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ORIGINAL RESEARCH

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GINS2 regulates epithelial mesenchymal transformation and cell cycle in endometrial carcinoma by stimulating ERK/MAPK signaling

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Abstract

Endometrial cancer (EC) is one of the three main gynecological cancers. Identifying new therapeutic targets and further elucidating the molecular mechanisms of EC tumorigenesis have important implications for women's health. The Go-Ichi-Ni-San (GINS) family, which includes four subunits (GINS1–4), has specific functions in DNA replication and cell cycle. The Cancer Genome Atlas (TCGA) data showed that GINS2 transcription level is upregulated in endometrial cancer tissue. However, the possible role of GINS2 in EC progression is still unknown. Herein, we explored the role of GINS2 in EC. We noticed that GINS2 was overexpressed in EC cells. GINS2 knockdown suppressed the proliferation of EC cells and induced cell cycle arrest. We further noticed that GINS2 knockdown restrained the Epithelial mesenchymal transformation (EMT) of EC cells. Mechanically, its downregulation suppressed the extracellular regulated protein kinase (ERK)/Microtubule-Associated Protein Kinase (MAPK) pathway, thereby suppressing EC progression. Thus, GINS2 has the potential to act as a therapeutic target for EC.

Keywords

Endometrial cancer (EC); GINS2; Cell cycle; EMT; ERK/MAPK pathway

1. Introduction

Endometrial cancer (EC) is one of the three main gynecological cancers [1]. Its incidence is on the rise and tends to be younger due to the increased obesity and decreased physical activity [2, 3]. Since most endometrial cancers are adenocarcinoma, they are not very sensitive to radiotherapy, so surgery is the main treatment and radiotherapy, chemotherapy and other comprehensive treatments are also used. Compared to other cancers, EC has a five-year total survival rate of more than 80% and has received less public attention. However, advanced and relapsed disease is difficult to treat, and the prognosis for these patients is poor, with an estimated survival of less than 1 year [4]. Therefore, identifying new therapeutic targets and further elucidating the molecular mechanisms of EC tumorigenesis and progression may be of great importance for women's health.

The Go-Ichi-Ni-San (GINS) family, which includes four subunits (GINS1–4), has specific functions in DNA replication and cell cycle, and plays a crucial role in chromosome development [5]. GINS2 is involved in oncogenesis of a variety of cancers [6, 7]. For example, GINS2 promotes EMT in pancreatic cancer by stimulating ERK/MAPK axis [6]. Knockdown of GINS2 restrains lung cancer cell growth and induces cycle arrest by inhibiting signal transducer and activator of transcription (STAT) signaling pathway [7]. Recent studies showed GINS2 protein is upregulated in endometrial tissues, and its high expression is correlated with more advanced pathological T staging, lymphatic infiltration and poor clinical outcome [8, 9]. In addition, TCGA data showed that GINS2 transcription level is upregulated in endometrial cancer tissue, indicating that GINS2 may be related to the progression of endometrial cancer. Therefore, its function needs further study.

This study aimed to investigate the role of GINS2 in EC. Our findings showed that GINS2 regulates EMT and cell cycle of EC by ERK/MAPK axis.

2. Materials and methods

2.1 Antibodies and plasmids

Anti-GINS2 (1:200 for Immunohistochemistry, IHC, 1:500 for Immunoblot, ab197123, abcam), anti-Vimentin (1:500, ab92547), anti-E-cadherin (1:500, ab231303), anti-p-ERK1 (1:1000, ab131438), anti-ERK1 (1:1000, ab32537), anti-p-c-Jun N-terminal kinase (JNK) 1 (1:500, ab215208), anti-JNK1 (1:500, ab110724) anti-p-p38 (1:1000, ab178867) anti-p38 (1:1000, ab170099), anti- glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1:3000, ab8245) were purchased from indicated companies.

The plasmids of pc-GINS2 were constructed in our lab. The

GINS2 siRNAs were bought from Riobio (Guangzhou, China).

2.2 Immunohistochemistry

EC tissues were collected from the patients receiving surgical resection in our hospital. The sections were deparaffinized by xylene as well as rehydrated by ethanol series. Sections were then fixed with 4% paraformaldehyde (PFA) at 25 °C for 30 min and 2% bovine serum albumin (BSA, ST023, Beyotime, Beijing, China) for 30 min. Sections were then incubated with antibody for 2 h. Sections were incubated with biotinylated secondary antibody (1:200; NB7158, Novus Biologicals, Littleton, CO, USA) for another 1 h.

2.3 Cell culture and transfection

The human endometrial epithelial cell line Endometrial Epithelial Cells (EEC), and 4 types of EC cell lines, including Human Endometrial Adenocarcinoma 1B (HEC1B), RL-952, Ishikawa and KLE cells, were all bought from American Type Culture Collection (ATCC).

2.4 Immunoblot assay

Radio Immunoprecipitation Assay (RIPA) lysate was added to fully lysate cells to extract protein. Samples were separated by Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis (SDS-PAGE), and further transferred onto the polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% milk for 1 h, and then the corresponding primary antibodies were added. All membranes were subsequently treated with primary antibodies for 1.5 h, and subsequently incubated with secondary antibodies for another 1 h.

2.5 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-diphenytetrazoliumromide (MTT) assay

1×10^3 EC cells per plate were plated into 96-well plates and maintained for 48 h. Cells were incubated with the MTT for 4 h. Then the cells were dissolved with 150 μL Dimethyl sulfoxide (DMSO) and optical density (OD) 90 value was measured by using a microplate reader (8.0, BD Biosciences, Inc., Franklin Lake, NJ, USA).

2.6 Colony formation assay

Cells were plated into the 6-well plates (1000 cell per well) and maintained in media (10% Fetal bovine serum, FBS) for 14 days at 37 °C. Then cells were fixed with PFA for 20 min and 0.1% crystal violet for 20 min.

2.7 Transwell assay

The cells were allowed to migrate into the transwell for 24 h. The invaded cells on the upper chamber were fixed, stained with 2% crystal violet and images were captured.

2.8 Wound-healing assay

A 10-μL pipette tip was used to create a scratch. Images were captured at 0 and 24 h to determine the width of wound closure. The width of wound closure was the percentage of the healed area divided by the total scratch area.

2.9 Flow cytometry analysis

The cells were washed with PBS. Subsequently cells were fixed using 70% ethanol at −20 °C for 2 h. Subsequently stained with PI at 4 °C and the cells at different phases were measured using FACSCalibur flow cytometer (8.0, BD Biosciences, Inc., Franklin Lake, NJ, USA) and CellQuest Pro 5.1 (BD Biosciences, Inc., Franklin Lake, NJ, USA).

2.10 Statistics

Data were analyzed using GraphPad 8.0 software (Graphpad, La Jolla, CA, USA). Error bars represent mean ± standard deviation (SD). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, siGINS2 *vs.* Si-Negative control (NC), ^$p < 0.05$, ^^$p < 0.01$, ^^^$p < 0.001$, pc-GINS2 *vs.* vector.

3. Results

3.1 GINS2 was overexpressed in human EC tissues as well as cells

To investigate the role of GINS2 in the progression of EC, we detected its expression levels in GeneExpression Profiling Interactive Analysis (GEPIA) database through bioinformation analysis. Interestingly, we noticed that the GEPIA database showed high expression of GINS2 in 174 tumor tissues (Fig. 1a). TCGA database also indicated the high transcript per million (TPM) of GINS2 in EC tissues (Fig. 1b). IHC assays were then performed to detect the protein expression of GINS2 in human EC tissues collected from patients. Consistently, the increased expression levels of GINS2 were detected in EC tissues (Fig. 1c). The protein levels of GINS2 were examined in human endometrial epithelial cell line EEC, and 4 types of EC cell lines, including HEC1B, RL-952, Ishikawa and KLE cells though Immunoblot assays. The results confirmed the high expression of GINS2 in EC cells (Fig. 1d). Therefore, GINS2 was highly expressed in human EC.

3.2 GINS2 promotes the proliferation of EC cells

The GINS2 plasmids pc-GINS2 and GINS2 siRNAs were transfected into EC cell lines, including HEC1B and Ishikawa cells. Through Immunoblot assays, the transfection of pc-GINS2 plasmids obviously increased its expression (Fig. 2a). Whereas siGINS2 transfection obviously downregulated the expression of GINS2 in HEC1B and Ishikawa cells (Fig. 2a). Through MTT assays, the overexpression of GINS2 induced the proliferation of HEC1B and Ishikawa cells (Fig. 2b), whereas its knockdown suppressed cell viability, with the decreased OD490 value (Fig. 2b). In addition, through colony formation assays, the overexpression of GINS2 increased the colony numbers of HEC1B and Ishikawa cells, whereas its knockdown decreased the colony numbers (Fig. 2c).

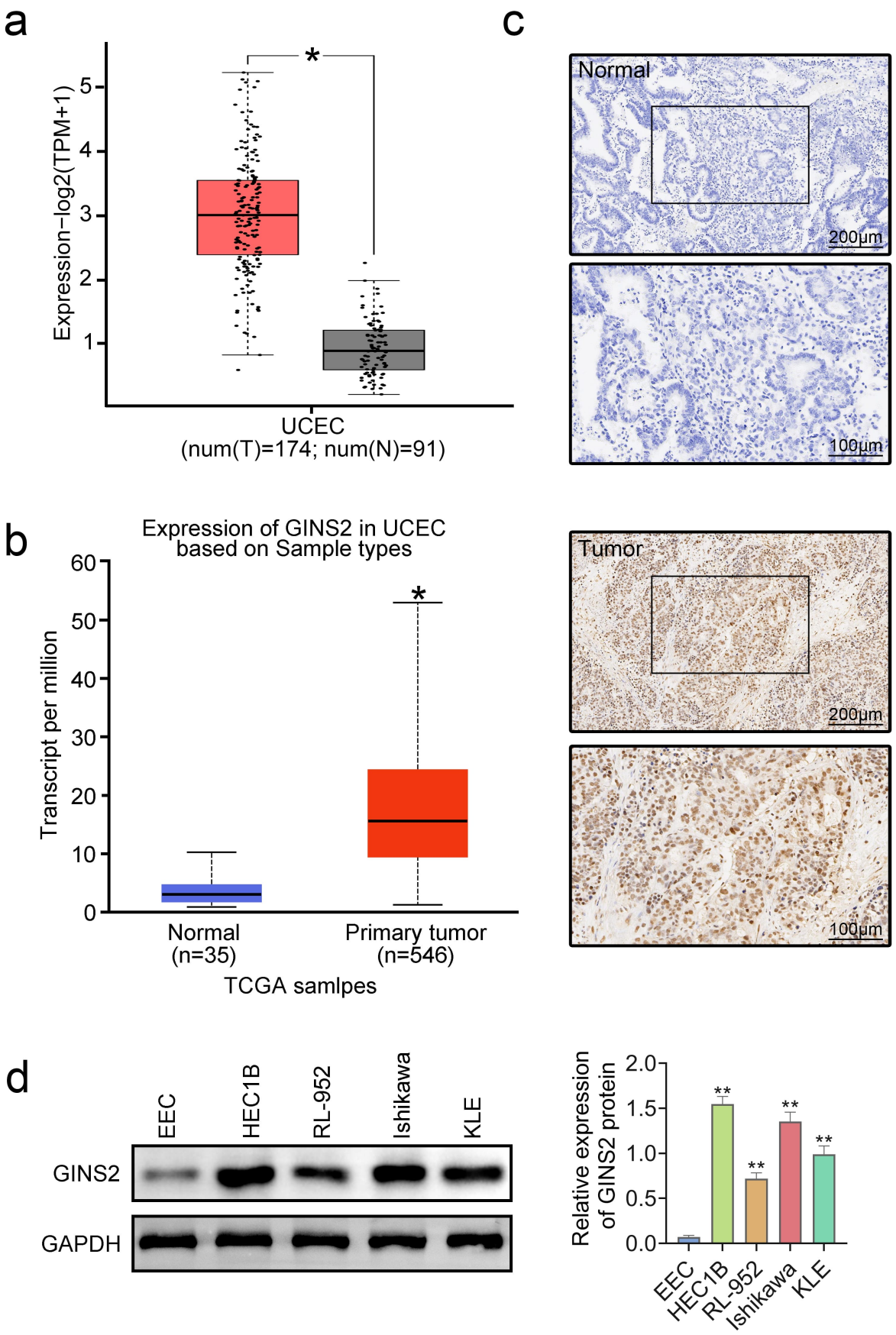

FIGURE 1. GINS2 was overexpressed in human EC tissues as well as cells. (a) The high levels of GINS2 in 174 tumor tissues. (b) The high transcript per million of GINS2 in EC tissues. (c) GINS2 expression in representative EC tissue and adjacent tissue. (d) GINS2 expression in EEC, HEC1B, RL-952, Ishikawa and KLE cells. Data are presented as mean ± SD, *$p < 0.05$, **$p < 0.01$. GINS2, Go-Ichi-Ni-San 2.

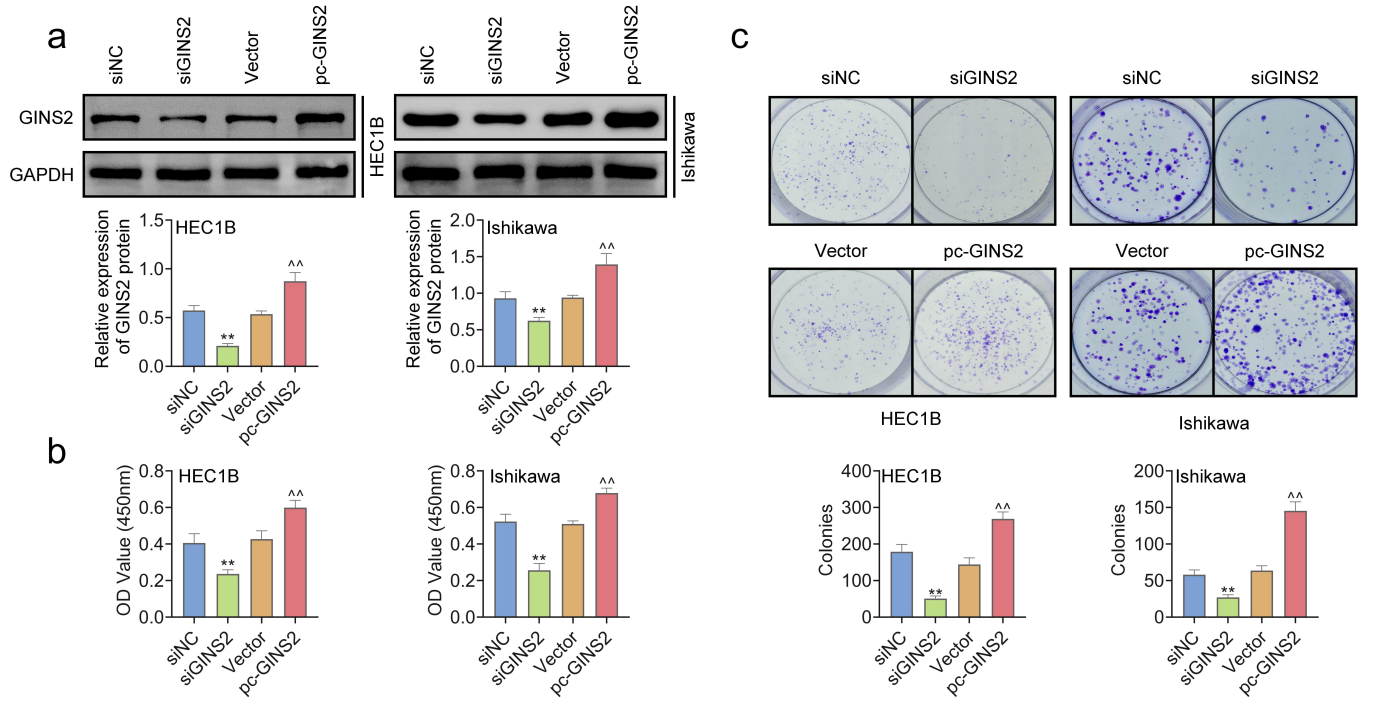

FIGURE 2. GINS2 promotes the proliferation of EC cells *in vitro*. (a) Immunoblot showed GINS2 levels in HEC1B as well as Ishikawa cells transfected with the indicated plasmids or siRNAs. (b) MTT assays showed the proliferation capacity HEC1B and Ishikawa cells, and the OD490 value was measured. (c) Colony formation assays showed the colony numbers. Data are presented as mean ± SD, $^{**}p < 0.01$, siGINS2 *vs.* siNC, $^{\wedge\wedge}p < 0.01$, pc-GINS2 *vs.* vector. NC, negative control; siNC, si Negative Control; MTT, 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di- phenytetrazoliumromide.

3.3 GINS2 mediates the cell cycle of EC cells

We then investigated whether GINS2 affected the cell cycle of EC cells through flow cytometry analysis. Interestingly, GINS2 overexpression increased the percentage of HEC1B and Ishikawa cells at S phase, whereas its knockdown decreased the percentage HEC1B and Ishikawa cells at S phase (Fig. 3).

3.4 GINS2 depletion suppressed the motility as well as EMT of EC cells

Through transwell assays, we noticed that the knockdown of GINS2 restrained EC cell invasion, with the decreased invasive cell numbers, whereas its overexpression promoted cell invasion of HEC1B and Ishikawa cells (Fig. 4a). Similarly, wound healing assays showed that downregulation of GINS2 inhibited the migration of HEC1B and Ishikawa cells, and its overexpression stimulated the migration of EC cells, with the decreased wound width (Fig. 4b). We found that GINS2 knockdown suppressed the expression of Vimentin and increased E-cadherin expression in EC cells, while GINS2 overexpression increased Vimentin expression and decreased E-cadherin (Fig. 4c).

3.5 GINS2 activates ERK/MAPK axis in EC cells

Through Immunoblot assays, GINS2 overexpression increased the phosphorylation of ERK, JNK and p38 in Ishikawa cells (Fig. 5). Additionally, knockdown of GINS2 suppressed the levels of ERK, JNK and p38 phosphorylation in Ishikawa cells (Fig. 5), further confirming the regulation of GINS2 on this pathway. Therefore, GINS2 activates ERK/MAPK axis in EC cells.

4. Discussion

EC is a malignant tumor originating from endometrial glands, and most of them are adenocarcinomas [10]. The incidence of EC is higher than cervical cancer and ranks as the first gynecological malignant tumor [1]. The high incidence age is 58–61 years old, accounting for 20%–30% of the malignant tumors of genital tract, and the incidence is increasing in recent years [2]. The risk factors of EC include long-term continuous estrogen stimulation, obesity, hypertension, diabetes, infertility or sterility, menopause and other physical factors and genetic factors [11]. For this disease, surgery is the main treatment, and radiotherapy is an important adjuvant therapy [12]. Targeted therapy is also an important option to improve prognosis [13]. In this study, through TCGA bioinformation analysis and Immunoblot, we noticed that GINS2 was overexpressed in EC cells. GINS2 contributed to the progression of EC.

Through MTT, colony formation and flow cytometry analysis, we noticed that GINS2 knockdown suppressed EC proliferation and stimulated cell cycle arrest. Further through wound healing, transwell assay and Immunoblot, we confirmed that GINS2 knockdown suppressed the motility and EMT of EC cells. These data confirmed the role of GINS2 in EC progression.

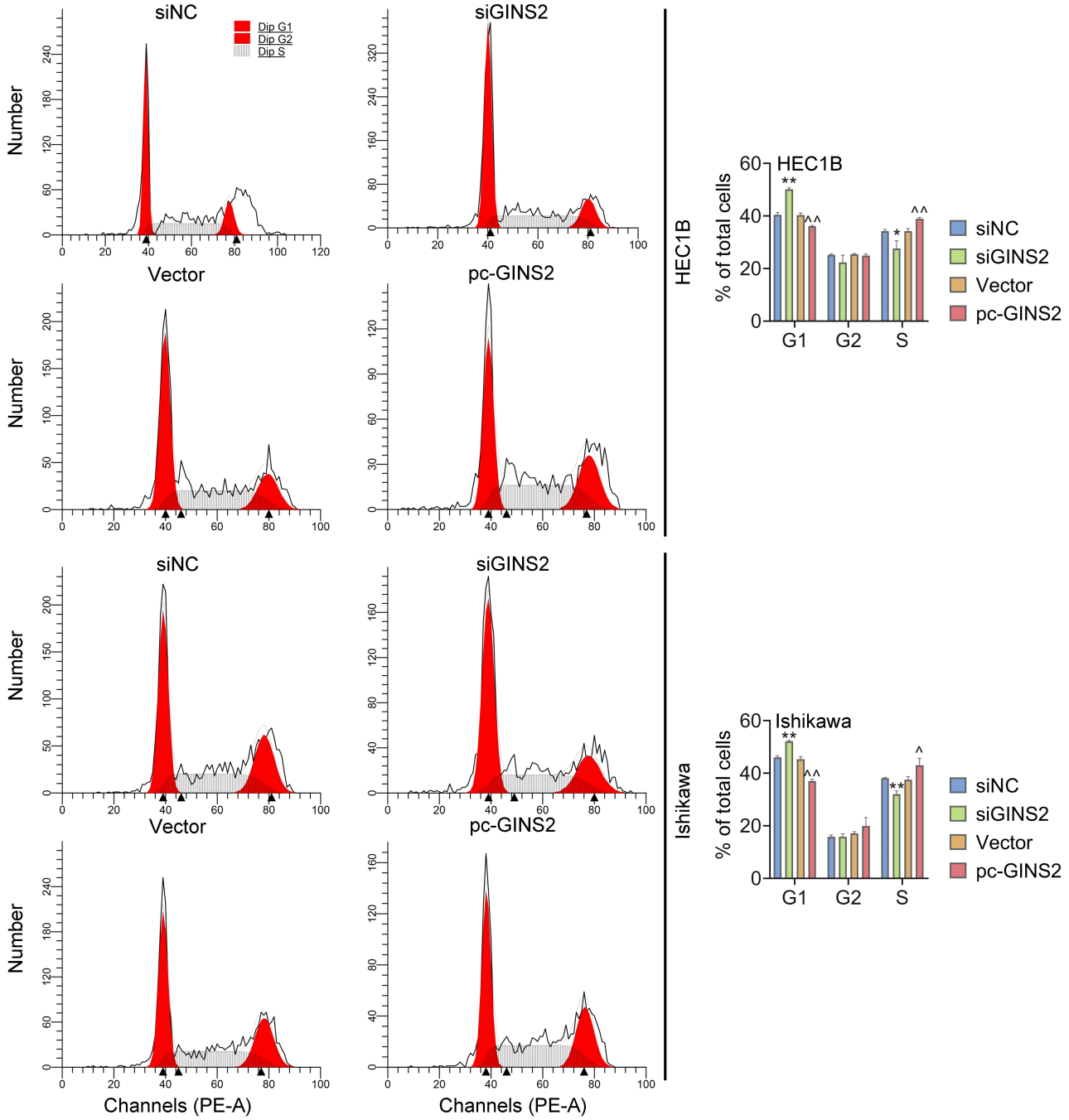

FIGURE 3. GINS2 mediates the cell cycle of EC cells. The cell cycle of HEC1B and Ishikawa cells. Data are presented as mean ± SD, *$p < 0.05$, **$p < 0.01$, siGINS2 *vs.* siNC, ^$p < 0.05$, ^^$p < 0.01$, pc-GINS2 *vs.* vector. NC, negative control; siNC, si negative Control; HEC1B, Human Endometrial Adenocarcinoma 1B; GINS2, Go-Ichi-Ni-San 2.

GINS2 is a subunit of GINS complex family that consists of GINS complex subunit 1 (PSF1), and GINS complex subunit 5 (SID5) [8]. The essence of Gins complex is a replicating helicase [14]. The GINS complex family is a regulatory gene necessary for cell growth and chromosome replication. GINS complex initiates the ring structure and is involved in DNA replication and cell cycle initiation by regulating multifunctional proteins, growth factor signaling and receptor molecules in eukaryotes [15]. GINS2 is highly expressed in many malignant solid tumors, which is closely related to the growth of cancer cells, and it affects a variety of biological characteristics by participating in the regulation of cell cycle [16–18]. Similarly, here we also confirmed the role of GINS2 in the proliferation and cell cycle of EC cells. The previous study also showed that GINS2 affected the apoptosis of tumor cells. Therefore, we next should confirm its effects on the apoptosis of EC cells.

Notably, previous study indicated that GINS2 promoted epithelial mesenchymal transition (EMT) in pancreatic cancer [6]. Similarly, we found that knockdown of GINS2 inhibited the activity of ERK/MAPK pathway in EC cells. The ERK/MAPK pathway is crucial in the development of various tumors [6]. There are three key target molecules in this pathway: small G protein Ras, and downstream Raf kinase,

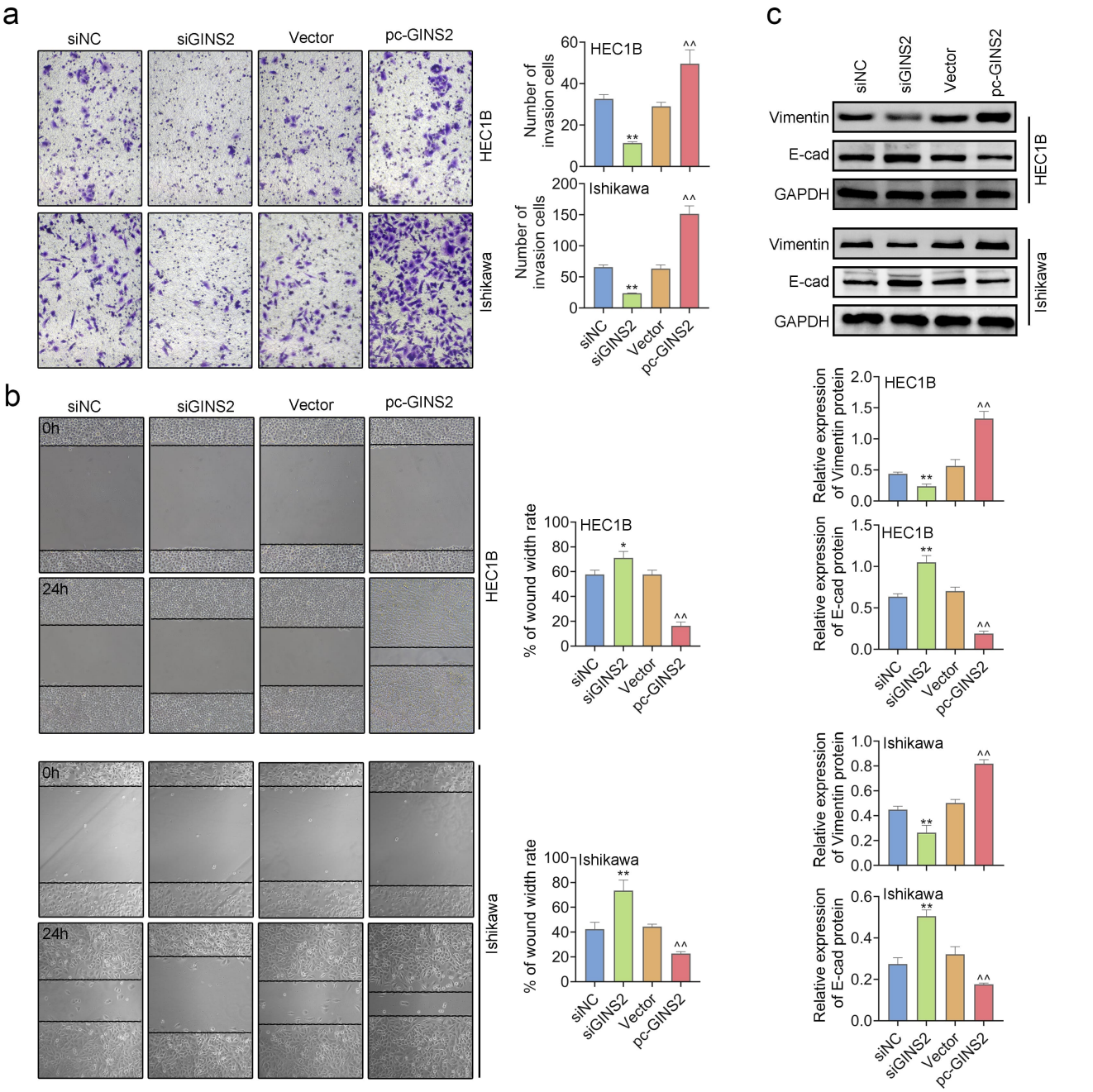

FIGURE 4. GINS2 depletion suppressed the motility as well as EMT of EC cells. (a) Transwell assays showed the effects of GINS2 on the invasion of HEC1B and Ishikawa cells. The invasive cell numbers were calculated. (b) Wound healing assays showed the effects of GINS2 on cell migration. The wound width rate was calculated. (c) Immunoblot showed the expression of Vimentin as well as E-cadherin in HEC1B and Ishikawa cells. Data are presented as mean ± SD, $*p < 0.05$, $**p < 0.01$, siGINS2 *vs.* siNC, $\hat{}\hat{}p < 0.01$, pc-GINS2 *vs.* vector. NC, negative control; siNC, si negative Control; GINS2, Go-Ichi-Ni-San 2; HEC1B, Human Endometrial Adenocarcinoma 1B.

Mitogen activates protein kinase (MEK) 1/2 and ERK1/2 [19]. Activation of the ERK/MAPK pathway will promote tumor progression and metastasis [19]. Our study showed that GINS2 influenced EC progression through this pathway, but the more detailed molecular mechanism remains to be further investigated. MAPK-ERK pathway abnormalities are present in several cancer types, including pancreatic, biliary, colorectal, lung, ovarian and endometrial cancers. The MAPK-ERK pathway is highly conserved in evolution. However, the precise regulation of the MARK pathway still needs study on downstream key proteins and targets, and validation in animals.

5. Conclusions

In conclusion, we found that knockdown of GINS2 inhibited the proliferation of endometrial cancer cells, induced cell cycle arrest and suppressed EMT *via* ERK/MAPK pathway.

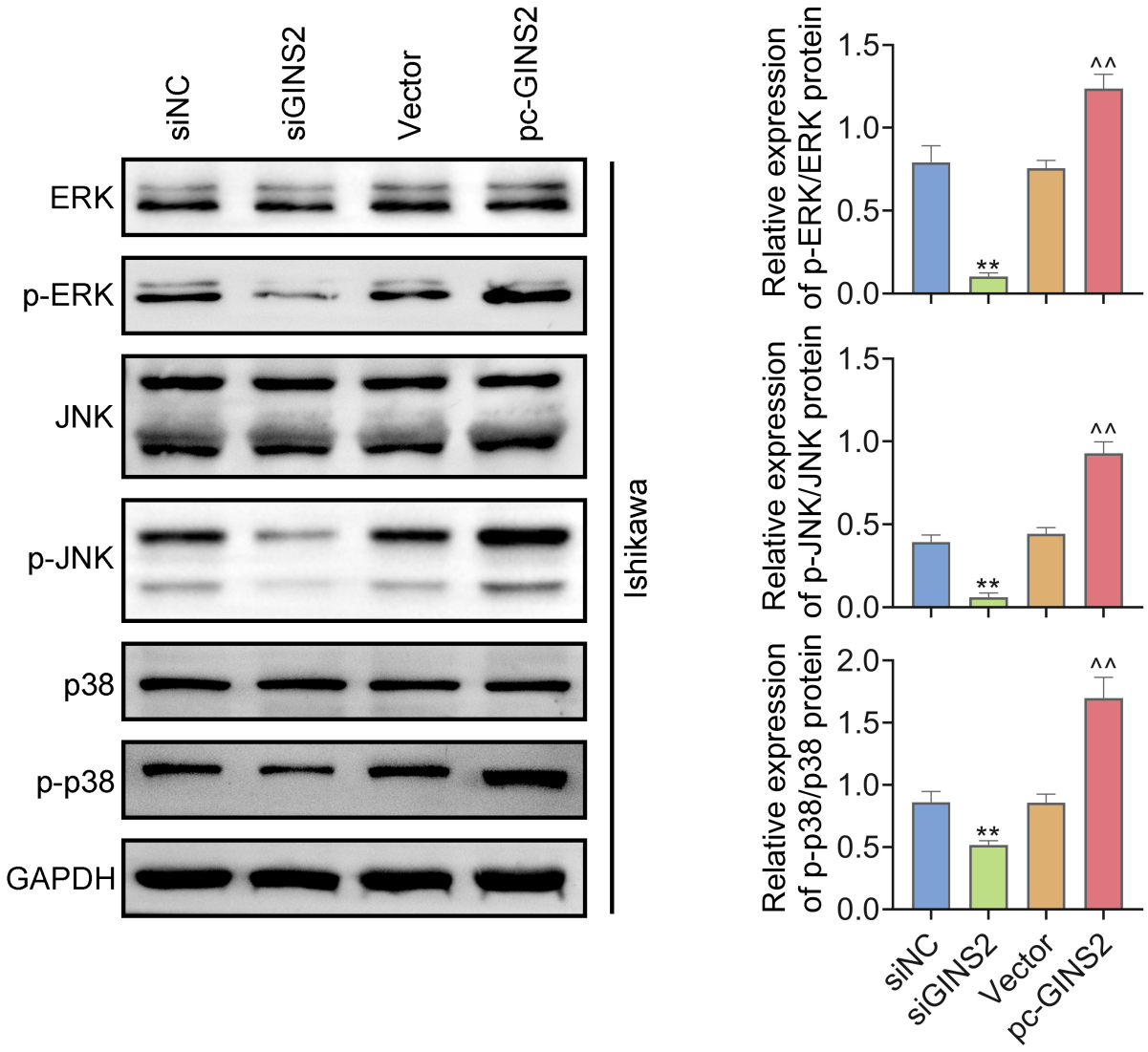

FIGURE 5. GINS2 activates ERK/MAPK signaling pathway in EC cells. The expression as well as phosphorylation levels of ERK, JNK and p38 in Ishikawa cells. Data are presented as mean ± SD, $^{**}p < 0.01$, siGINS2 *vs.* siNC, $^{\wedge\wedge}p < 0.01$, pc-GINS2 *vs.* vector. NC, negative control; siNC, si Negative Control; GINS2, Go-Ichi-Ni-San 2.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

MZ, WH, YLS—designed the study and carried them out, supervised the data collection, analyzed the data, interpreted the data, prepare the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies with human participants or animals performed by any of the authors.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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