading buffer and boiling for 5 min. Protein expression from total extracts and immunoprecipitates were then determined by western blot analysis, as aforementioned.

Immunofluorescence and confocal microscopy. HeLa cells were cultured to 80% confluence prior to fixation with 4% formaldehyde at room temperature for 10 min, following which the cells were blocked with 5% bovine serum albumin (VWR International, LLC) for 1 h at room temperature. Anti-α-tubulin antibody (1:200; cat. no. 11224-1-AP; ProteinTech Group, Inc.) was then applied to the cells at 4˚C overnight. Following washing with PBS three times, the cells were incubated with fluorescently-labeled secondary antibodies (Alexa Fluor® 594; 1:400; cat. no. A-11012; Invitrogen; Thermo Fisher Scientific, Inc.) for 2 h at room temperature. Subsequently, the cells were washed with PBS three times and stained with DAPI (1 μg/ml; Nanjing KeyGen Biotech Co., Ltd.) at room temperature for 3 min. Images were obtained at x600 magnification and cells were analyzed with a confocal microscope (Leica Microsystems, Inc.).

Cell survival assay. HeLa cells were cultured to 50% confluence and then incubated with nocodazole (1 μM) for 36 h. Afterwards, cells were collected by centrifugation (200 x g for 5 min at room temperature) and resuspended in PBS. Cell viability was determined using a trypan blue exclusion assay as follows: 0.1 ml trypan blue solution (0.4%; Gibco; Thermo Fisher Scientific, Inc.) was added to 0.9 ml cell suspension, mixed and loaded into a hemocytometer, the cells were immediately examined under a microscope (Olympus IX53; Olympus Corporation) and the number of total cells and stained cells were counted. The cell viability was calculated as [1.00-(number of stained cells/number of total cells)] x100%.

Cell cycle analysis. Cell cycle analysis was performed using a kit from Nanjing KeyGen Biotech Co., Ltd. HeLa cells in the exponential growth phase were collected by centrifugation (200 x g for 5 min at room temperature) and fixed in ice-cold 75% ethanol for 2 h at 4˚C. Then, the cells were treated with propidium iodide (50 μg/ml) and RNase A (100 μg/ml) at room temperature for 30 min and analyzed using a flow cytometer (BD FACSCalibur; BD Biosciences). The cell cycle was analyzed with MODFIT LT 3.0 software (Verity Software House, Inc.).

Metaphase spread preparation. MEFs in the exponential growth phase were treated with colcemid (0.1 μg/ml; VWR International, LLC) for 2 h. Then, the cells were trypsinized and harvested by centrifugation at 200 x g at room temperature for 5 min. The collected cells were suspended in 8 ml 75 mM KCl solution at 37˚C for 20 min. Following pre-fixation with 1 ml fresh pre-cold fixation solution (methanol: Acetic acid at a ratio of 3:1) for 2 min at room temperature, the cells were collected by centrifugation at 200 x g for 5 min at room temperature. The cells were resuspended and fixed in 15 ml

cold fixation solution for 20 min at room temperature three times. Following centrifugation (200 x g for 8 min at room temperature), the cells were resuspended in 400 μl fixation solution, and 50 μl cell suspension was applied to slides and air-dried. The slides were stained with DAPI (1 μg/ml) at room temperature for 3 min. Images were obtained at x1000 magnification and cells were analyzed with a fluorescence microscope (Carl Zeiss AG).

Statistical analysis. All statistical analyses were performed using GraphPad Prism v.5.0 (GraphPad Software, Inc.). Data were presented as the mean ± standard deviation. A paired Student's t-test was used for comparisons of two groups and one-way analysis of variance for comparisons of multiple groups. The post-hoc Newman-Keuls test was used for pairwise comparisons in multiple groups. A χ^2 test was used to compare chromosome numbers: The metaphase spread was prepared and the chromosome numbers of each cell were counted in two categories ($PTEN^+$ and $PTEN^-$ MEFs). The chromosome numbers were then divided into two different categories [euploid (40) vs. aneuploid (≠40) or tetraploid (80) vs. non-tetraploid (≠80)]. The cell numbers of each category were counted and then used for statistical analysis. The data were considered to be categorical variables, and were obtained by counting the frequency of the different categories. P<0.05 was considered to indicate a statistically significant difference.

Results

PTEN-deficient cells undergo G2/M arrest and are resistant to spindle disruption. PTEN serves various functions in different stages of the cell cycle, including modulating G1 cell cycle progression, controlling DNA replication and regulating chromosome congression (11,23-25). To additionally investigate the function of PTEN in mitosis, the expression of PTEN in HeLa cells was knocked down using 2 PTEN-targeted shRNAs, shPTEN1 and shPTEN2. Western blot analysis revealed that the protein expression levels of PTEN decreased significantly in these cells (Fig. 1A). Additionally, PTEN knockdown arrested the cells in G2/M phase; the population of cells in G2/M phase increased from 12.88±0.40% in the control group to 19.20±0.88 and 17.64±1.61%, in the shPTEN1 and shPTEN2 PTEN knockdown groups, respectively (P<0.05; Fig. 1B). This suggested potential defects in the cell cycle checkpoints within PTEN-deficient cells.

The SAC is responsible for metaphase-to-anaphase transition. Once the SAC is activated, cells are arrested in metaphase until all sister chromatids have correctly attached to microtubules. To evaluate the function of the SAC in PTEN-knockdown cells, cell viability following nocodazole treatment was analyzed. Nocodazole is an anti-microtubule agent that halts cells at prometaphase by activating the SAC; prolonged nocodazole treatment may result in cell death by apoptosis (26,27). As presented in Fig. 1C, nocodazole suppressed the viability of control cells, while PTEN-knockdown cells were markedly resistant to nocodazole treatment. The results revealed that PTEN knockdown alleviated the effects of nocodazole, suggesting that the SAC was inhibited. These results indicated that PTEN deficiency may induce G2/M arrest, which may be associated with dysregulations in the assembly spindle checkpoint. As both of the PTEN-targeted shRNAs had similar effects, only shPTEN1 was selected for subsequent analyses.

Loss of PTEN leads to aberrant chromosome segregation. To additionally investigate the effects of PTEN loss on mitosis, chromosome segregation during mitosis was analyzed via DAPI staining. Examination of the mitotic cells revealed that the PTEN-knockdown cells exerted notable chromosome mis-segregation, which was associated with lagging chromosomes and the formation of anaphase bridges. In addition, deletion of PTEN significantly increased the percentage of lagging chromosomes in anaphase from 10.84±1.74 to 20.37±2.87%. Furthermore, the percentage of anaphase bridges increased from 10.39±1.37 to 21.66±4.09%; tripolar segregation was also observed in PTEN-knockdown cells (Fig. 2A). The results indicated that PTEN silencing impeded normal chromosome segregation.

Proper spindle assembly is important for accurate chromosome segregation. Therefore, the spindle apparatus in PTEN-knockdown cells was analyzed via immunofluorescence staining. Numerous abnormal spindles (asymmetric or incomplete) were observed in PTEN-knockdown cells (Fig. 2B). Aberrant mitosis usually results in erroneous chromosome inheritance. Therefore, the effects of PTEN loss on chromosome numbers were determined in $PTEN^{+/+}$ and $PTEN^{-/-}$ MEFs. Metaphase chromosome spreading was induced, following which the number of chromosomes was determined. Compared with wild-type MEFs, the frequency of tetraploidy in the $PTEN^{-/-}$ MEFs was notably increased. Aneuploidy was also detected in the $PTEN^{-/-}$ MEFs, but not in wild-type MEFs (Fig. 2C). Additionally, $PTEN^{-/-}$ MEFs exhibited abnormal morphology and premature sister chromatid separation (Fig. 2D). These results indicated that PTEN deficiency may induce aberrant spindle formation and chromosome segregation, leading to inaccurate chromosome inheritance.

MAD2 is downregulated in PTEN-deficient cells. MAD2 is essential for proper spindle formation and correct chromosome segregation. Several studies have indicated that the dysregulated expression of MAD2 may induce cell cycle arrest, aberrant chromosomal segregation, premature sister chromatid separation, aneuploidy and polyploidy (28-30). Therefore, immunostaining of the control and PTEN-knockdown HeLa cells was performed using MAD2 antibodies to determine whether PTEN silencing affected protein expression. Western blot analysis revealed that the protein expression levels of MAD2 were notably decreased in asynchronous PTEN-deficient cells (Fig. 3A). Then, cells were treated with nocodazole for 20 h to synchronize the cells. Under this condition, the protein level of PTEN was not changed significantly (Fig. S1A), and significant decreases in the protein expression levels of MAD2 were detected in the cells transfected with PTEN shRNA (Fig. 3B). These results indicated that PTEN deficiency may decrease the expression of MAD2 protein in asynchronous and synchronous cells, suggesting that the underlying mechanism may be independent of the cell cycle. Additionally, the protein expression levels of cyclin B1, a downstream target of MAD2 and important factor involved

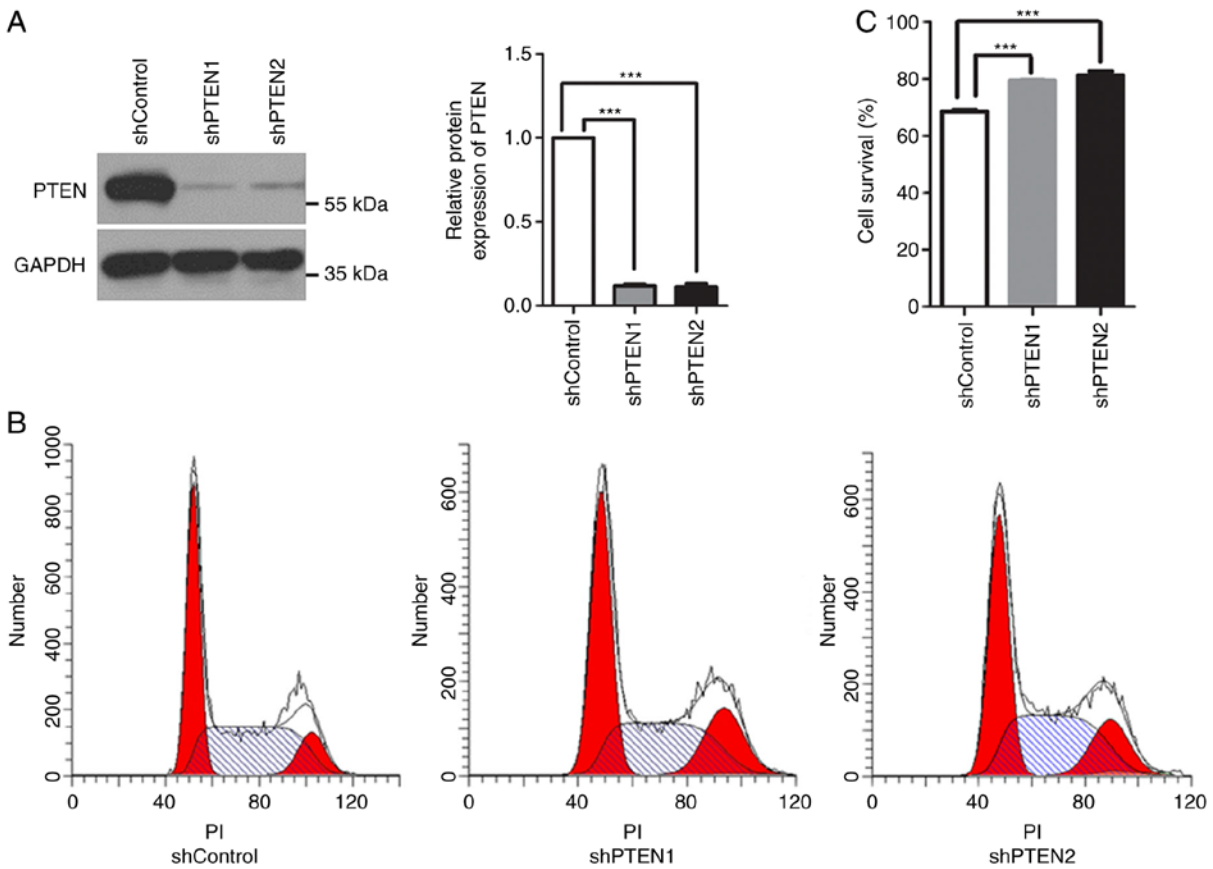

Figure 1. PTEN-deficient cells undergo G2/M arrest and are resistance to spindle disruption. (A) HeLa cells were transfected with control and 2 PTEN-targeted shRNAs, respectively. PTEN expression levels were detected by western blot analysis. (B) HeLa cells transfected with control or PTEN-specific shRNAs were harvested for cell cycle analysis by flow cytometry. (C) The rate of cell survival for control and PTEN-knockdown HeLa cells incubated with nocodazole for 36 h. All data were obtained from 3 independent experiments. ***P<0.001 vs. shControl group. PTEN, phosphatase and tensin homolog; sh, short hairpin RNA.

in the initiation of anaphase, were analyzed. The results of the western blot analysis demonstrated that cyclin B1 expression was decreased in PTEN-knockdown asynchronous cells, but also in nocodazole-treated cells (Fig. 3B), which may disrupt the spindle checkpoint and lead to impaired mitosis. These data additionally confirmed that the loss of PTEN may induce mitotic defects by disrupting spindle checkpoint activity.

PTEN knockdown leads to instability of MAD2 protein. To investigate how PTEN regulates MAD2 protein expression, cells were treated with CHX, a protein synthesis inhibitor, following which the expression of MAD2 was examined. Western blot analysis revealed that, following CHX treatment, the expression levels of MAD2 were decreased in cells transfected with PTEN shRNA, but not in the control cells (Fig. 3C), indicating that MAD2 was prone to degradation in PTEN-knockdown cells. In addition, cells were treated with MG132, a proteasome inhibitor. The expression levels of MAD2 was notably recovered following treatment with MG132 for 4 h in PTEN-knockdown cells, while the protein level of PTEN was not altered following MG132 treatment (Figs. 3D and S1B). The ubiquitination levels of MAD2 were also investigated using a ubiquitination assay. The results indicated that PTEN knockdown increased the ubiquitination levels of MAD2 compared with control cells (Fig. 3E). Collectively, these results suggested that PTEN deficiency may induce MAD2 degradation via the ubiquitin-proteasome pathway.

Recovery of MAD2 expression partially ameliorates impaired mitosis. To investigate whether PTEN loss-induced MAD2 degradation was responsible for aberrant mitosis, MAD2 was overexpressed in PTEN-knockdown cells. A His-tagged MAD2 expression plasmid was transfected into HeLa cells and PTEN-knockdown cells; western blot analysis confirmed that MAD2 was markedly overexpressed (Figs. 4A and S2). The protein expression levels of cyclin B1 were also increased in MAD2-overexpression cells, suggesting recovery of the spindle assembly checkpoint (Fig. 4A). Additionally, ectopic expression of MAD2 decreased cell viability following nocodazole treatment compared with the shPTEN group (Fig. 4B). Furthermore, restoration of MAD2 expression significantly decreased the number of binucleated and multinucleated cells observed in the PTEN-knockdown groups, but to a lesser extent compared with in the control group (Fig. 4C), which indicated that MAD2 may decrease the polyploidization caused by PTEN loss. These data suggested that abnormal chromosome segregation caused by PTEN knockdown may be mediated by MAD2.

Discussion

PTEN is important in chromosome protection and tumor suppression (5,8,14,31). In the present study, it was demonstrated that the basal expression of PTEN was essential for MAD2 stability and chromosome segregation. The ectopic

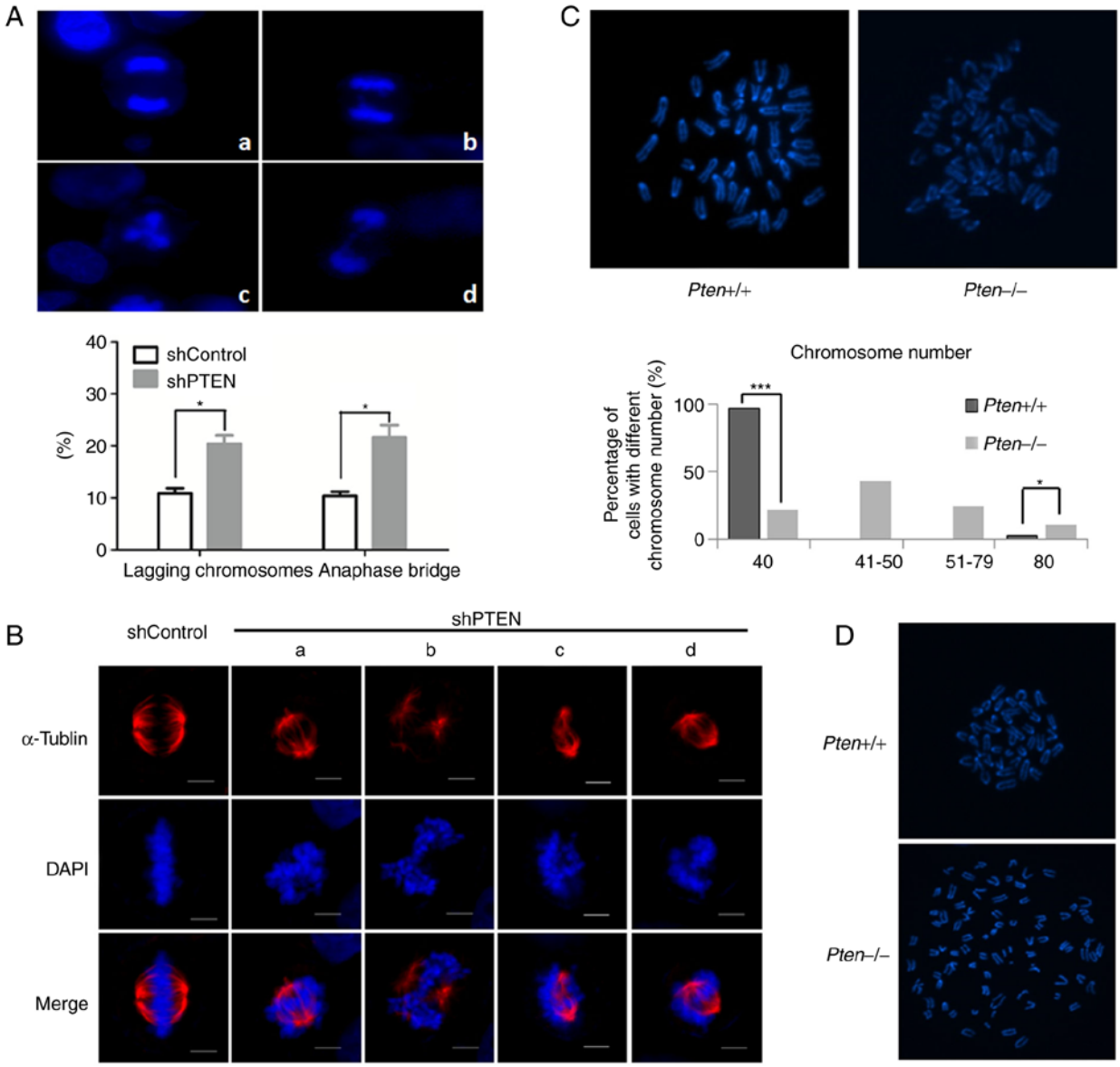

Figure 2. Loss of PTEN leads to aberrant chromosome segregation. (Top panel) Nuclei of mitotic cells were stained with DAPI. (A-a) Normal chromosome segregation. (A-b) Lagging chromosome. (A-c) Tripolar segregation. (A-d) anaphase bridge. (Bottom panel) Statistical analysis of lagging chromosomes and anaphase bridges. A total of 80 anaphase cells were counted. Among these anaphase cells, cells with lagging chromosome or anaphase bridge were counted. The percentage was calculated as the lagging chromosome number (anaphase bridge number)/anaphase number. *P<0.05 vs. short hairpin RNA-control group. (B) Representative images of α-tubulin-stained cells exhibiting (left panel) normal spindle formation and (panels a-d) aberrant spindle formation in metaphase. (C) Metaphase spreads were prepared from PTEN$^{+/+}$ and PTEN$^{-/-}$ MEFs. Chromosome numbers of 71 PTEN$^{+/+}$ MEFs and 100 PTEN$^{-/-}$ MEFs were counted. Then the cells were divided into four groups according to their chromosome numbers (40; 41-50; 51-79; and 80). The percentage of each group was calculated as cell numbers in each group/total cell numbers (71 PTEN$^{+/+}$ MEFs or 100 PTEN$^{-/-}$ MEFs) and analyzed via a χ^2 test. *P<0.05 and ***P<0.001 vs. PTEN$^{+/+}$ MEFs. (D) Representative images of normal metaphase spread in PTEN$^{+/+}$ MEFs and premature sister chromatid separation within PTEN$^{-/-}$ MEFs stained by DAPI. MEFs, mouse embryonic fibroblasts; PTEN, phosphatase and tensin homolog.

expression of MAD2 ameliorated PTEN loss-induced mitotic defects, which additionally confirmed the important role of MAD2 in mitosis. These data indicated that PTEN serves a key role in chromosome segregation by maintaining accurate mitosis. The results of the present study also revealed a novel function of PTEN in regulating the expression of MAD2 in mitosis.

Regulation upstream of MAD2 has been studied previously: It has been demonstrated that MAD2 may be regulated by E2F1, Myc and RE1 silencing transcription factor at the transcriptional level (32,33); however, how the cellular expression of MAD2 is regulated at the post-transcriptional level requires further investigation. Withaferin A was suggested to induce the degradation of MAD2, yet the molecular mechanisms remain unknown (34). SMAD specific E3 ubiquitin protein ligase 2 (Smurf2) has been suggested to inhibit the ubiquitination of MAD2 (35), but the underlying mechanism requires additional study. In the present study, the regulator PTEN was examined, and was determined to maintain the stability of MAD2. The mechanism underlying the regulation of MAD2 degradation mediated by PTEN was not explored, but Akt has been recently demonstrated to induce the phosphorylation and degradation of Smurf2 (36). Therefore, we hypothesized that the deletion of PTEN may activate Akt,

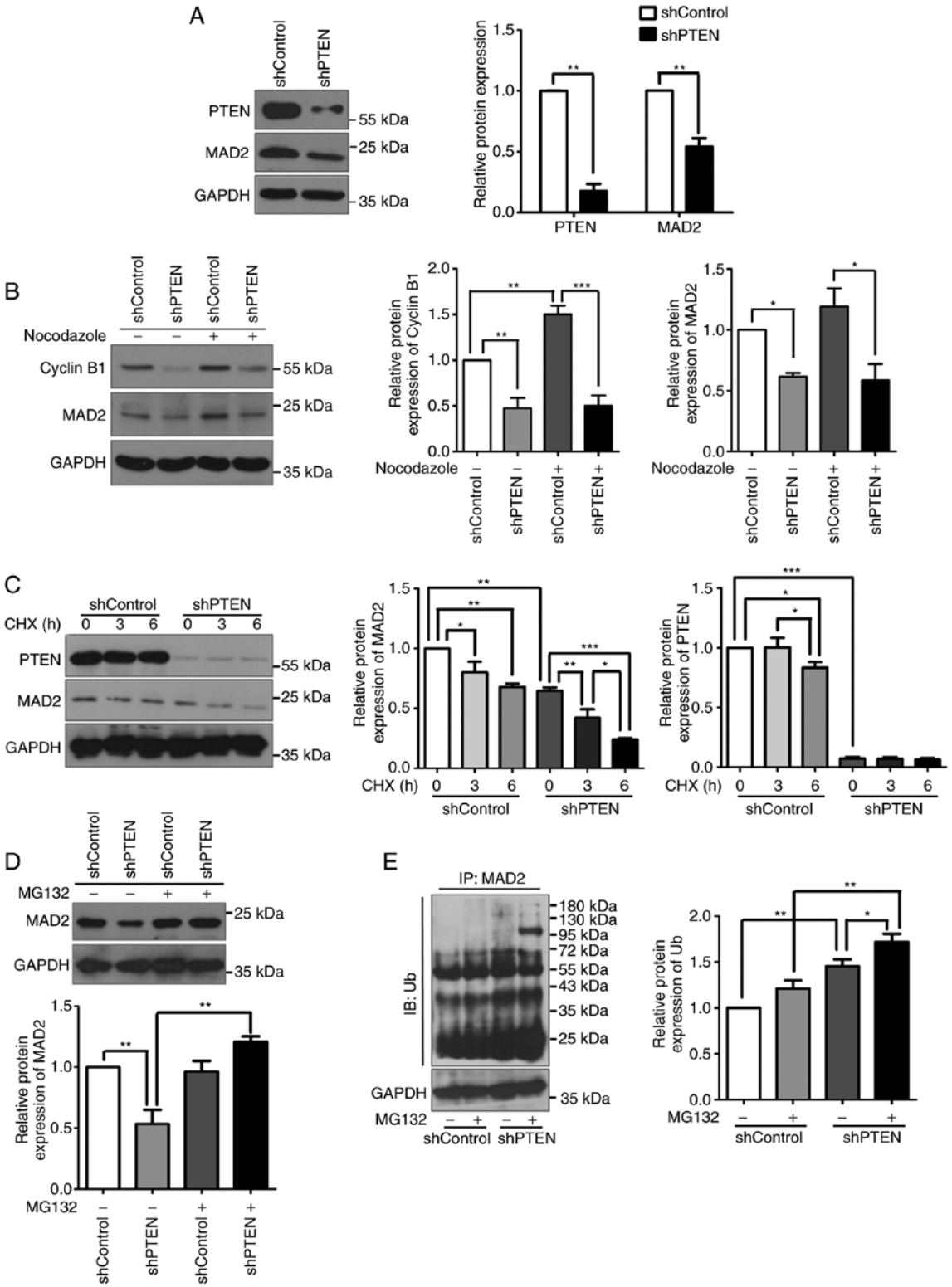

Figure 3. MAD2 is downregulated in PTEN-deficient cells. (A) MAD2 expression was evaluated using cell lysates from control and PTEN-knockdown cells by western blot analysis. (B) The protein expression levels of MAD2 and cyclin B1 in control and PTEN-knockdown HeLa cells cultured with or without nocodazole were detected by western analysis. (C) Control and PTEN-knockdown HeLa cells cultured with or without CHX were harvested at 0, 3 and 6 h after treatment. Then, the protein expression levels of MAD2 were evaluated using western blot analysis. (D) Control and PTEN-knockdown HeLa cells cultured with or without MG132 were analyzed for MAD2 expression by western blot analysis. (E) Ubiquitin was overexpressed in control and PTEN-knockdown HeLa cells. Following MG132 treatment, the cells were harvested for analysis via a ubiquitination assay. All data were obtained from 3 independent experiments. *P<0.05, **P<0.01 and ***P<0.001. MAD2, mitotic arrest deficient 2; PTEN, phosphatase and tensin homolog; sh, short hairpin RNA; Ub, ubiquitin; CHX, cycloheximide; IP, immunoprecipitates; IB, immunoblot.

resulting in decreased Smurf2 expression, which may lead to the degradation of MAD2. Further investigation is required to validate this hypothesis regarding the PTEN-Smurf2-MAD2 axis and other mechanisms.

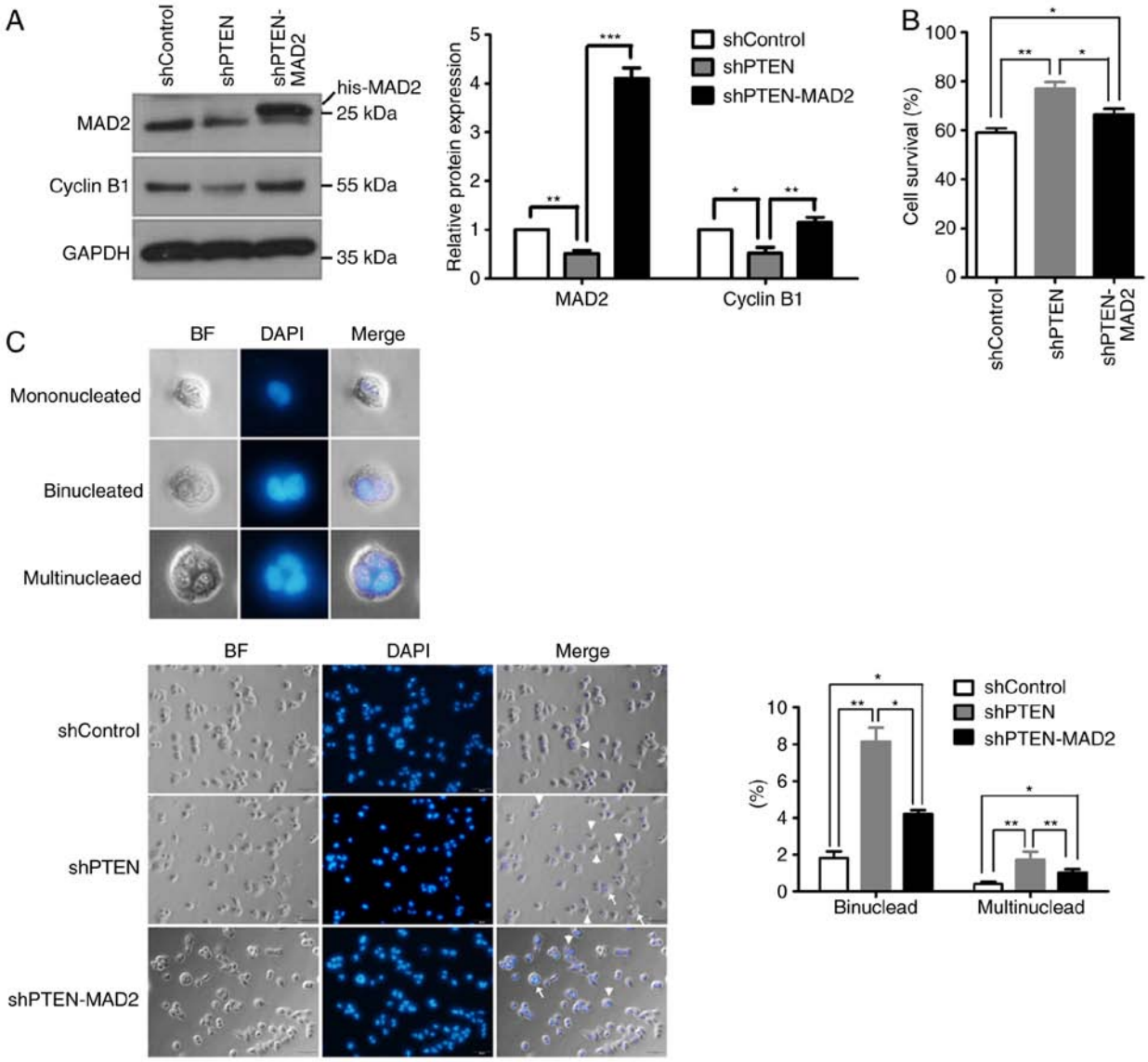

Figure 4. Recovery of MAD2 expression partially ameliorates impaired mitosis. (A) PTEN-knockdown HeLa cells were transfected with His-tagged MAD2 expression plasmids prior to the analysis of MAD2 and cyclin B1 expression by western blot analysis. (B) The rate of cell survival for control, PTEN-knockdown and His-MAD2-overexpression PTEN-knockdown HeLa cells incubated with nocodazole for 36 h was calculated. (C) (Left panel) Representative images indicating the general morphology of mononucleated, binucleated and multinucleated cells at high magnification. (Middle panel) Representative images demonstrating cells of the shControl, shPTEN and shPTEN-MAD2 groups stained by DAPI. Binucleated cells (arrowhead) and multinucleated cells (arrow) are marked in each image. (Right panel) Statistical analysis of the percentage of binucleated or multinucleated cells in the different groups. A total of 500 cells were counted. Among these cells, binucleated or multinucleated cells were counted. The percentage was calculated as binucleated cell number (or multinucleated cell number)/total cell number. All data were obtained from 3 independent experiments, $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$. BF, bright field; MAD2, mitotic arrest deficient 2; PTEN, phosphatase and tensin homolog; sh, short hairpin RNA; his-MAD2, His-tagged MAD2 expression plasmid.

The results of the present study suggested that restoration of MAD2 may partially rescue the mitotic defects induced by PTEN loss, which indicated that MAD2 may not be the only protein responsible for the PTEN-associated functions in mitosis. PTEN may target different protein substrates that control mitosis. Notably, it has been demonstrated that PTEN may control the spindle architecture and chromosome congression by regulating Eg5 (25). Polo like kinase 1 is another target of PTEN involved in the regulation of chromosome stability during mitosis (37). In addition, PTEN serves a role in cytokinesis by dephosphorylating PIP3 (38). Additional targets of PTEN may be identified in the future, which may improve understanding regarding the function of PTEN in mitosis.

PTEN is a multifunctional tumor suppressor gene. Numerous studies have indicated that PTEN may regulate the cell cycle through different mechanisms (39-41). In the present study, PTEN knockdown was determined to cause G2/M arrest in HeLa cells by downregulating MAD2 and cyclin B1. Conversely, Song *et al* (9) revealed that nuclear PTEN interacted with APC/C and enhanced the activity of APC-fizzy-related protein homolog (CDH1), which facilitated the ubiquitination of cyclin B1, decreasing its expression. It appears that the results of the present study are contradictory to these data, but this may be due to the various functions of PTEN. For example, PTEN may regulate cyclin B1 through a variety of mechanisms in different cell cycle phases. In metaphase, PTEN maintains the levels of cyclin B1 by stabilizing

MAD2 to ensure accurate chromosome segregation. In addition, from anaphase to G1 phase, PTEN downregulates cyclin B1 by enhancing APC-CDH1 activity to prevent the abnormal accumulation of cyclin B1. These contradictory results suggest the different functions of PTEN in the various phases of the cell cycle. Furthermore, Song *et al* (9) investigated the function of nuclear PTEN and indicated that the functions of this protein were independent of its phosphatase activity, whereas the present study explored the function of total PTEN. The present study proposed that PTEN functions may be attributed to the dephosphorylation of p-Akt. This suggests the different functions of nuclear PTEN, based on C-terminal protein binding activity, and cytoplasmic PTEN, based on N-terminal phosphatase activity.

Abnormal mitosis is a hallmark of cancer, which makes it a common target for anticancer therapies. For example, microtubule-targeting agents (MTAs) are now applied to treat cancer clinically; MTAs arrest cancer cells in mitosis, resulting in their death (42,43); however, a proportion of cancer cells may develop resistance to MTAs, which restricts its application (44). The present study revealed that PTEN deficiency may disrupt the function of SAC and protect cells against the damage caused by MTAs. As a result, MTAs may be less effective to cancer cells with PTEN deficiency. Cyclin B1/cyclin-dependent kinase 1 (CDK1) is an additional target for cancer therapy (45,46); agents against these targets may inhibit their activity and induce cell cycle arrest. However, the results of the present study indicated that PTEN knockdown may lead to the downregulation of cyclin B1, which may also attenuate the anti-cancer activity of cyclin B1/CDK1 inhibitors. Therefore, the expression levels of PTEN may be associated with the effectiveness of these types of anti-cancer drugs. Therefore, to ensure the effectiveness of treatment, the expression of PTEN in patients may be evaluated prior to the administration of these agents. The results of the present study may aid the selection of anti-mitotic therapeutic agents under conditions of PTEN deficiency.

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Availability of data and materials

The datasets used and/or analyzed during the curernt study are available from the corresponding author on reasonable request.

Authors' contributions

ZSu and JL performed the majority of the experiments, analyzed the data and prepared the manuscript. MW and ML performed experiments. LB and ZSh analyzed the data. LH and YW participated in the conception and design of the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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