SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 18: 6752-6758, 2019

YAP promotes gastric cancer cell survival and migration/invasion via the ERK/endoplasmic reticulum stress pathway

HAIBIN LIU[1], DONG MEI[2], PENGCHENG XU[3], HAISHENG WANG[3] and YAN WANG[1]

[1]Department of Gastrointestinal Surgery, China-Japan Friendship Hospital, Beijing 100029; [2]Department of Pharmacy, Beijing Children's Hospital, Capital Medical University, Beijing 100045; [3]Department of Pharmaceutical Engineering, College of Pharmacy, Inner Mongolia Medical University, Hohhot, Inner Mongolia 010110, P.R. China

Received June 1, 2019; Accepted October 3, 2019

DOI: 10.3892/ol.2019.11049

Abstract. Yes-associated protein (YAP) has been reported to serve an important role in gastric cancer cell survival and migration. However, the underlying mechanism remains unclear. The aim of present study was to identify the underlying mechanism through which Yap sustains gastric cancer viability and migration. The results of the present study demonstrated that YAP expression was upregulated in gastric cancer MKN-28/74 cells compared with normal gastric GES-1 cells. Functional studies revealed that silencing of YAP inhibited gastric cancer MKN-28/74 cell viability and invasion. Mechanistically, YAP may promote gastric cancer cell survival and migration/invasion by inhibiting the endoplasmic reticulum (ER) stress pathway. In addition, YAP may regulate ER stress by activating the ERK signaling pathway. The results of the present study suggested that YAP may be a tumor promoter in gastric cancer and act through the ERK/ER stress pathway; therefore, YAP may have potential implications for new approaches to gastric cancer therapy.

Introduction

Gastric cancer is one of the most common malignant tumors worldwide with an occurance rate of 10.79% (1). Gastric cancer is the fifth leading cause of cancer-associated mortality in both the male and female population worldwide with a mortality rate of 8.8% (2). Despite the continuous development of comprehensive diagnosis and treatment technologies in recent years, the 5-year survival rate for patients with advanced gastric cancer is still >30% (3-5). The reasons for this are complex, and one of the most important issues is that gastric cancer cells are prone to survival and migration/invasion (6-8). Therefore, it is worthwhile to explore the mechanism of gastric cancer cell survival and migration/invasion for early intervention, late treatment and improvement of treatment outcomes.

Endoplasmic reticulum (ER) is the primary site of protein folding, modification and assembly, as well as intracellular Ca^{2+} storage in eukaryotic cells (9,10). Under stressed conditions, misfolded or unfolded protein aggregation and imbalances in Ca^{2+} levels in the ER lumen occur, and the cell enters a state termed ER stress (11-13). If the stress persists or the stress damage exceeds the ability of cell survival and protection, the ER stress-dependent apoptosis pathway is activated, leading to apoptosis (14-16). Recent studies have suggested that ER stress-mediated cell migration/invasion is closely associated with the occurrence and development of gastric cancer (17-20). However, the initiator of ER stress that regulates gastric cancer cell survival and migration/invasion remains unknown.

Yes-associated protein (YAP) is involved in the regulation of cell proliferation, organ development and the occurrence of tumors (21-23). Previous studies have demonstrated that YAP is abnormally expressed in breast, ovarian and other types of cancer, and its expression levels are associated with stage and prognosis of patients with tumors (24-27). Upregulation of YAP has been observed in gastric cancer and is associated with the clinicopathological characteristics of patients with gastric cancer (28,29). In addition, YAP integrates ER stress to control liver size and tumorigenesis, suggesting a potential connection between YAP and ER stress (29,30). Therefore, the present study hypothesized that YAP may reduce gastric cancer cell survival and migration through the activation of ER stress.

Materials and methods

Cell culture and treatments. The gastric cancer MKN-28/74 cells and normal gastric GES-1 cells were purchased from the American Type Culture Collection. The MKN28 cell line has been reported as cross-contaminated with MKN74; thus, it is referred to as MKN-28/74 throughout the present study (31). MKN-28/74 cells were cultured in RPMI-1640 medium (Nacalai Tesque, Inc.) supplemented with 10% fetal bovine serum (FBS; HyClone; GE Healthcare Life Sciences) at 37˚C in a 5% CO_2 humidified incubator; GES-1 cells were cultured in DMEM (HyClone; GE Healthcare Life Sciences)

Correspondence to: Professor Yan Wang, Department of Gastrointestinal Surgery, China-Japan Friendship Hospital, 2 Yinghua Road, Beijing 100029, P.R. China
E-mail: drwangyan@126.com

Key words: yes-associated protein, gastric cancer, ERK, endoplasmic reticulum stress

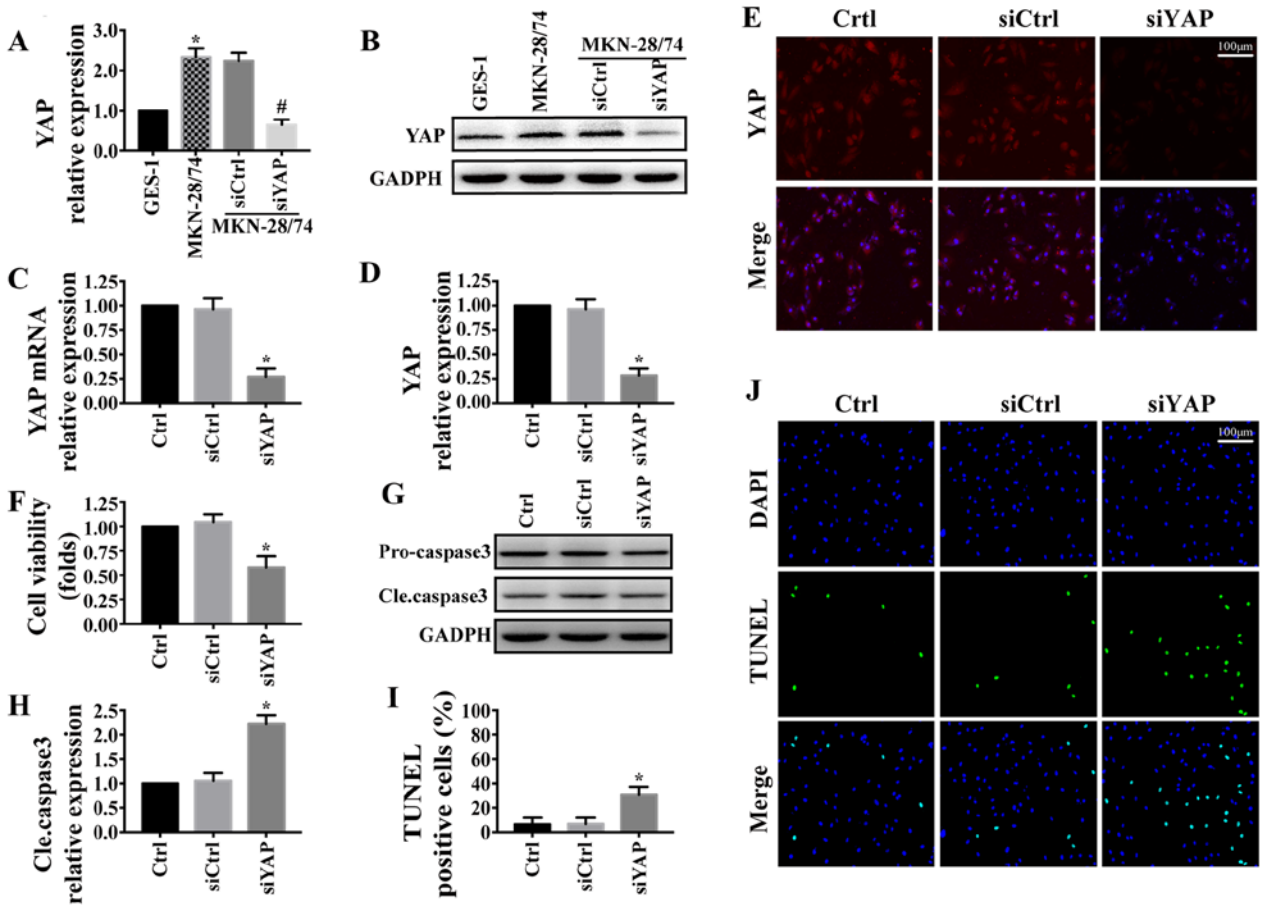

Figure 1. YAP affects viability and apoptosis in gastric cancer MKN-28/74 cells. (A and B) The protein level of YAP was measured in gastric cancer MKN-28/74 cell and normal gastric GES-1 cells. (C-E) siYAP transfection efficiency was confirmed by (C) reverse transcription-quantitative PCR and (D and E) immunofluorescence assays. (F) MTT assay was used to measure MKN-28/74 cell viability following YAP knockdown. (G and H) Western blotting was used to detect the expression of caspase-3 and cleaved caspase-3. (I and J) TUNEL staining was performed to determine the effect of YAP on apoptosis in MKN-28/74 cells. *P<0.05 vs. GES-1 or Ctrl. #P<0.05 vs. MKN-28/74. YAP, yes-associated protein; siYAP, small interfering RNA targeting YAP; siCtrl, control small interfering RNA; cle, cleaved; Ctrl, untransfected control.

containing 10% FBS (HyClone; GE Healthcare Life Sciences) at 37˚C in a 5% CO_2 humidified incubator (32). Tunicamycin (TM; 100 nM; Sigma-Aldrich; Merck KGaA) and 4-phenylbutyrate (10 mM; Sigma-Aldrich; Merck KGaA), the agonist and antagonist for ER stress, respectively, were added to the medium for 12 h. MKN-28/74 cell were pre-treated with PD98059 (10 μM) for 24 h at 37˚C.

Transfection. To evaluate the functional role of YAP, small interfering (si)RNA was used to knockdown its expression. siYAP (5'-GCGACATTCAGGGUGACUAUU-3') and non-targeting sequences (siCtrl; 5'-UUCUCCGAACGUGUCACGU-3') were purchased from GenePharma Co., Ltd. (33). A total of 20 nM siYAP or siCtrl was used to transfect MKN-28/74 cells (2x10^6 cells/well) with Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.) for 48 h in 6-well plates, and the transfection efficiency was determined by western blotting.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from the MKN-28/74 cells using an RNeasy kit (Beyotime Institute of Biotechnology) and reverse transcribed using One-step RT-PCR kit (cat. no., AE311-02; Beijing Transgen Biotech Co., Ltd.) at 37˚C for 30 min according to the manufacturer's protocol (34). qPCR was performed using the SYBR Green RT-PCR kit (Takara Bio, Inc.) according to the manufacturer's protocol. The thermocycling conditions were as follows: 95˚C for 5 min; followed by 40 cycles of 95˚C for 40 sec, 60˚C for 30 sec and 72˚C for 30 sec. GAPDH was selected as an internal control. The following primers were used for PCR: YAP forward, 5'-AAGGCTTGACCCTCGTTT-3' and reverse, 5'-CTGCTGCTGCTGGTTTGA-3'; and GAPDH forward, 5'-GTCAACGGATTTGGTCGTATTG-3' and reverse, 5'-CATGGGTGGAATCATATTGGAA-3'. Fold-changes in mRNA expression were calculated using the $2^{-\Delta\Delta Cq}$ method (35).

Western blotting. The MKN28/74 cells (5x10^6) was homogenized and sonicated in a lysis buffer (Beyotime Institute of Biotechnology). Protein concentrations were detected using a BCA Protein Quantification kit, according to the manufacturer's protocol. The proteins (50 μg) were separated by 10% SDS-PAGE and then transferred onto polyvinylidene difluoride membranes. The membrane was blocked with 5% non-fat dry milk for 1 h at room temperature and incubated with specific primary antibodies overnight at 4˚C. The

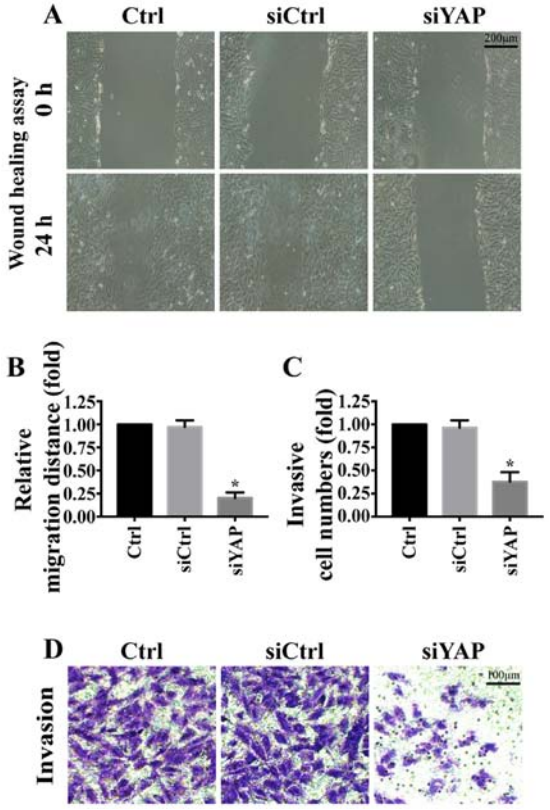

Figure 2. YAP inhibition is associated with cell migration and invasion. (A) Knockdown of YAP significantly reduced wound closure rates in gastric cancer MKN-28/74 cells compared with those in the Ctrl group. (B) Relative migration distance. (C) Knockdown of YAP reduced the numbers of migrated gastric cancer MKN-28/74 cells compared with those in the Ctrl group. (D) Transwell chamber assay. *P<0.05 vs. Ctrl. YAP, yes-associated protein; siYAP, small interfering RNA targeting YAP; siCtrl, control small interfering RNA; Ctrl, untransfected control.

primary antibodies used were as follows: YAP (1:1,000; Cell Signaling Technology, Inc.; cat. no. 14074), pro-caspase-3 (1:1,000; Abcam; cat. no. ab13847), cleaved caspase-3 (1:1,000; Abcam; cat. no. ab49822), glucose-regulated protein 78 kDa (GRP78; 1:1,000; Abcam; cat. no. ab21685), GADPH (1:1,000; Abcam; cat. no. ab8245), pro-caspase-12 (1:1,000; Abcam; cat. no. ab8117), cleaved caspase-12 (1:1,000; Cell Signaling Technology, Inc.; cat. no. 2202), C/EBP homologous protein (CHOP; 1:1,000; Abcam; cat. no. ab11419), ERK (1:1,000; Cell Signaling Technology, Inc.; cat. no. 4695), phosphorylated (p-) ERK (1:1,000; Cell Signaling Technology, Inc.; cat. no. 4370). The blots were detected with an enhanced chemiluminescence substrate kit (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The bands were scanned and quantified by ImageJ version 1.47 software (National Institutes of Health) (36).

Immunofluorescence staining. Following transfection treatment, the MKN28/74 cell ($0.5x10^6$ cells/well) were fixed with 3.7% paraformaldehyde for 10 min at room temperature and subsequently blocked with 5% bovine serum albumin (Sigma-Aldrich; Merck KGaA) in PBS for 1 h at room temperature. Cells were incubated with primary antibodies for 4 h at room temperature. The primary antibodies used were YAP (1:500; Cell Signaling Technology, Inc.; cat. no. 14074) and CHOP (1:500; Abcam; cat. no. ab11419). DAPI (5 mg/ml; Sigma-Aldrich; Merck KGaA) was used to stain the nuclei at room temperature for 3 min. A total of 5 randomly selected fields of view were used per smaple and images were captured with a laser confocal microscope (magnification, x600; TcS SP5; Leica Microsystems, Inc.).

Cell invasion and migration. Following transfection treatment, cell invasion was analyzed using a Transwell chamber assay as previously described (37). Briefly, cells ($1x10^6$ cells/well) were suspended in RPMI-1640 medium containing 10% FBS and seeded into the upper chambers.

Cell migration was analyzed using a wound-healing assay and cells were cultured with RPMI-1640 medium in 12-well plates. Once cells reached >80% confluency, a sterile pipette tip was used to evenly scratch the 12-well plate. Following cell attachment, a straight line was gently scratched in the cell layer with a 200 μl pipette tip, and the cells were washed with PBS (pH 7.4) three times. The relative wound closure was imaged under a light microscope (magnification, x100; Leica Microsystems, Inc.) at 0 and 24 h. The wound was measured using ImageJ 1.74v software (National Institutes of Health).

MTT assay and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL). The MKN-28/74 cells were seeded into 96-well plates at $8x10^3$ cells/well and incubated overnight. Following transfection treatment, MTT (5 mg/ml) was added to each well and incubated for 4 h. The insoluble formazan was collected and dissolved in dimethylsulfoxide, and the optical density value was measured with a scanning spectrophotometer at a wavelength of 570 nm.

The TUNEL assay was used for the detection of apoptosis. A one-step TUNEL kit (Beyotime Institute of Biotechnology) was used for TUNEL staining. The MKN-28/74 cells ($1x10^6$ cells) were incubated with fluorescein-dUTP (Invitrogen; Thermo Fisher Scientific, Inc.) to stain the apoptotic cell nuclei and with DAPI (5 mg/ml) to stain all cell nuclei at room temperature for 3 min. Images were captured with a laser confocal microscope (magnification, x600; TcS SP5; Leica Microsystems, Inc.). The number of TUNEL-positive cells was calculated by counting at least five random fields of view as the ratio of the experimental samples to the control samples (untransfected cells).

Statistical analysis. All analyses were performed with SPSS 20.0 software (IBM Corp.). Experiments were repeated three times and data are presented as the means ± standard error of the mean. Statistical analyses were performed using one-way analysis of variance with the Bonferroni test for post hoc comparisons. P<0.05 was considered to indicate a statistically significant difference.

Results

YAP is upregulated in gastric cancer MKN-28/74 cells and promotes cell survival. The expression levels of YAP were detected by western blotting in MKN-28/74 gastric cancer cells and GES-1 normal gastric cells. The results demonstrated that YAP was significantly upregulated in gastric cancer MKN-28/74 cells compared with GES-1 cells (Fig. 1A and B).

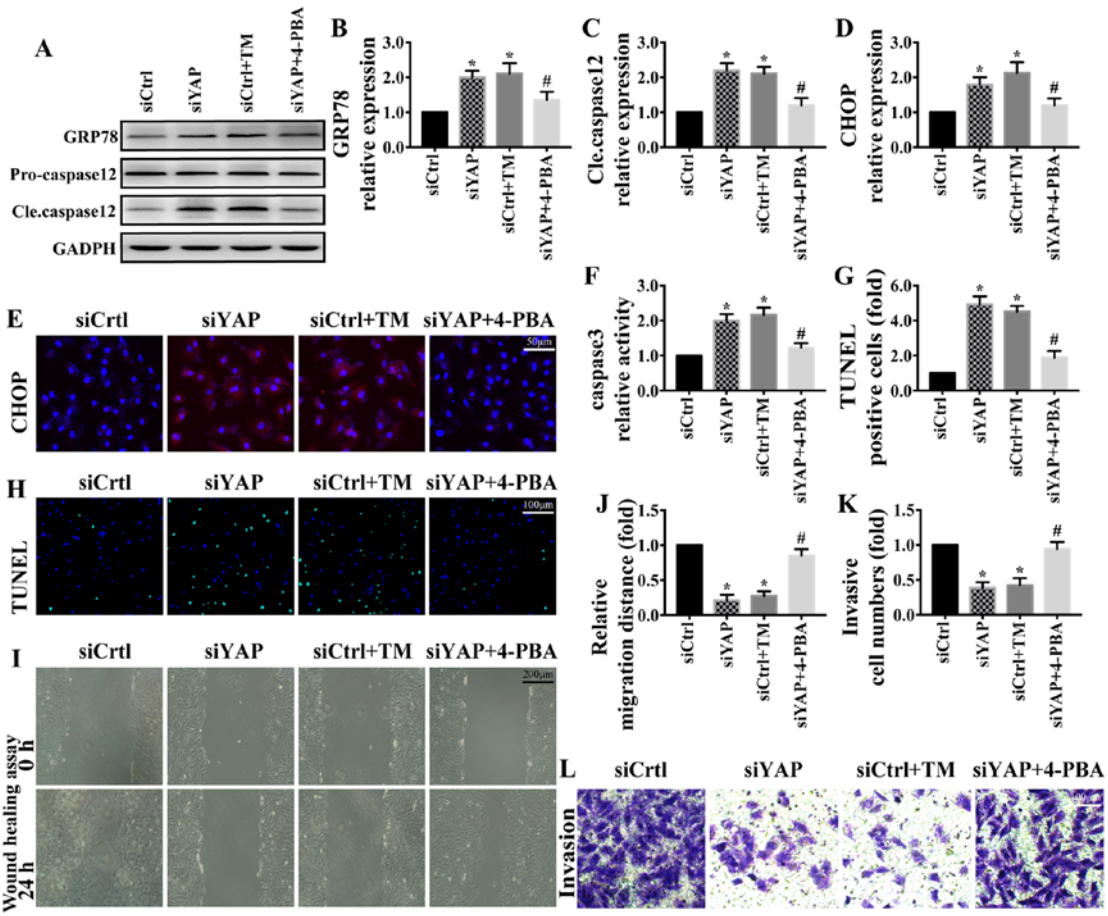

Figure 3. YAP promotes MKN-28/74 cell survival and migration through the inhibition of ER stress. (A) Protein levels of (B) GRP-78 and (C) Cle.caspase12 were evaluated via western blotting. (D) Knockdown of YAP also reduced expression of CHOP. (E) Expression of CHOP was measured by immunofluorescence assay. (F) Caspase3 activity assay and (G) TUNEL staining were performed to determine the effects of ER stress on MKN-28/74 cell apoptosis. (H) TUNEL staining. (I) Wound-healing assay. (J) ER stress reduced the wound closure rates in gastric cancer MKN-28/74 cells. (K and L) Transwell assay was used to detect the invasive ability of MKN-28/74 cells. *P<0.05 vs. siCtrl; #P<0.05 vs. siYAP. YAP, yes-associated protein; siYAP, small interfering RNA targeting YAP; siCtrl, control small interfering RNA; Ctrl, control; ER, endoplasmic reticulum.

To confirm the role of YAP in the progression gastric cancer, siYAP was transfected into MKN-28/74 cells to knockdown the expression of YAP. The transfection efficiency was detected by western blotting (Fig. 1A and B), RT-qPCR (Fig. 1C) and immunofluorescence (Fig. 1D and E). The results demonstrated that siYAP, but not siCtrl, significantly inhibited the expression of YAP in gastric cancer MKN-28/74 cells compared with untransfected cells. The effect of YAP on MKN-28/74 cell viability was investigated. The results of the MTT assay demonstrated that YAP knockdown significantly reduced the viability of MKN-28/74 cells (Fig. 1F). In addition, the inhibition of YAP expression increased the expression of cleaved caspase-3 (Fig. 1G and H) and the number of TUNEL-positive cells (Fig. 1I and J) in gastric cancer MKN-28/74 cells. These results suggested that YAP was upregulated in gastric cancer MKN-28/74 cells and promoted cell survival by inhibiting apoptosis.

YAP is associated with MKN-28/74 cell migration and invasion. The role of YAP in MKN-28/74 cell migration and invasion was further investigated. Knockdown of YAP significantly reduced wound closure rates in the wound-healing assay (Fig. 2A and B). In addition, compared with the control group, knockdown of YAP reduced the invasive ability of gastric cancer MKN-28/74 cells (Fig. 2C and D). These results suggested that YAP promoted MKN-28/74 cell migration and invasion.

YAP promotes MKN-28/74 cell survival and migration/invasion through the inhibition of ER stress. ER stress serves a critical role in the progression of cancer (38,39). To determine the underlying mechanism by which YAP may regulate gastric cancer MKN-28/74 cell survival and metastasis, the present study focused on ER stress. TM, the activator of ER stress, was used to induce ER stress in MKN-28/74 cells transfected with siCtrl. 4-phenylbutyrate (4-PBA), the inhibitor of ER stress, was used to inhibit ER stress in YAP-knockdown MKN-28/74 cells. Western blotting (Fig. 3A-C) and immunofluorescence (Fig. 3D and E) were used to determine the changes in ER stress markers. Compared with the siCtrl group, knockdown of YAP contributed to the upregulation of GRP78, CHOP and cleaved caspase-12; similar results were observed following TM treatment in the siCtrl group. However, the upregulation of ER stress markers was partially reversed by 4-PBA (Fig. 3A-E). These results suggested that YAP knockdown was associated with ER stress. In addition,

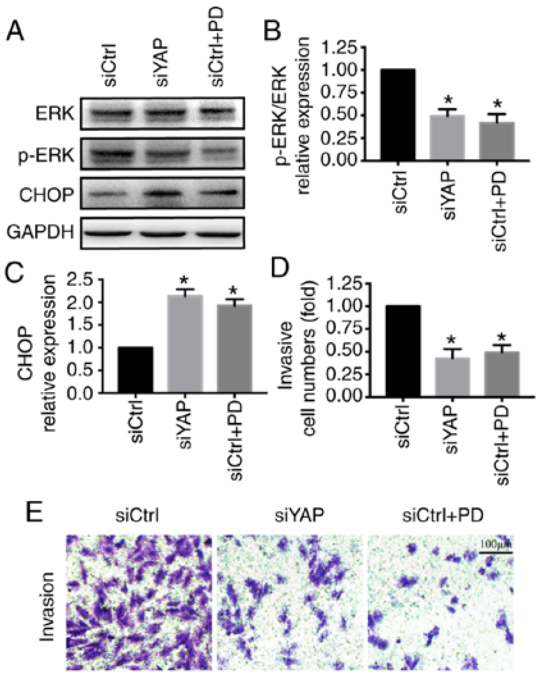

Figure 4. The ERK pathway is involved in YAP-mediated endoplasmic reticulum stress inhibition. (A-C) The protein levels of ERK, p-ERK and CHOP were evaluated by western blotting. PD98059 was used to inhibit the ERK pathway in MKN-28/74 cells transfected with siCtrl. (D and E) Transwell assay was used to detect the invasive ability of MKN-28/74 cells. *P<0.05 vs. siCtrl. YAP, yes-associated protein; siYAP, small interfering RNA targeting YAP; siCtrl, control small interfering RNA; Ctrl, control; p-, phosphorylated; PD, PD98059.

ER stress activation was associated with apoptosis activation (Fig. 3F-H) and the inhibition of migration/invasion (Fig. 3I-L). By contrast, inhibiting ER stress with 4-PBA in YAP-knockdown cells promoted cell survival and invasion. These results indicated that YAP promoted gastric cancer MKN-28/74 cell survival and migration/invasion through the regulation of ER stress.

YAP regulates ER stress via the ERK pathway. Finally, experiments were performed to determine how YAP inhibited ER stress. The ERK pathway has been reported to be involved in YAP-associated functions and ER stress inhibition (40,41). PD98059, an inhibitor of the ERK pathway, was used to inhibit the ERK pathway in MKN-28/74 cells transfected with siCtrl. The activation of the ERK pathway was assessed by western blotting. Compared with the control group, YAP knockdown inhibited ERK phosphorylation, similar to PD98059 treatment (Fig. 4A). The inhibition of the ERK pathway by siYAP promoted the activation of ER stress as indicated by the upregulation of CHOP and reduced cell invasion (Fig. 4). These results suggested that the ERK pathway may contribute to the YAP-induced ER stress inhibition.

Discussion

Previous studies have demonstrated that YAP is essential for gastric cancer cell survival and migration/invasion (42-44). However, the underlying mechanism remains unclear. The present study proposes a novel underlying mechanism by which YAP regulates gastric cancer MKN-28/74 cell survival and metastasis. The results of the present study demonstrated that: i) YAP was upregulated in gastric cancer MKN-28/74 cells compared with normal gastric GES-1 cells; ii) YAP promoted gastric cancer MKN-28/74 cell survival and migration/invasion by inhibiting ER stress; iii) YAP may regulate ER stress by activating the ERK pathway. The present study provides a new target for the treatment of gastric cancer that may affect cancer cell survival and metastasis. A limitation of the present study was that only one gastric cancer cell line was used. Additional cell lines will be used in our future study, to confirm the results.

In eukaryotic cells, the ER is responsible for protein synthesis and calcium storage; perturbations in the ER function, a process termed ER stress, have been reported to be involved in cancer initiation, growth and metastasis in the majority of solid tumors (45,46). However, the role of ER stress in tumorigenesis and development is still controversial. Previous studies have demonstrated that ER stress is a tumor suppressor, and the activation of ER stress inhibits gastric cancer cell survival and migration (19,47,48). However, a number of studies have suggested that ER stress can promote tumor development (49,50). Induction of ER stress protects gastric cancer cell apoptosis during cisplatin and doxorubicin treatment via the p38 MAPK pathway (51).

Recent studies have identified an association between YAP and ER stress. The activated Hippo-YAP signaling pathway promoted neuron survival in the TNFα-induced microenvironment by inhibiting ER stress (52). In addition, downregulation of YAP evoked ER stress and contributed to myocyte death in isoproterenol-induced myocardial infarction (53). The results of the present study are consistent with previous studies. However, the exact mechanism by which YAP controls ER stress remains unknown. The results of the present study suggested that YAP may inhibit ER stress via the ERK pathway. Thus, these results provide valuable information on the role of YAP and ER stress in tumorigenesis.

In the present study, the critical role of YAP in the progression of gastric cancer was identified. A recent study demonstrated that YAP regulates gastric cancer survival and migration through SIRT1/Mfn2/mitophagy (42). The results of the present study demonstrated that YAP may function via the ERK/ER stress pathway in gastric cancer survival and metastasis. To the best of our knowledge, this is the first identification of YAP functions involved in ER stress and the ERK pathway in the development of gastric cancer. However, *in vivo* experiments and clinical data are required to support these results.

In conclusion, the results of the present study identified the important role of YAP in gastric cancer cell migration and survival. YAP promoted gastric cancer MKN-28/74 cell survival and migration/invasion via the ERK/ER stress pathway. These results suggested that the YAP/ERK/ER stress pathway may be a potential target for the treatment of gastric cancer.

Acknowledgements

Not applicable.

Funding

This work was supported in part by Inner Mongolia Autonomous Region Natural Science Foundation (grant no., 2016MS0847), Scientific Research Planning Project of Health and Family Planning Commission of Inner Mongolia Autonomous Region (grant no., 201701048) and Science and Technology Innovation Guidance Project of Inner Mongolia Autonomous Region (grant no., KCBJ2018021).

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

HL and DM conceived and designed the study, performed the experiments, analyzed and interpreted the data and wrote the manuscript. PX, HW and YW were involved in data analysis and interpretation.

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.

References

1. Karimi P, Islami F, Anandasabapathy S, Freedman ND and Kamangar F: Gastric cancer: Descriptive epidemiology, risk factors, screening, and prevention. Cancer Epidemiol Biomarkers Prev 23: 700-713, 2014.
2. Zhang XY and Zhang PY: Gastric cancer: Somatic genetics as a guide to therapy. J Med Genet 54: 305-312, 2017.
3. Hamashima C: Current issues and future perspectives of gastric cancer screening. World J Gastroenterol 20: 13767-13774, 2014.
4. Japanese Gastric Cancer Association: Japanese gastric cancer treatment guidelines 2014 (ver. 4). Gastric Cancer 20: 1-19, 2017.
5. Sano T: Gastric cancer: Asia and the world. Gastric Cancer 20 (Suppl 1): S1-S2, 2017.
6. Rahman R, Asombang AW and Ibdah JA: Characteristics of gastric cancer in Asia. World J Gastroenterol 20: 4483-4490, 2014.
7. Goldenring JR: The AGA/Funderburg award in gastric cancer: Twenty-five years of advances in gastric cancer research. Gastroenterology 152: 1262-1266, 2017.
8. Bekaii-Saab T and El-Rayes B: Identifying and targeting cancer stem cells in the treatment of gastric cancer. Cancer 123: 1303-1312, 2017.
9. Song S, Tan J, Miao Y and Zhang Q: Crosstalk of ER stress-mediated autophagy and ER-phagy: Involvement of UPR and the core autophagy machinery. J Cell Physiol 233: 3867-3874, 2018.
10. Sarvani C, Sireesh D and Ramkumar KM: Unraveling the role of ER stress inhibitors in the context of metabolic diseases. Pharmacol Res 119: 412-421, 2017.
11. Coppola-Segovia V, Cavarsan C, Maia FG, Ferraz AC, Nakao LS, Lima MM and Zanata SM: ER stress induced by tunicamycin triggers alpha-Synuclein oligomerization, dopaminergic neurons death and locomotor impairment: A New model of Parkinson's disease. Mol Neurobiol 54: 5798-5806, 2017.
12. Rahmati M, Moosavi MA and McDermott MF: ER stress: A therapeutic target in rheumatoid arthritis? Trends Pharmacol Sci 39: 610-623, 2018.
13. Hetz C: The unfolded protein response: Controlling cell fate decisions under ER stress and beyond. Nat Rev Mol Cell Biol 13: 89-102, 2012.
14. Smith M and Wilkinson S: ER homeostasis and autophagy. Essays Biochem 61: 625-635, 2017.
15. Koga T, Suico MA, Shimasaki S, Watanabe E, Kai Y, Koyama K, Omachi K, Morino-Koga S, Sato T, Shuto T, *et al*: Endoplasmic reticulum (ER) stress induces Sirtuin 1 (SIRT1) expression via the PI3K-Akt-GSK3β signaling pathway and promotes hepatocellular injury. J Biol Chem 290: 30366-30374, 2015.
16. Mohamed E, Cao Y and Rodriguez PC: Endoplasmic reticulum stress regulates tumor growth and anti-tumor immunity: A promising opportunity for cancer immunotherapy. Cancer Immunol Immunother 66: 1069-1078, 2017.
17. Papaioannou A and Chevet E: Driving Cancer tumorigenesis and metastasis through UPR signaling. Curr Top Microbiol Immunol 414: 159-192, 2018.
18. Kim C and Kim B: Anti-cancer natural products and their bioactive compounds inducing ER stress-mediated apoptosis: A review. Nutrients 10: pii: E1021, 2018.
19. Zhang B, Han H, Fu S, Yang P, Gu Z, Zhou Q and Cao Z: Dehydroeffusol inhibits gastric cancer cell growth and tumorigenicity by selectively inducing tumor-suppressive endoplasmic reticulum stress and a moderate apoptosis. Biochem Pharmacol 104: 8-18, 2016.
20. Chen W, Zou P, Zhao Z, Chen X, Fan X, Vinothkumar R, Cui R, Wu F, Zhang Q, Liang G and Ji J: Synergistic antitumor activity of rapamycin and EF24 via increasing ROS for the treatment of gastric cancer. Redox Biol 10: 78-89, 2016.
21. Panciera T, Azzolin L, Cordenonsi M and Piccolo S: Mechanobiology of YAP and TAZ in physiology and disease. Nat Rev Mol Cell Biol 18: 758-770, 2017.
22. Andl T, Zhou L, Yang K, Kadekaro AL and Zhang Y: YAP and WWTR1: New targets for skin cancer treatment. Cancer Lett 396: 30-41, 2017.
23. Zanconato F, Cordenonsi M and Piccolo S: YAP/TAZ at the roots of cancer. Cancer Cell 29: 783-803, 2016.
24. Sorrentino G, Ruggeri N, Zannini A, Ingallina E, Bertolio R, Marotta C, Neri C, Cappuzzello E, Forcato M, Rosato A, *et al*: Glucocorticoid receptor signalling activates YAP in breast cancer. Nat Commun 8: 14073, 2017.
25. Xia Y, Chang T, Wang Y, Liu Y, Li W, Li M and Fan HY: YAP promotes ovarian cancer cell tumorigenesis and is indicative of a poor prognosis for ovarian cancer patients. PLoS One 9: e91770, 2014.
26. Hall CA, Wang R, Miao J, Oliva E, Shen X, Wheeler T, Hilsenbeck SG, Orsulic S and Goode S: Hippo pathway effector Yap is an ovarian cancer oncogene. Cancer Res 70: 8517-8525, 2010.
27. Brown JS, O'Carrigan B, Jackson SP and Yap TA: Targeting DNA repair in cancer: Beyond PARP inhibitors. Cancer Discov 7: 20-37, 2017.
28. Pan Z, Tian Y, Zhang B, Zhang X, Shi H, Liang Z, Wu P, Li R, You B, Yang L, *et al*: YAP signaling in gastric cancer-derived mesenchymal stem cells is critical for its promoting role in cancer progression. Int J Oncol 51: 1055-1066, 2017.
29. Ma L, Cui J, Xi H, Bian S, Wei B and Chen L: Fat4 suppression induces Yap translocation accounting for the promoted proliferation and migration of gastric cancer cells. Cancer Biol Ther 17: 36-47, 2016.
30. Zhu P, Xue J, Zhang ZJ, Jia YP, Tong YN, Han D, Li Q, Xiang Y, Mao XH and Tang B: Helicobacter pylori VacA induces autophagic cell death in gastric epithelial cells via the endoplasmic reticulum stress pathway. Cell Death Dis 8: 3207, 2017.
31. Yokozaki H: Molecular characteristics of eight gastric cancer cell lines established in Japan. Pathol Int 50: 767-777, 2000.
32. Zhang XR, Wang SY, Sun W and Wei C: Isoliquiritigenin inhibits proliferation and metastasis of MKN28 gastric cancer cells by suppressing the PI3K/AKT/mTOR signaling pathway. Mol Med Rep 18: 3429-3436, 2018.
33. Noto A, De Vitis C, Pisanu ME, Roscilli G, Ricci G, Catizone A, Sorrentino G, Chianese G, Taglialatela-Scafati O, Trisciuoglio D, *et al*: Stearoyl-CoA-desaturase 1 regulates lung cancer stemness via stabilization and nuclear localization of YAP/TAZ. Oncogene 36: 4573-4584, 2017.
34. Zhu P, Hu S, Jin Q, Li D, Tian F, Toan S, Li Y, Zhou H and Chen Y: Ripk3 promotes ER stress-induced necroptosis in cardiac IR injury: A mechanism involving calcium overload/XO/ROS/mPTP pathway. Redox Biol 16: 157-168, 2018.

35. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
36. Li R, Yang HQ, Xi HL, Feng S and Qin RH: Inhibition of CDH17 gene expression via RNA interference reduces proliferation and apoptosis of human MKN28 gastric cancer cells. Int J Oncol 50: 15-22, 2017.
37. Ye H, Wang WG, Cao J and Hu XC: SPARCL1 suppresses cell migration and invasion in renal cell carcinoma. Mol Med Rep 16: 7784-7790, 2017.
38. Liu SH, Lee WJ, Lai DW, Wu SM, Liu CY, Tien HR, Chiu CS, Peng YC, Jan YJ, Chao TH, *et al*: Honokiol confers immunogenicity by dictating calreticulin exposure, activating ER stress and inhibiting epithelial-to-mesenchymal transition. Mol Oncol 9: 834-849, 2015.
39. Yan Y, Liu S, Li M, Zhao Y, Shao X, Hang M and Bu X: Recombinant Newcastle disease virus expressing human IFN-λ1 (rL-hIFN-λ1)-induced apoptosis of A549 cells is connected to endoplasmic reticulum stress pathways. Thorac Cancer 9: 1437-1452, 2018.
40. Kennedy D, Mnich K, Oommen D, Chakravarthy R, Almeida-Souza L, Krols M, Saveljeva S, Doyle K, Gupta S, Timmerman V, *et al*: HSPB1 facilitates ERK-mediated phosphorylation and degradation of BIM to attenuate endoplasmic reticulum stress-induced apoptosis. Cell Death Dis 8: e3026, 2017.
41. Zhang Y, Yuan J, Zhang X, Yan F, Huang M, Wang T, Zheng X and Zhang M: Angiomotin promotes the malignant potential of colon cancer cells by activating the YAP-ERK/PI3K-AKT signaling pathway. Oncol Rep 36: 3619-3626, 2016.
42. Yan H, Qiu C, Sun W, Gu M, Xiao F, Zou J and Zhang L: Yap regulates gastric cancer survival and migration via SIRT1/Mfn2/mitophagy. Oncol Rep 39: 1671-1681, 2018.
43. Jiao S, Wang H, Shi Z, Dong A, Zhang W, Song X, He F, Wang Y, Zhang Z, Wang W, *et al*: A peptide mimicking VGLL4 function acts as a YAP antagonist therapy against gastric cancer. Cancer Cell 25: 166-180, 2014.
44. Qiao Y, Chen J, Lim YB, Finch-Edmondson ML, Seshachalam VP, Qin L, Jiang T, Low BC, Singh H, Lim CT and Sudol M: YAP regulates actin dynamics through ARHGAP29 and promotes metastasis. Cell Rep 19: 1495-1502, 2017.
45. Cubillos-Ruiz JR, Bettigole SE and Glimcher LH: Tumorigenic and immunosuppressive effects of endoplasmic reticulum stress in cancer. Cell 168: 692-706, 2017.
46. Dumartin L, Alrawashdeh W, Trabulo SM, Radon TP, Steiger K, Feakins RM, di Magliano MP, Heeschen C, Esposito I, Lemoine NR and Crnogorac-Jurcevic T: ER stress protein AGR2 precedes and is involved in the regulation of pancreatic cancer initiation. Oncogene 36: 3094-3103, 2017.
47. Chen PP, Ma XY, Lin Q, Xu HL, Shi HX, Zhang HY, Xiao J, Geng FN and Zhao YZ: Kangfuxin promotes apoptosis of gastric cancer cells through activating ERstress and autophagy. Mol Med Rep 16: 9043-9050, 2017.
48. Kim JL, Lee DH, Na YJ, Kim BR, Jeong YA, Lee SI, Kang S, Joung SY, Lee SY, Oh SC and Min BW: Iron chelator-induced apoptosis via the ER stress pathway in gastric cancer cells. Tumour Biol 37: 9709-9719, 2016.
49. Hersey P and Zhang XD: Adaptation to ER stress as a driver of malignancy and resistance to therapy in human melanoma. Pigment Cell Melanoma Res 21: 358-367, 2008.
50. Ye J and Koumenis C: ATF4, an ER stress and hypoxia-inducible transcription factor and its potential role in hypoxia tolerance and tumorigenesis. Curr Mol Med 9: 411-416, 2009.
51. Feng R, Zhai WL, Yang HY, Jin H and Zhang QX: Induction of ER stress protects gastric cancer cells against apoptosis induced by cisplatin and doxorubicin through activation of p38 MAPK. Biochem Biophys Res Commun 406: 299-304, 2011.
52. Hou S, Wang L and Zhang G: Mitofusin-2 regulates inflammation-mediated mouse neuroblastoma N2a cells dysfunction and endoplasmic reticulum stress via the Yap-Hippo pathway. J Physiol Sci 69: 697-709, 2019.
53. Ashokkumar R, Jamuna S, Sakeena Sadullah MS and Niranjali Devaraj S: Vitexin protects isoproterenol induced post myocardial injury by modulating hipposignaling and ER stress responses. Biochem Biophys Res Commun 496: 731-737, 2018.