GINS2 regulates the proliferation and apoptosis of colon cancer cells through PTP4A1

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Abstract. Colon cancer is associated with high death rates worldwide and poses a serious threat to public health. GINS complex subunit 2 (GINS2) serves a carcinogenic role in many cancers, including gastric adenocarcinoma, ovarian cancer and pancreatic cancer. However, the specific function of GINS2 in the development of colon cancer has not been described in detail. The present study aimed to clarify the role of GINS2 in colon cancer. A Cell Counting Kit-8 assay, EdU staining, TUNEL and flow cytometry analyses were performed to determine the levels of cell viability, proliferation and apoptosis and to evaluate the cell cycle. Through the analysis of BioGrid, a Protein-Protein Interaction database, it was hypothesized that protein tyrosine phosphatase 4A1 (PTP4A1) is a protein that might interact with GINS2, which was then validated using a co-immunoprecipitation assay. mRNA and protein levels were measured using reverse transcription-quantitative PCR and western blotting, respectively. The results of the present study demonstrated that GINS2 expression levels were increased in colon cancer cells. Furthermore, GINS2 knockdown inhibited the proliferation of colon cancer cells, while the levels of cell cycle arrest and apoptosis were increased. By interacting with PTP4A1, GINS2 promoted the expression of PTP4A1, a novel p53 target. GINS2 knockdown was increased, while PTP4A1 overexpression decreased the protein level of p53. Notably, PTP4A1 overexpression partly reversed the effects of GINS2 downregulation on colon cancer cells. Therefore, the present study demonstrated that GINS2 regulated the proliferation and apoptosis of colon cancer cells through PTP4A1/p53 pathway, highlighting that GINS2 may serve as a novel molecular marker for colon cancer prevention and therapy.

Introduction

Colon cancer is a common, highly malignant type of cancer characterized by a high morbidity rate and a poor prognosis (1). According to statistics obtained from the American Cancer Society, human colon cancer has an incidence rate of 10.2% and a mortality rate of 9.2% worldwide, ranking it as the fourth most common cancer (2,3). Although advances have been made in the current treatment options available for colon cancer, the associated mortality rates continue to increase and the current 5-year survival rate remains low (4,5). Risk factors associated with colon cancer include age and diet; however, the overexpression of oncogenes and the inactivation of tumor suppressor genes remain the most important contributors (6). At present, conventional therapies, such as surgery, chemotherapy and antibody therapies have been adopted to protect against colon cancer (7). However, these methods exhibit low levels of effectiveness in clinical practice (8). Thus, the present study aimed to investigate the mechanisms underlying the development and progression of colon cancer, which may help to determine effective therapeutic options.

GINS complex subunit 2 (GINS2), also known as PSF2, belongs to the GINS family and acts as a vital participant in both DNA duplication and cell cycle progression (9,10). In addition, GINS2 has been found to serve a key role in the tumorigenesis of various cancers (11). For example, GINS2 is overexpressed in breast cancer cell lines and GINS2 knockdown inhibited breast cancer growth and metastasis (12). In addition, Yan *et al* (13) demonstrate that GINS2-regulated cell proliferation and apoptosis in human epithelial ovarian cancer. GINS2 is highly expressed in colon cancer (14,15), but the specific role is yet to be fully elucidated.

The results of previous studies demonstrated that phosphatase of regenerating liver 1 (PTP4A1) is upregulated in numerous tumor cells and is involved in promoting both cell migration and invasion (16-18). The analysis of BioGrid (https://thebiogrid.org/), a protein-protein interaction database, suggested that PTP4A1 could interact with GINS2. Results from a previous study demonstrate that PTP4A1 is highly expressed in colon cancer, but not in normal colon tissues or

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colonic adenomas (19). In addition, GINS2 inhibition regulates the proliferation and apoptosis of non-small cell lung cancer cells through p53 (20). PTP4A1 is considered as a novel p53 target and PTP4A1 inhibition increases p53 expression level (21).

Therefore, the present study aimed to explore the role of GINS2 in the proliferation and apoptosis of colon cancer and its potential regulatory mechanism on the PTP4A1/p53 pathway.

Materials and methods

Cell culture, treatment and transfection. Normal human intestinal epithelial cell line (HIEC-6) and colon cancer cell lines (HCT116, LS174T, HCT8 and SW620) were purchased from the American Type Culture Collection. Cells were incubated in DMEM supplemented with 10% FBS (both from Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen; Thermo Fisher Scientific, Inc.) at 37°C in a humidified atmosphere with 5% CO_2.

To knockdown GINS2, short hairpin RNA (shRNA)-GINS2-1/2 and its negative control (shRNA-NC) were designed and synthesized by Shanghai GenePharma Co., Ltd. and shRNA fragments were cloned into a lentiviral GV493 vector (Shanghai GeneChem Co., Ltd.). The target sequences were as follows: shRNA-GINS2-1, 5'-GATTAA CCTGAACAAAGA-3'; shRNA-GINS2-2, 5'-ATCAAC ACCAGCGGGACTTTC-3'; shRNA-NC, 5'-TTTCTCCGA ACGTGTCACGT-3'. To overexpression PTP4A1, the PTP4A1 coding sequence was synthesized and cloned into a lentiviral GV492 vector. The empty GV492 vector was considered as a negative control (Ov-NC). When 293T cells were cultured to 60-70% confluency, according to the manufacturer's protocol, a total of 1 μg lentiviral plasmid, 1 μg 3rd generation viral packaging vectors and 5 μl Lipofectamine® 2000 transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.) in serum-free DMEM was incubated for 15 min at room temperature and then added into 293T cells to amplify at 37°C for 6 h. After transfection for 48 h, the supernatant containing virus was collected by centrifugation at 10,000 x g for 4 h at 4°C. HCT116 cells at the 3rd passage were infected with lentiviral vectors at an MOI of 50 in the presence of 5 μg/ml polybrene (MilliporeSigma) for 24 h at 37°C. Subsequently, the medium was replaced with fresh medium and the stable cells were selected with 2 μg/ml puromycin for 3 days. The transfection efficiency was evaluated by reverse transcription-quantitative (RT-q) PCR and western blotting.

RT-qPCR. Total RNA was extracted from $1x10^6$ cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and subsequently reverse transcribed into cDNA using the PrimeScript RT Reagent kit (Takara Biotechnology Co., Ltd.) according to the manufacturer's protocols under the following conditions: 37°C for 15 min and at 85°C for 5 sec. The synthesized cDNA was used as a template for PCR, which was performed using an ABI 7000 quantitative PCR instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.) using the SYBR Green PCR kit (Applied Biosystems; Thermo Fisher Scientific, Inc.). The following thermocycling conditions were used for the qPCR: Initial denaturation at 95°C for 6 min;

followed by 40 cycles of denaturation at 95°C for 15 sec, annealing at 60°C for 30 sec and extension at 72°C for 45 sec and a final extension at 72°C for 5 min. The sequences of the gene primers were as follows: GINS2 forward, 5'-CAGAAA TGTCGCCTGCTCC-3' and reverse, 5'-GGATTTCGTCTG CCTTCG-3'; PTP4A1 forward, 5'-ATTGAAGGTGGAATG AAATACGAAG-3' and reverse, 5'-TACTTCTCCAAATAC AGAAGTTGCT-3'; and GAPDH forward, 5'-ACCTGACCT GCCGTCTAGAAAA-3' and reverse, 5'-TTGAAGTCAGAG GAGACCACCTG-3'. Relative mRNA expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method (22) and normalized to the internal reference gene GAPDH.

Western blotting. Total protein was extracted from the cells using RIPA lysis buffer (Beyotime Institute of Biotechnology) and subsequently quantified using BCA kits (Thermo Fisher Scientific, Inc.). Proteins (30 μg/lane) were separated on a 10% gel by SDS-PAGE and subsequently transferred onto PVDF membranes. The membranes were blocked with 5% non-fat milk for 1 h at room temperature and the proteins were incubated with primary antibodies against GINS2 (1:500; cat. no. ab197123; Abcam), PTP4A1 (1:400; cat. no. ab121185; Abcam), p21 (1:1,200; cat. no. ab109520; Abcam), cyclin D1 (1:200; cat. no. ab16663; Abcam), Bcl2 (1:1,500; cat. no. ab182858; Abcam), Bax (1:1,500; cat. no. ab182733; Abcam), poly (ADP-ribose) polymerase (PARP) (1:1,000; cat. no. ab191217; Abcam), p53 (1:5,000; cat. no. ab32389; Abcam) and GAPDH (1:2,500; cat. no. ab9485; Abcam) at 4°C overnight. Following primary incubation, the membranes were incubated with an HRP-conjugated goat anti-rabbit secondary antibody (1:20,000; cat. no. ab205718; Abcam) for 2 h at room temperature. Protein bands were visualized using enhanced chemiluminescence (MilliporeSigma) and imaging system (Tanon-5200; Tanon Science and Technology Co., Ltd.). The gray values of the bands were semi-quantified using ImageJ software (version 1.0; National Institutes of Health) and the protein expression was normalized against GAPDH.

Cell Counting Kit-8 (CCK-8). Transfected cells ($5x10^3$ cells/well) were inoculated into 96-well plates. CCK-8 reagent (10 μl/well; Beyotime Institute of Biotechnology) was added and the cells were incubated at 37°C for 2 h. Subsequently, cell viability at 24, 48 or 72 h was evaluated using a microplate reader at 450 nm.

5-ethynyl-2'-deoxyuridine (EdU) staining. Transfected cells ($5x10^4$ cells/well) were inoculated into 96-well plates and incubated with 20 μM EdU (Thermo Fisher Scientific, Inc.) at 37°C for 2 h. Subsequently, cells were fixed and permeated using 4% paraformaldehyde for 30 min and 0.5% Triton X-100 for 10 min at room temperature, respectively. The cells were stained with Cell-Light™ EdU Apollo® 488 *In Vitro* Imaging kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Cells were observed using a fluorescence microscope (magnification, x200; Nikon Corporation).

Flow cytometry. The harvested cells were fixed overnight with 75% ethanol at 4°C and subsequently cultured with propidium iodide (PI)/RNase staining buffer at 37°C for 30 min in the
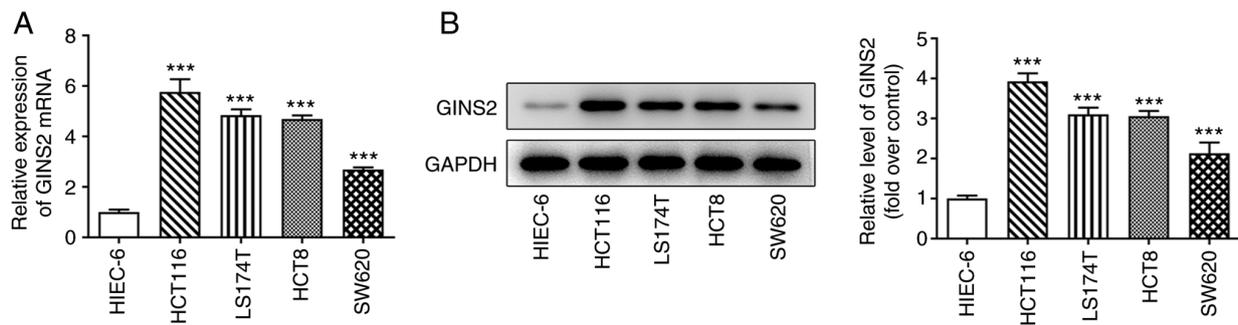
Figure 1. GINS2 is overexpressed in colon cancer cells. (A) GINS2 mRNA expression levels in colon cancer cells were detected using reverse transcription-quantitative PCR. (B) GINS2 protein expression levels in colon cancer cells were detected using western blotting analysis. ***P<0.001 vs. HIEC-6. GINS2, GINS complex subunit 2.

dark. The cell cycle analysis was performed using a BD FACSCalibur flow cytometer (BD Biosciences). The distribution of cell cycle, that is, the cell percentages at the G_0/G_1, S and G_2/M phases, were quantified using FlowJo software (version 7.0; FlowJo LLC).

TUNEL staining. Cell apoptosis was determined using the TUNEL Apoptosis Assay kit (Beyotime Institute of Biotechnology). Briefly, fixation and permeabilization of HCT116 cells were performed using 4% paraformaldehyde for 20 min and 0.1% Triton X-100 for 10 min at room temperature, respectively. Subsequently, cells were incubated with the TUNEL reaction solution at 37°C for 1 h in the dark and the nuclei were stained with DAPI (5 μg/ml) for 5 min at room temperature. A total of five visual fields were randomly selected and apoptotic cells were observed on glass coverslips under a fluorescence microscope (magnification, x200; Nikon Corporation).

Co-immunoprecipitation (Co-IP) assay. The interaction between GINS2 and PTP4A1 was determined using a Co-IP assay. Briefly, the harvested cells were fully lysed using 1 ml Co-RIPA buffer (Applygen Technologies, Inc.) and then lysates were pre-cleared with 30 μl Protein A/G PLUS-Agarose (Santa Cruz Biotechnology, Inc.) at 4°C for 1 h. After centrifuging the lysate, taking the supernatant and removing the beads in the supernatant, immunoprecipitation with antibodies against GINS2 (1:50; cat. no. sc-376595; Santa Cruz Biotechnology, Inc.), PTP4A1 (1:30; cat. no. sc-365659; Santa Cruz Biotechnology, Inc.) and IgG (1:50; cat. no. sc-69786; Santa Cruz Biotechnology, Inc.) was performed with shaking at 4°C overnight. The protein A/G-Sepharose beads were added to the samples and shaken at 4°C for 4 h. Following centrifugation at 800 x g for 5 min at 4°C, the supernatant was removed and the beads were washed three times (800 μl/time) with pre-cooled Co-RIPA buffer to obtain protein samples for western blotting analysis.

Statistical analysis. All experiments were performed in triplicate. All data are presented as the mean ± standard deviation and statistical analysis was performed using SPSS 20.0 (IBM Corp.). Unpaired Student's t-tests were performed to compare the differences between two groups and one-way ANOVA

followed by Tukey's post hoc test was used for multiple comparisons. P<0.05 was considered to indicate a statistically significant difference.

Results

GINS2 is overexpressed in colon cancer cell lines. The expression levels of GINS2 were measured in all colon cancer cells. As depicted in Fig. 1A and B, compared with HIEC-6, the GINS2 mRNA and protein expression levels were increased in colon cancer cell lines and the levels were highest in HCT116 cells. Subsequently, the HCT116 cell line was selected for the following experiments.

GINS2 knockdown inhibits the proliferation of HCT116 cells. HCT116 cells were transfected with shRNA-GINS2 plasmids for GINS2 knockdown and this was subsequently detected using RT-qPCR and western blotting. The results displayed in Fig. 2A and B demonstrated that the mRNA and protein expression levels of GINS2 were significantly reduced in shRNA-GINS2-1 and shRNA-GINS2-2 cells compared with shRNA-NC. GINS2 expression levels were lowest in HCT116 cells transfected with the shRNA-GINS2-1 plasmid. Thus, the shRNA-GINS2-1 plasmid was used for subsequent experiments. The results of the CCK-8 assay demonstrated that, compared with cells transfected with shRNA-NC, the viability of HCT116 cells was markedly reduced following GINS2 knockdown (Fig. 2C). Similarly, the results of the EdU staining assay demonstrated that transfection with the shRNA-GINS2 plasmid notably inhibited the levels of cell proliferation, compared with HCT116 cells transfected with shRNA-NC (Fig. 2D). In summary, GINS2 promoted colon cancer cell proliferation.

GINS2 knockdown promotes HCT116 cell cycle arrest. The cell cycle was evaluated using flow cytometry. As demonstrated in Fig. 3A, the number of cells in the G_0/G_1 phase was increased, while those in S phase and G_2 phase were decreased following GINS2 knockdown, compared with cells transfected with shRNA-NC. In addition, western blotting was performed to determine the expression levels of cell cycle-associated proteins and the results demonstrated that shRNA-GINS2 reduced the levels of cyclin D1 expression, while p21 expression was increased, compared with cells transfected with
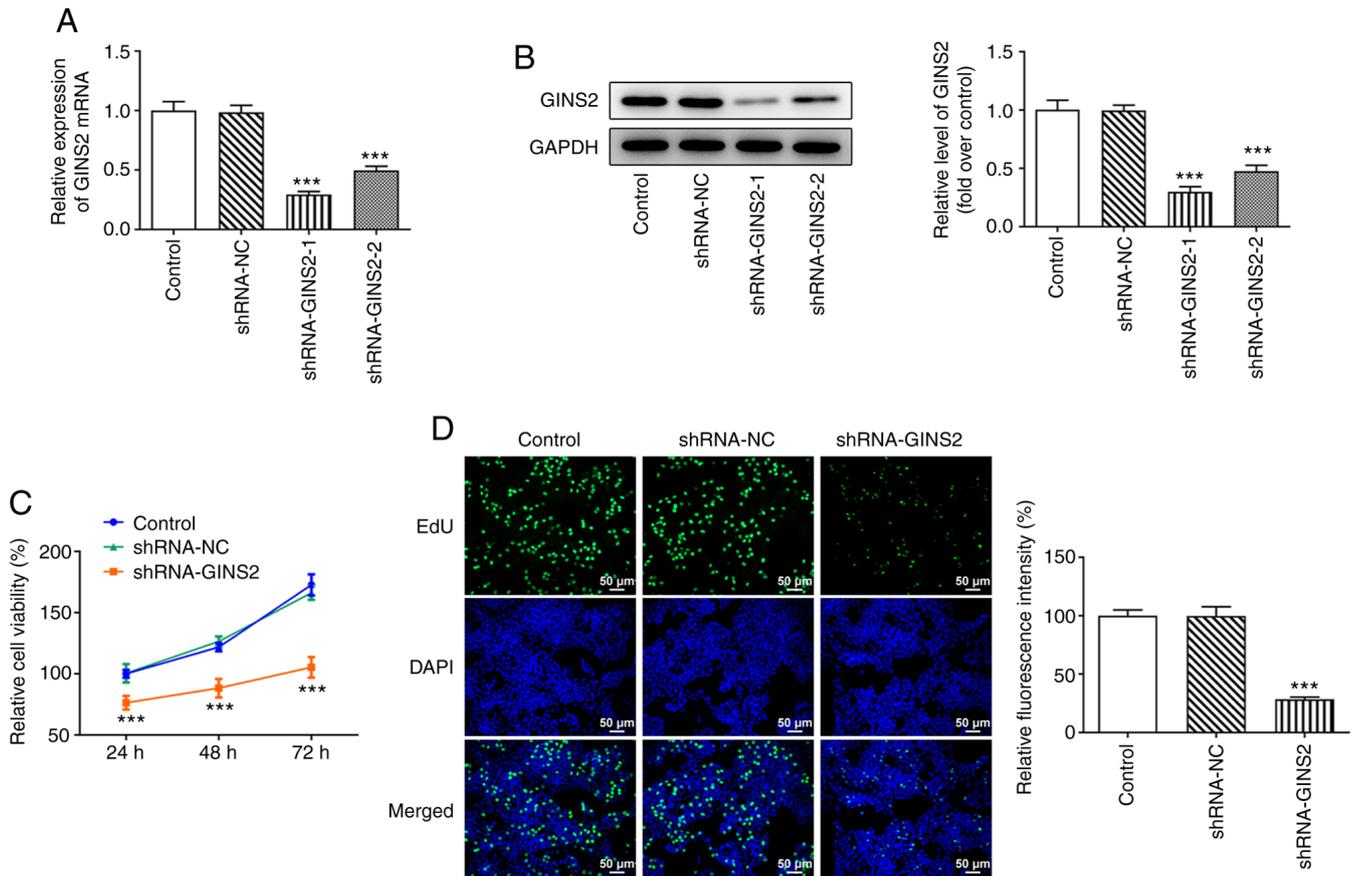
Figure 2. GINS2 knockdown inhibits the proliferation of HCT116 cells. GINS2 (A) mRNA and (B) protein levels in GINS2-knockdown HCT116 cells were detected using reverse transcription-quantitative PCR and western blotting analyses, respectively. (C) Cell Counting Kit-8 assay was performed to detect the viability of GINS2-knockdown HCT116 cells. (D) EdU staining was performed to detect the proliferation of GINS2-knockdown HCT116 cells. ***P<0.001 vs. shRNA-NC. GINS2, GINS complex subunit 2; shRNA, short hairpin RNA; NC, negative control.

shRNA-NC (Fig. 3B). Collectively, these results demonstrated that GINS2 promoted colon cancer cell cycle progression.

GINS2 knockdown promotes colon cancer cell apoptosis. TUNEL staining was performed to determine the effects of GINS2 on HCT116 cell apoptosis. As demonstrated in Fig. 4A, cell apoptosis was markedly increased following transfection with the shRNA-GINS2 plasmids. In addition, the levels of apoptosis-associated proteins, such as Bcl2, Bax and cleaved PARP, were measured using western blotting. The results of the present study demonstrated that the expression levels of Bcl2 were notably reduced following GINS2 knockdown, but the expression levels of Bax and cleaved PARP/PARP were increased, compared with cells transfected with shRNA-NC (Fig. 4B).

GINS2 knockdown activates the p53 pathway through PTP4A1. To determine the expression levels of PTP4A1 in colon cancer cells, RT-qPCR was performed. The results demonstrated that the mRNA expression levels of PTP4A1 were increased in HCT116, LS174T, HCT8 and SW620 cells, compared with the with HIEC-6 cells (Fig. 5A). In addition, a Co-IP assay was performed to verify the targeted binding of GINS2 and PTP4A1. The results of Co-IP demonstrated that GINS2 could interact with PTP4A1 (Fig. 5B). In addition, compared with shRNA-NC, shRNA-GINS2 reduced

PTP4A1 expression level and increased p53 expression level (Fig. 5C).

Following transfection with Ov-PTP4A1 plasmid, the mRNA and protein levels of PTP4A1 were detected using RT-qPCR and western blotting, respectively. Compared with cells transfected with Ov-NC, PTP4A1 expression levels were increased in Ov-PTP4A1 (Fig. 5D). In addition, the results displayed in Fig. 5E demonstrated that shRNA-GINS2 upregulated the expression levels of p53, which were subsequently reversed following transfection with the Ov-PTP4A1 plasmid.

GINS2 knockdown regulates cell proliferation, cycle arrest and apoptosis in colon cancer cells through PTP4A1/p53 pathway. As displayed in Fig. 6A and B, the shRNA-GINS2-mediated decrease in cell proliferation was increased following PTP4A1 overexpression. In addition, shRNA-GINS2 markedly decreased the number of cells in the G_0/G_1 phase and decreased the number of cells in the S phase and G_2 phase. Notably, this effect was inhibited following PTP4A1 overexpression (Fig. 6C), which highlighted that the cell cycle arrest induced by shRNA-GINS2 was subsequently restored following PTP4A1 overexpression. The results of the TUNEL assay revealed that apoptosis was increased in shRNA-GINS2; however, this effect was inhibited following transfection with Ov-PTP4A1 (Fig. 6D). Furthermore, the western blotting analysis results demonstrated that the shRNA-GINS2-mediated
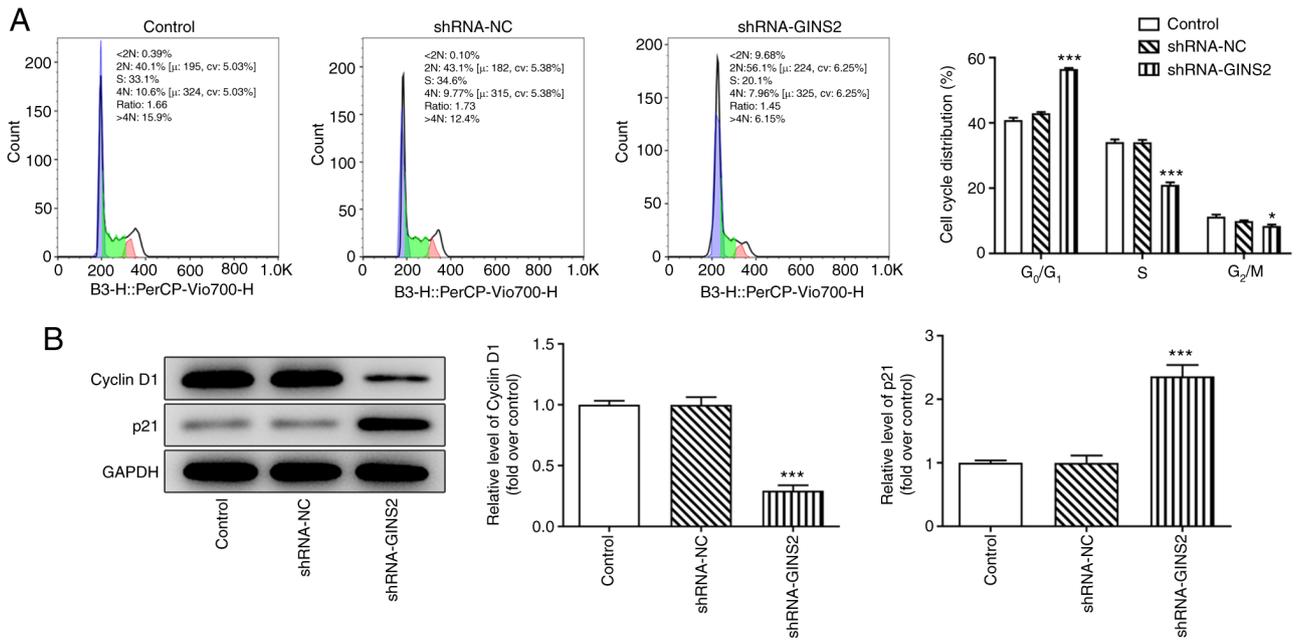
Figure 3. GINS2 knockdown promotes cell cycle arrest in colon cancer. (A) Flow cytometry analysis was performed to examine the cell cycle distribution of GINS2-knockdown HCT116 cells. (B) Western blotting was performed to detect the protein expression levels of p21 and cyclin D1. $^*P<0.05$, $^{***}P<0.001$ vs. shRNA-NC. GINS2, GINS complex subunit 2; shRNA, short hairpin RNA; NC, negative control.
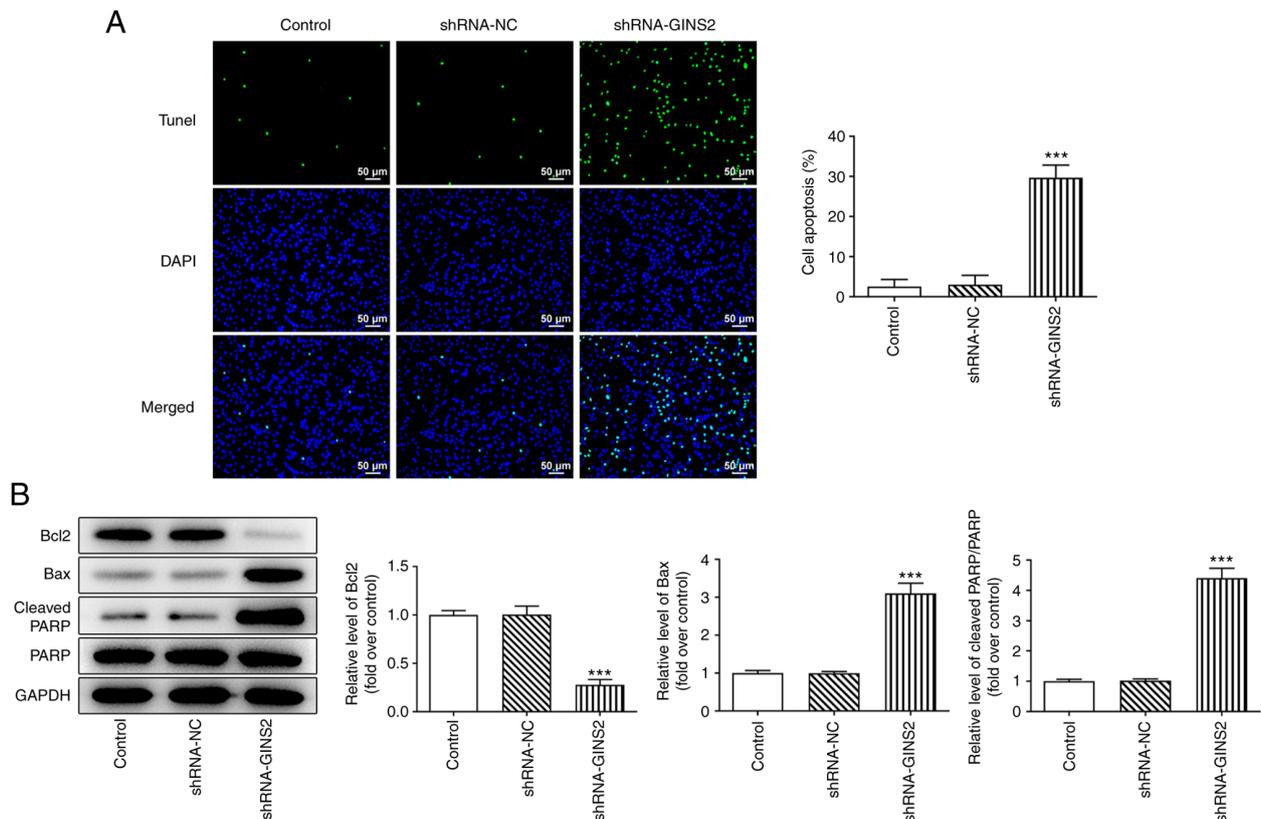

Figure 4. GINS2 knockdown promotes the apoptosis of colon cancer cells. (A) TUNEL staining was performed to detect the apoptosis of GINS2-knockdown HCT116 cells. (B) Western blotting was performed to detect the protein expression levels of Bcl2, Bax, cleaved PARP and PARP. $^{***}P<0.001$ vs. shRNA-NC. GINS2, GINS complex subunit 2; shRNA, short hairpin RNA; NC, negative control; PARP, poly ADP-ribose polymerase.

reduction in the protein expression levels of cyclin D1 and Bcl2 and the increased protein expression levels of p21, Bax, cleaved PARP/PARP, were rescued following PTP4A1 overexpression (Fig. 6E and F). These results indicated that GINS2 modulated colon cancer cell proliferation, cycle arrest and apoptosis through the PTP4A1/p53 pathway (Fig. 6G).
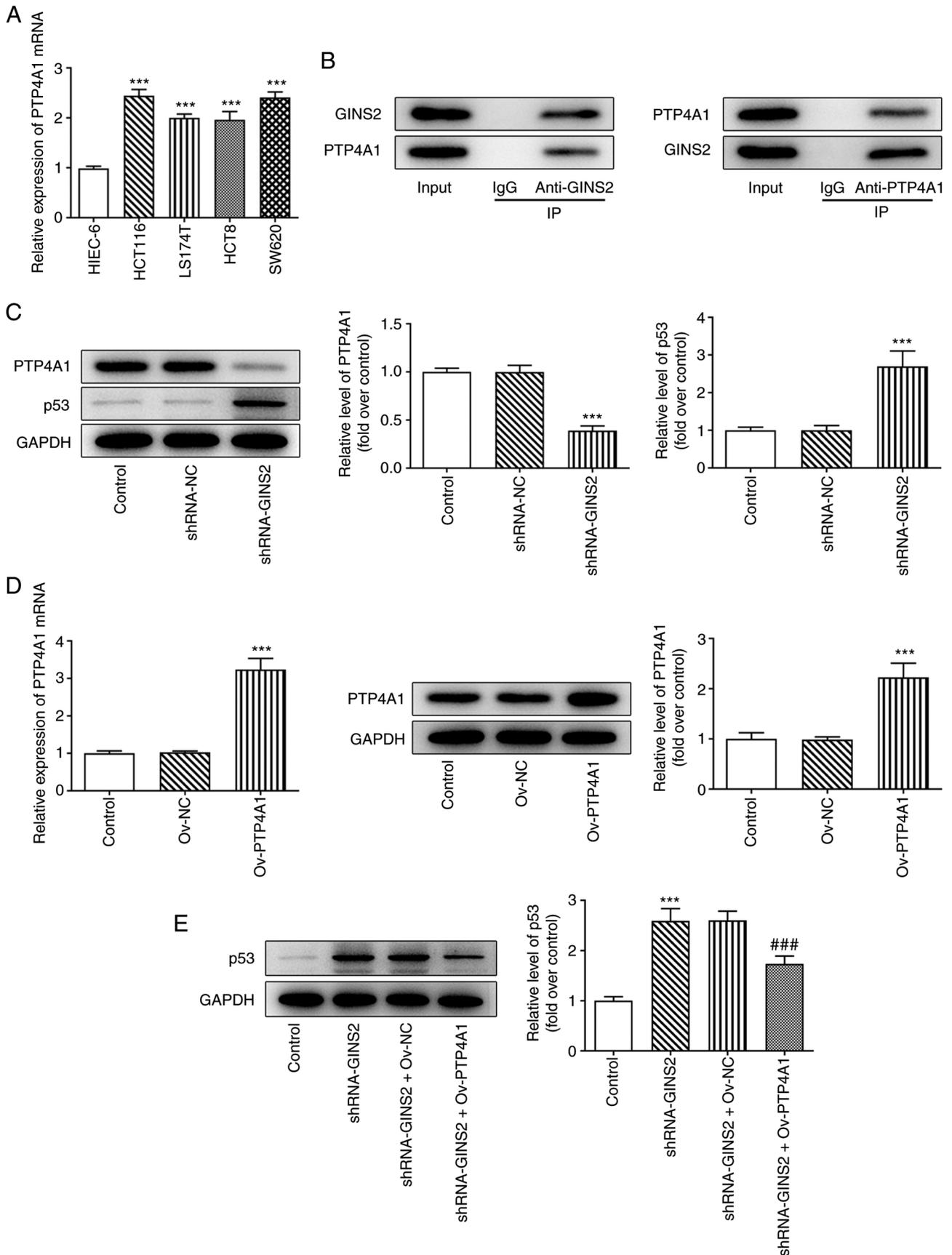
Figure 5. GINS2 knockdown activates the p53 pathway through PTP4A1. (A) RT-qPCR was performed to detect the PTP4A1 mRNA expression level in colon cancer cells. ***P<0.001 vs. HIEC-6. (B) The binding of GINS2 and PTP4A1 was detected using a co-immunoprecipitation assay. (C) mRNA and protein expression levels of PTP4A1 and p53 were detected using RT-qPCR and western blotting, respectively. ***P<0.001 vs. shRNA-NC. (D) PTP4A1 mRNA and protein expression levels were detected using RT-qPCR and western blotting, respectively. ***P<0.001 vs. Ov-NC. (E) p53 protein expression levels were detected using western blotting analysis. ***P<0.001 vs. Control; ###P<0.001 vs. shRNA-GINS2 + Ov-NC. GINS2, GINS complex subunit 2; PTP4A1, protein tyrosine phosphatase 4A1; RT-qPCR, reverse transcription-quantitative PCR; shRNA, short hairpin RNA; NC, negative control; Ov, overexpression.
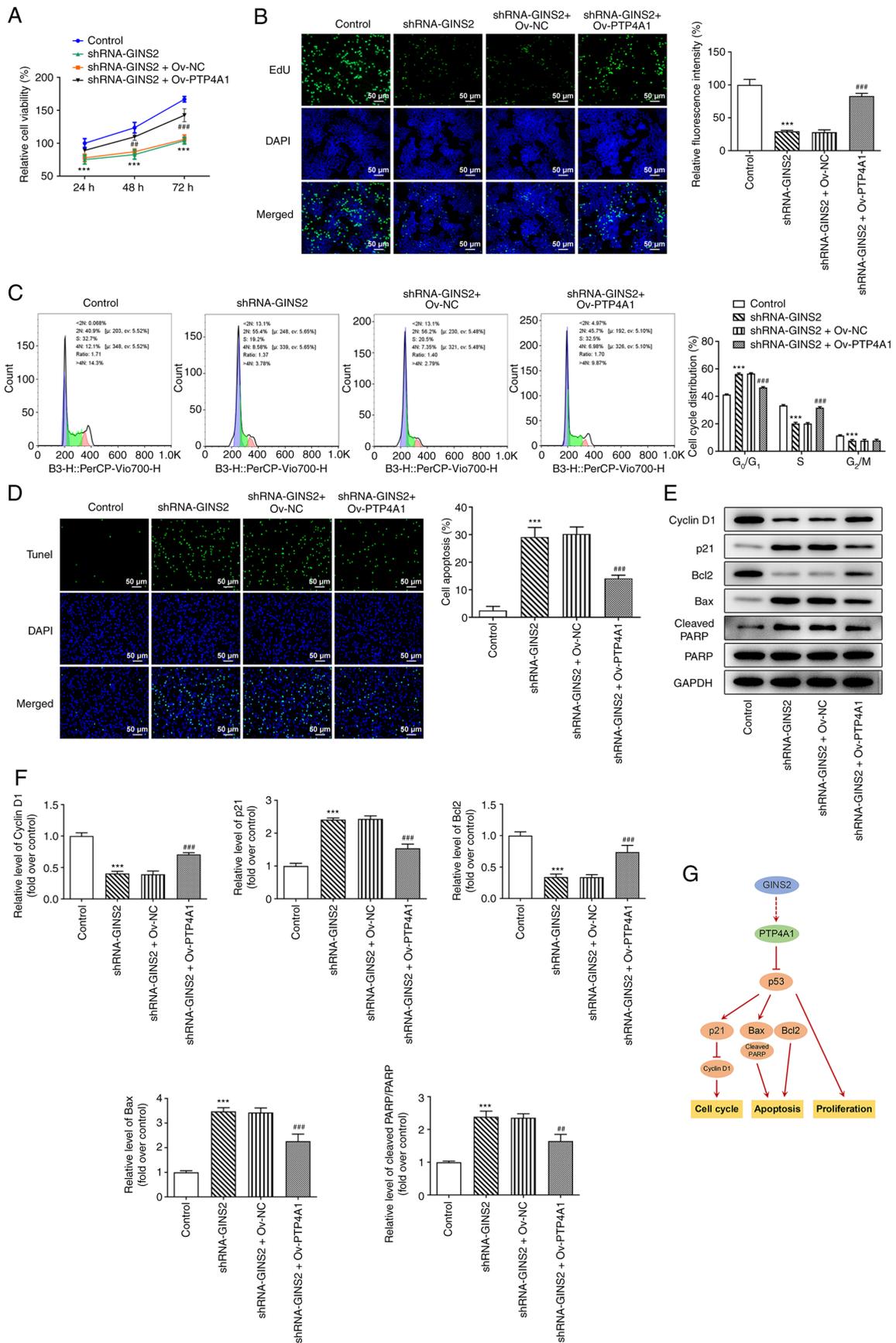
Figure 6. GINS2 knockdown regulates the proliferation, cycle arrest and apoptosis through the PTP4A1/p53 pathway. (A) Cell viability was detected using a Cell Counting Kit-8 assay. (B) Cell proliferation was detected using EdU staining. (C) Flow cytometry was performed to examine cell cycle distribution. (D) Cell apoptosis was detected using a TUNEL assay. (E) Western blotting analyses were performed and (F) quantified to detect the protein expression levels of cyclin D1, p21, Bcl2, Bax, cleaved PARP and PARP. (G) Schematic depiction of regulatory mechanism underlying colon cancer proliferation, cycle arrest and apoptosis via GINS2/PTP4A1/p53 pathway. $^{***}P<0.001$ vs. Control; $^{##}P<0.01$, $^{###}P<0.001$ vs. shRNA-GINS2 + Ov-NC. GINS2, GINS complex subunit 2; PTP4A1, protein tyrosine phosphatase 4A1; shRNA, short hairpin RNA; NC, negative control; Ov, overexpression; PARP, poly (ADP-ribose) polymerase.

Discussion

Novel indicators for determining the prognosis of patients with colon cancer are required for the development of effective treatment options (1). GINS2 has been identified as a crucial regulator in cell cycle progression (23). It has also been reported that GINS2 promotes cell proliferation and apoptosis desensitization (24). The present results demonstrated that GINS2 knockdown inhibited the proliferation and induced cell cycle arrest and apoptosis in colon cancer. GINS2 may serve a key role in colon cancer.

The cell cycle is a complex process that provides the tumor cell with the opportunity to repair its damaged DNA (25). This process is regulated by numerous protein families, including cyclin-dependent kinases (CDKs), which drive cell cycle progression (26,27). Cyclin D1, in association with CDK4/6, functions as an important regulator of the cell cycle (28). As a CDK inhibitor, p21 mainly serves a suppressive role in cell cycle progression (29). Apoptosis, also known as programmed cell death, is a physiological process that occurs in multicellular organisms (30). Chen *et al* (31) demonstrate that apoptosis acts as a critical participant in numerous biological processes, including tissue development and organ formation. Downregulation of GINS2 induces cell cycle arrest and apoptosis in lung cancer A549 cells (32). Silencing of GINS2, which is overexpressed in melanoma, inhibits cell proliferation and increases apoptosis in A375 cells (33). GINS2 interference induces cell cycle arrest and apoptosis of pancreatic cancer via the MAPK/ERK pathway (34). The results of the present study demonstrated that GINS2 knockdown induced cell cycle arrest and promoted apoptosis in colon cancer. In addition, the results of the present study also demonstrated that GINS2 knockdown reduced the expression levels of the anti-apoptotic protein Bcl2, but increased the expression levels of the pro-apoptotic proteins Bax and cleaved PARP.

PTP4A1 expression is increased in numerous cancers, including intrahepatic cholangiocarcinoma (18), non-small cell lung cancer (35) and cervical cancer (36). According to the Biogrid database, GINS2 has the ability to interact with PTP4A1, which was subsequently confirmed using a Co-IP assay. In addition, PTP4A1 expression levels were increased in colon cancer cells and the increased levels of PTP4A1 expression were decreased following transfection with the shRNA-GINS2 plasmids.

p53, a tumor suppressor, is described as a co-immunoprecipitating protein by Kress *et al* (37). Through the transcription regulation of downstream target genes involved in cell cycle arrest, apoptosis, DNA repair and metabolism, p53 exerts multiple biological functions in human diseases (38,39). In addition, p53 influences the sensitivity of colon cancer cells to bleomycin (40). Mutant p53 is shown to promote angiogenesis in colon cancer, leading to a poor prognosis (41). As a regulatory target gene of p53, PTP4A1 activates p53 expression (21). The results of the present study demonstrated that GINS2 knockdown led to the activation of the p53 pathway through PTP4A1. Additionally, rescue experiments confirmed that the GINS2 knockdown-mediated suppression in cell proliferation and the increased levels of cell cycle arrest and apoptosis in colon cancer cells, were restored following PTP4A1 overexpression. These results highlighted that GINS2 knockdown exerted its regulatory effects on the proliferation, cycle arrest and apoptosis of colon cancer cells via regulation of the PTP4A1/p53 pathway. However, a key limitation of the present study was the lack of detection of GINS2 and PTP4A1 expression in clinical samples. Therefore, future studies should validate the results of the present study by performing some *in-vivo* experiments.

In conclusion, it was demonstrated that GINS2 regulated the proliferation, cell cycle progression and apoptosis of colon cancer cells through PTP4A1, indicating that GINS2 may serve as a biomarker for the development of novel therapies for colon cancer.

Availability of data and materials

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

Authors' contributions

ZL designed the study and supervised the project. HH and LY performed the experiments and analyzed data. HH drafted the manuscript. All authors participated in the revision of the manuscript. ZL and HH confirm the authenticity of all the raw data. 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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