Pathology - Research and Practice 215 (2019) 152471

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

The potential role of TNFAIP3 in malignant transformation of gastric carcinoma

Bing Du[a], Mingna Liu[a], Chunhong Li[b], Xinyu Geng[a], Xu Zhang[a], Dandan Ning[a], Meiyan Liu[b,*]

[a] Department of Gastroenterology, Second Affiliated Hospital of Harbin Medical University, 148 Baojian Road, Nangang District, Harbin, 150081, People's Republic of China

[b] Department of Internal Medicine, Harbin Medical University Cancer Hospital, 6 Baojian Road, Nangang District, Harbin, 150040, People's Republic of China

ARTICLE INFO

Keywords:
Gastric cancer
TNFAIP3
EMT
Proliferation
Migration
Invasion

ABSTRACT

Tumor necrosis factor alpha-induced protein 3 (TNFAIP3), a deubiquitinating enzyme, plays an essential regulatory role in inflammation, immune responses and tumorigenesis. Our present study indicates that TNFAIP3 is required for the ubiquitination degradation of epithelial–mesenchymal transition (EMT) related transcription factors Snail and ZEB1, which further altered the expression of EMT-related marker proteins and eventually contributing to the malignant phenotype and poorer prognosis of gastric carcinoma. Depletion of TNFAIP3 attenuated the capacity of proliferation, migration and invasion of gastric cancer cells in vitro. Taken together, these findings propose a pathway linking the TNFAIP3 to the EMT-mediated metastatic process in gastric cancer, which provides a viable strategy regarding the interventions and prognostic analysis of gastric carcinoma in clinical practice.

1. Introduction

Globally, gastric cancer is the fifth leading cause of malignant tumor and the third leading cause of death, therefore making a significant contribution to the global health burden [6]. Due to the lack of therapy with enough specificity and sensitivity, the prognosis of gastric cancer is generally poor and unsatisfactory. Besides, gastric cancer is often diagnosed with late stage disease and accompanied by malignant metastasis, since the early symptoms of gastric cancer is not obvious. And the five-year survival rate for gastric cancer patients is reported to be less than 10 percent [7,13]. Therefore, it is strongly necessary to reveal the molecular mechanisms underlying the gastric cancer genesis and development, thus to identify effective therapeutic targets or prognosis biomarkers for patients with gastric cancer.

The epithelial–mesenchymal transition (EMT) is a biological process by which epithelial cells lose their polarity and adhesion between cells; also, the migratory and invasive properties of cells were markedly enhanced [5,11]. Therefore, EMT, characterized by suppressing the expression of epithelial markers and enhancing the expression of mesenchymal markers, as well as the EMT-related transcription factors like Snail and ZEB1 [3,9,17], is highly associated with cancer progression such as invasion and metastasis.

Tumor necrosis factor alpha-induced protein 3 (TNFAIP3), also referred to as A20, acts as a key regulator in NF-kappa B activation as well as TNF-mediated apoptosis [12]. It is reported that TNFAIP3 regulates intracellular protein expression via facilitating ubiquitin degradation [10]. Recent studies indicated that TNFAIP3 has cell- or tissue- specific expression patterns and functions, which may be ascribed to its site-specific ubiquitin modifying activity or ubiquitin-binding status with its target molecules [4,16]. Generally, TNFAIP3 is transiently expressed in a variety of cell types in response to inflammatory signals and immune responses. Despite knowledge of the roles of TNFAIP3 in inflammation and immune responses, its functions in gastric cancer are not yet fully understood, especially in the aspects of EMT or other neoplastic malignant processes.

Although the functions of TNFAIP3 are well studied in inflammation and immune responses, it remains unknown how TNFAIP3 contributes to gastric tumorigenesis, invasion and metastasis. In the present study, we uncover a mechanism where TNFAIP3 is involved in the ubiquitination degradation of EMT-related transcription factors (Snail and ZEB1), which further altered the expression of EMT marker proteins and subsequently affected the biological behaviors of gastric cancer cells.

* Corresponding author.
E-mail address: lmyan78@yeah.net (M. Liu).

https://doi.org/10.1016/j.prp.2019.152471
Received 11 December 2018; Received in revised form 14 May 2019; Accepted 23 May 2019

2. Materials and methods

2.1. Tissue samples

We collected a total of 32 cases of gastric cancer tissues and paired non-carcinoma tissues between May 2016 and February 2017 in the Harbin Medical University Cancer Hospital (Harbin, China). We selected 27 cases from the total 32 cases. There are 12 male patients and 15 female patients, with a medium age at 58 years old. No patients have received chemotherapy or radiotherapy before surgery, and all cases were diagnosed by pathology and immunohistochemistry. The present study has been approved by the ethical committee of Harbin Medical University Cancer Hospital and the written informed consents were obtained from all patients recruited.

2.2. Cell culture and transfection

Human gastric cancer cell lines SGC-7901, MGC-803, HGC-27, MKN-45, AGS and human gastric mucosal cell (GES-1) were all purchased from the Chinese Academy of Sciences Shanghai Institutes for Biological Sciences (Shanghai, China). Theses cell lines were cultured in RPMI1640 medium (Gibco, Grand Island, NY, USA) supplement with 10% fetal bovine serum (Gibco, Grand Island, NY, USA) at 37 °C with 5% CO_2 in a humidified incubator. Two plasmids carrying shRNA (pSUPER- TNFAIP3-shRNA) targeting TNFAIP3 respectively and the scrambled negative control (NC) were designed and constructed by RIBOBIO (Guangzhou, China) and then transfected into MGC-803 cells by Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA).

2.3. Western blotting

Proteins were extracted from tissues and cells with RIPA buffer (Thermo Fisher Scientific, Waltham, MA, USA) complemented with protease inhibitors (Roche, Mannheim, Germany). The concentration of protein was determined by using the NanoDrop 2000 (NanoDrop Technologies, Wilmington, DE). Briefly, 1 μl of each protein sample was added to the measured well, and the concentration of protein was recorded by the machine. For western blot analysis, equal amounts of protein (100 μg) were subjected to electrophoresis on 8% SDS-PAGE and transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% non-fat milk dissolved in PBST for 1 h at room temperature, then immunoblotted with the primary antibodies overnight at 4 °C. The primary antibodies included TNFAIP3 (#5630, Cell Signaling Technology, Inc., Beverly, MA, USA), E-Cadherin (#14472, Cell Signaling Technology, Inc., Beverly, MA, USA), **N**-Cadherin (#13116, Cell Signaling Technology, Inc., Beverly, MA, USA), Vimentin (#5741, Cell Signaling Technology, Inc., Beverly, MA, USA), Snail (#3879, Cell Signaling Technology, Inc., Beverly, MA, USA), ZEB1 (#3396, Cell Signaling Technology, Inc., Beverly, MA, USA), GAPDH (#5174, Cell Signaling Technology, Inc., Beverly, MA, USA). Subsequently, the membranes were incubated with fluorochrome labeled secondary antibody Alexa Fluor 800 (#A32730, Thermo Fisher Scientific, Inc., Waltham, MA, USA) or Alexa Fluor 700 (#A-11029, Thermo Fisher Scientific, Inc., Waltham, MA, USA). The bands were visualized by the Odyssey Infrared Imaging System (LI−COR Biosciences, Lincoln, NE, USA) and the band intensity was measured by Odyssey software version 1.2 (LI−COR Biosciences, Lincoln, NE, USA). The expression of proteins was all normalized to GAPDH.

2.4. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted using the Trizol reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions. 0.5 μg RNA of each sample was then reverse transcribed by the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Thermo Fisher Scientific, Inc., Waltham, USA). Afterwards, the cDNA was subjected to quantitative qRT-PCR using the Power SYBR Green PCR Master Mix (Roche Applied Science, Penzberg, Germany), and the detection was performed on the ABI 7500 Detection System (Life Technologies, NY, USA). GAPDH was used to normalize the expression of TNFAIP3 between different samples. The primer sequences for real time PCR were listed as follows: TNFAIP3 F 5′−CCGGTCTCTGTTAAC AAGTGG-3′, and R 5′- GAGTGTTACAGATATCCCATCGTC-3′; GAPDH F 5′-ATCACTGCCACCCAGAAGAC-3′, R5′-TTTCTAGACGGCAGGTC AGG-3′.

2.5. Proliferation assay

The Cell Count Kit 8 (CCK-8) were used to determine the cell proliferation according to the manufacturer's instructions. Briefly, cells were seeded in triplicate in 96-well plates at 1×10^4 cells per well and maintained for 24 h. After that, 10 μl CCK-8 working solution was added to each well and the plates were incubated in 37 °C for another 2 h. Absorbance was recorded at 450 nm using an Easy Reader 340 AT (SLT-Lab Instruments, Salzburg, Austria). The experiment was independently repeated three times.

2.6. Wound-healing assay

For the wound-healing assay, 1×10^5 cells were grown on a six-well plate until they reached 80% confluence. Then the monolayer cells were scratched with a sterile pipette tip. The image of cell migration was recorded at 0 h, 24 h after wound scratch at the same position. Three independent experiments were performed.

2.7. Cell invasion assay

Invasion assays were performed using Corning chambers (pore size, 8 μm, Corning, MA, USA) with Matrigel (Corning, MA, USA). In brief, cell suspensions (2×10^5) prepared in 200 μl media containing 2% FBS were added into upper chambers. While the bottom chamber contained medium with 15% FBS as a chemoattractant stimulus. After a 24 -h incubation at 37 °C, the non-invasive cells on the upper side of the membrane were gently removed with a cotton swab, and the cells that had migrated through the membrane filter were fixed in 4% paraformaldehyde. After that, the cells were stained with crystal violet (Beyotime Biotechnology, Shanghai city, China) for 15 min at room temperature. Finally, cells in three randomly selected microscopic fields (at 200 × magnification) were counted and photographed. All experiments were independently repeated in triplicate.

2.8. Statistical analysis

All quantitative data are expressed as the mean ± standard deviation (SD) and analyzed by using SPSS 17.0 (IBM; Armonk, NY, USA). The differential expression level of TNFAIP3 between gastric cancer tissues and adjacent normal tissues was evaluated by McNemar's chi-squared test. One-way analysis of variance (ANOVA) was used for statistical evaluation of the data between multiple groups with post hoc contrasts. Statistical significance was achieved when the p value is < 0.05.

3. Results

3.1. The expression of TNFAIP3 in gastric cancer tissues

Firstly, we analyzed the Oncomine datebases to compare the expression of TNFAIP3 in gastric carcinoma and normal tissues, and we found that the mRNA expression level of TNFAIP3 was statistically higher in gastric cancer tissues compared with normal tissues (Fig. 1A).

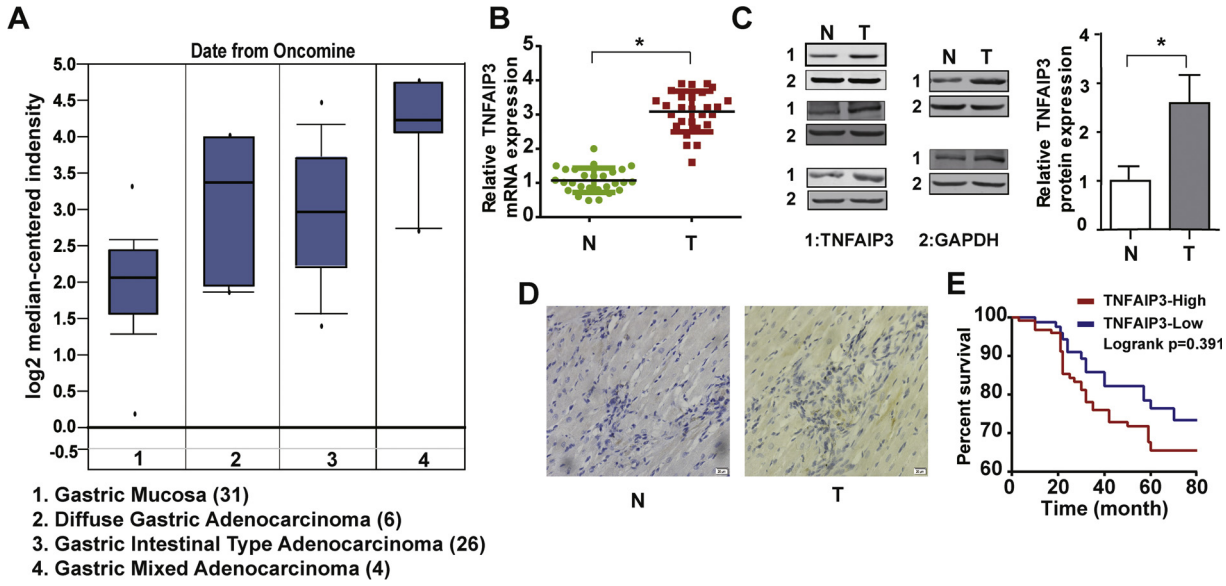

Fig. 1. Abnormal expression of TNFAIP3 in gastric carcinomas. (A) The expression pattern of TNFAIP3 in different gastric carcinomas and normal tissue. The date was from Oncomine datebase. (B) The mRNA expression level of TNFAIP3 in 27 cases of gastric cancer tissues and the adjacent noncancerous tissues determined by qRT- PCR. n = 27, * $p < 0.05$. (C) The representative western blot results of TNFAIP3 in gastric cancer tissues and the adjacent noncancerous tissues. n = 27, * $p < 0.05$. (D) The representative images of TNFAIP3 immunohistochemistry results. (E) The survival time of gastric cancer patients with different levels of TNFAIP3. p = 0.391.

Thus, we performed qRT-PCR (quantitative real-time PCR) and western blotting to investigate the expression status of TNFAIP3 in the 32 cases of gastric cancer tissues and the corresponding adjacent noncancerous tissues obtained from surgical resection specimens of gastric cancer patients. The results were in accordance with the date from Oncomine, the expression levels of both mRNA and protein were significantly elevated in the carcinoma tissues compared with normal tissues (Fig. 1).

3.2. The expression pattern of TNFAIP3 in gastric cancer cell lines

To explore the role of TNFAIP3 on the biological characteristics of gastric cancer cells, we first assessed the expression levels of endogenous TNFAIP3 in a panel of 5 gastric cancer cell lines (SGC-7901, MGC-803, HGC-27, MKN-45, AGS) and a normal human gastric mucosal cell (GES-1) by qRT-PCR and western blotting. Fig. 2 indicated that MGC-803 cell lines showed high endogenous TNFAIP3 expression in this group and therefore was selected for a loss-of-function study.

3.3. Knockdown of TNFAIP3 inhibits proliferation, migration and invasion of MGC-803 cells

Two plasmids carrying shRNA (pSUPER-TNFAIP3-shRNA) targeting TNFAIP3 respectively (shRNA1 and shRNA2) were transfected into MGC-803 cells, and then the expression of exogenous TNFAIP3 was determined by qRT-PCR and western blotting (Fig. 3A–B). The knockdown efficiency of shRNA2 plasmid on the target gene is superior to shRNA1 plasmid. Thus, the shRNA2 plasmid was selected for the following study. The results of CCK8 showed that the cell proliferation was decreased by about 20% compared with the control group (Fig. 3C). Meanwhile, TNFAIP3 depletion markedly reduced the migration and invasion capacity of MGC-803 cells (Fig. 3D–E). The statistical column charts represent the migration and invasion ability of MGC-803 cells in different experimental groups are shown in Fig. 3E–F. Collectively, these results provided evidence that TNFAIP3 silencing could inhibit the tumorigenicity of gastric cancer cell in vitro.

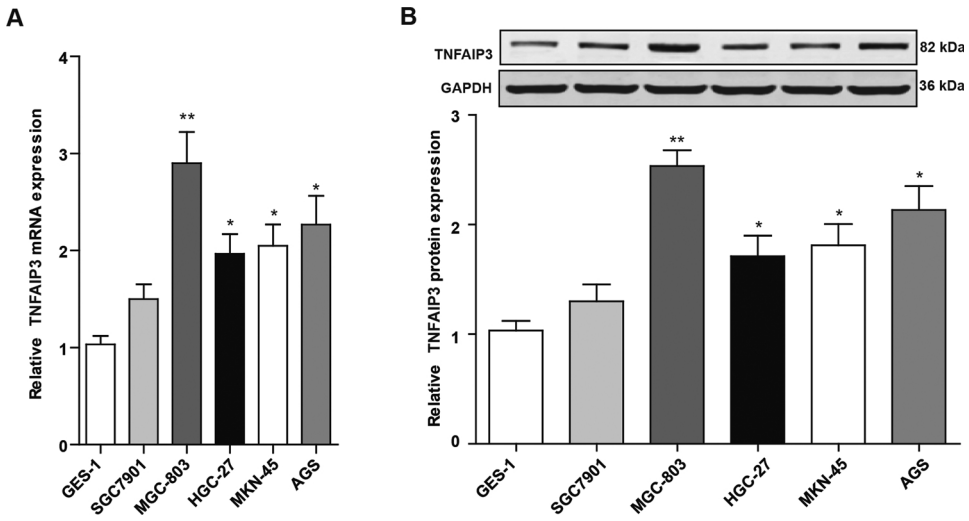

Fig. 2. The expression patterns of TNFAIP3 in gastric cancer cells. (A) The mRNA expression level of TNFAIP3 in a panel of 5 gastric cancer cell lines (SGC-7901, MGC-803, HGC-27, MKN-45, AGS) and a normal human gastric mucosal cell (GES-1) determined by qRT- PCR. n = 3, * $p < 0.05$ or ** $p < 0.01$ vs. GES-1.

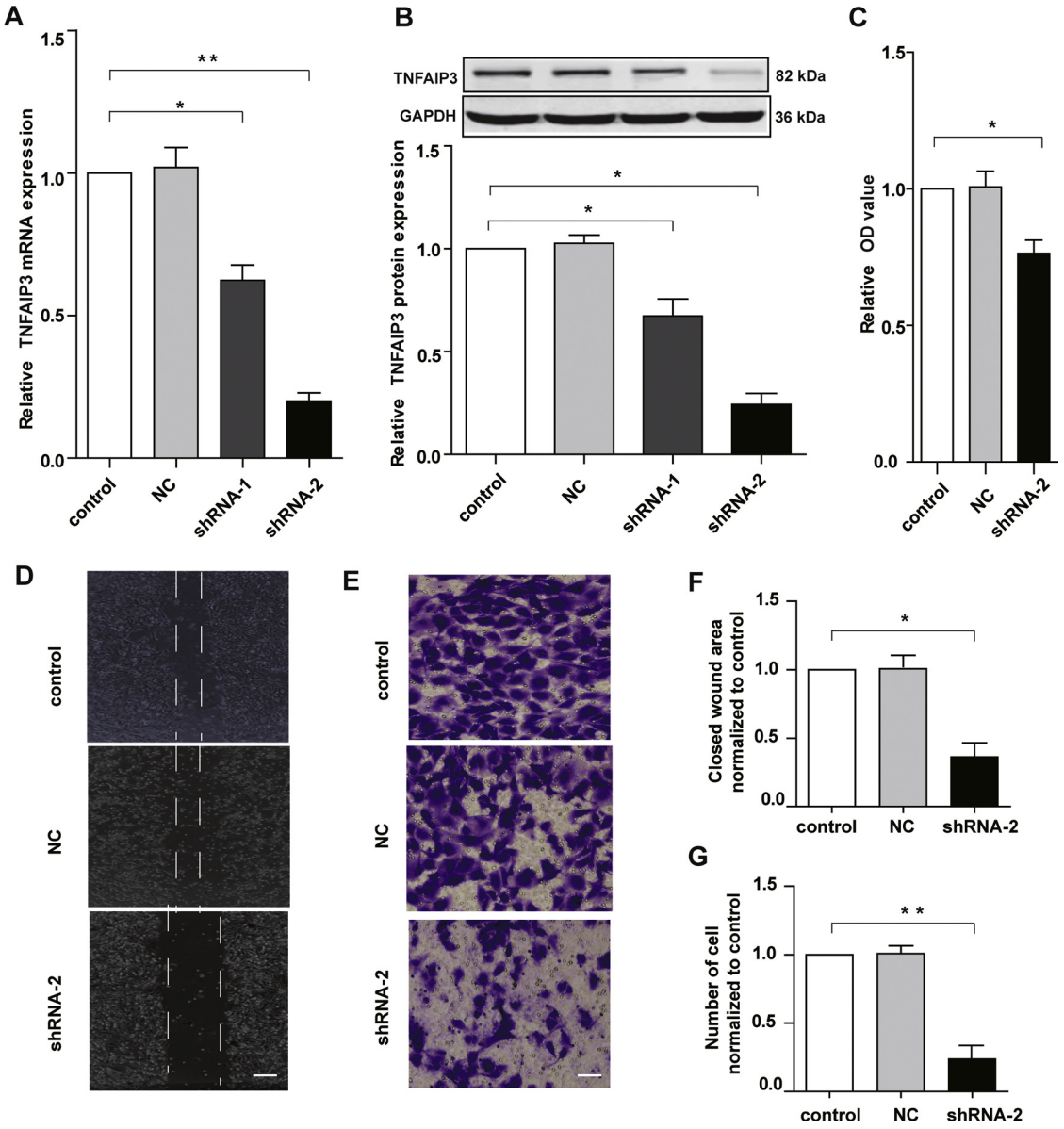

Fig. 3. TNFAIP3 silencing inhibits proliferation, migration and invasion of MGC-803 cells. (A) The efficiency of TNFAIP3 knockdown by shRNA1 and shRNA2 was confirmed by qRT- PCR. n = 3, * $p < 0.05$ or ** $p < 0.01$ vs. control. (B) The efficiency of TNFAIP3 knockdown by shRNA1 and shRNA2 confirmed by western blotting. n = 3, * $p < 0.05$ vs. control. (C) The proliferation of MGC-803 after transfection with negative control shRNA (NC) or TNFAIP3-shRNA (shRNA2) for 24 h determined by CCK8. n = 6, * $p < 0.05$ vs. control. (D) The migration ability of MGC803 cells assessed by scratch wound-healing assay. Scale bar 10 μm. (E) The invasion ability of MGC803 cells assessed by cell invasion assay. Scale bar 50 μm. (F) The statistical column chart represents the migration ability of MGC803 cells. n = 3, * $p < 0.05$ vs. control. (G) The statistical column chart represents the invasion ability of MGC803 cells. n = 3, ** $p < 0.01$ vs. control.

3.4. TNFAIP3 is involved in the process of EMT

Since epithelial-mesenchymal transition (EMT) is the key event in driving tumor migration and invasion, we further investigate the effects of TNFAIP3 silencing on the expression levels of EMT markers. Compared with the control cells, increase in E-cadherin protein expression and reduction in the protein expression of mesenchymal markers, including **N**-cadherin and vimentin, were seen in TNFAIP3-depleted MGC-803 cells. To investigate whether this variation caused by TNFAIP3 silencing is pre-transcriptional or post-transcriptional, qRT-PCR analysis was further performed. Interestingly, similar results were observed in the mRNA expression of CDH1, CDH2 and VIM, which encode E-cadherin, **N**-cadherin and vimentin, respectively (Fig. 4C). These observations prompted the investigation of EMT-related transcription factors including Snail and ZEB1.

3.5. TNFAIP3 is required for ubiquitination degradation of Snail and ZEB1

Based on our results that TNFAIP3 probably affects the expression of EMT markers through a pre-transcriptional regulatory mechanism, we investigated the expression of EMT-related transcription factors. As Fig. 5 showed, knockdown of TNFAIP3 dramatically reduced the expression of Snail and ZEB1, which were important EMT-related transcription factors. However, in the presence of the proteasome inhibitor MG132, the decreased Snail and ZEB1 expression was restored, which suggested that the reduction of EMT-related transcription factors (Snail and ZEB1) is probably achieved through TNFAIP3-mediated ubiquitination degradation. In addition, our hypothesis was further confirmed by the evidence that, 3-MA (inhibitor of PI3K) pre-treatment did not rescue the TNFAIP3 silencing induced decrease in the expression of Snail and ZEB1. Taken together, our results provide evidence that TNFAIP3 plays an important role in the ubiquitination degradation of

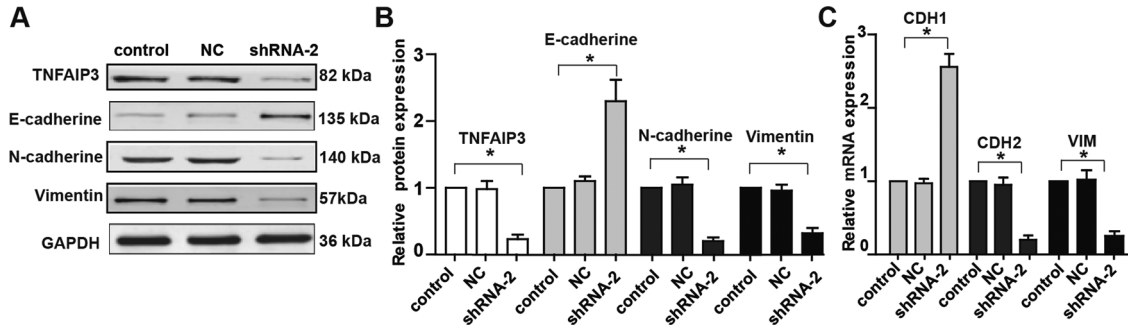

Fig. 4. Depletion of TNFAIP3 inhibits EMT. (A) Western blot analysis showed the decreased expression of epithelial makers (E-cadherin) and the increased expression of mesenchymal markers (N-cadherin and Vimentin). (B) The statistical column chart represents the relative expression of EMT-related proteins. n = 3, * $p < 0.05$ vs. control. (C) The statistical column chart represents the relative mRNA expression of CDH1, CDH2 and VIM determined by qRT- PCR, which encode E-cadherin, N-cadherin and vimentin, respectively. n = 3, * $p < 0.05$ vs. control.

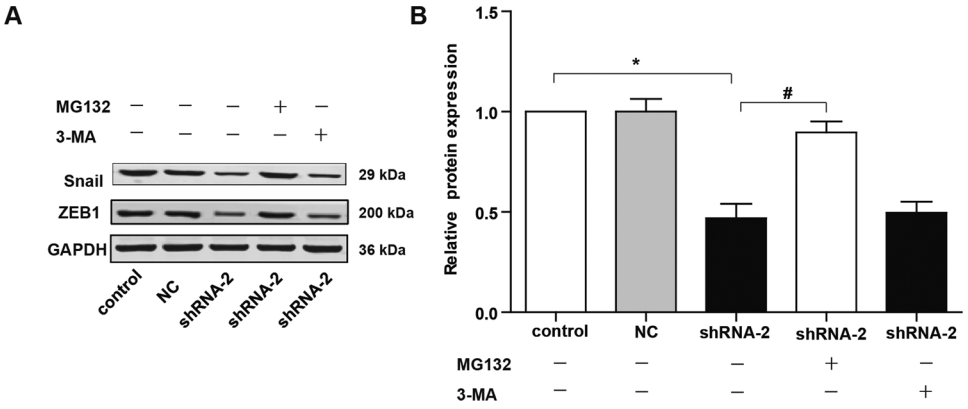

Fig. 5. TNFAIP3 affects EMT-related proteins through the ubiquitination pathway. (A) The protein expression of ZEB1 and Snail in the presence or absence of MG132 (proteasome inhibitor) or 3-MA (inhibitor of PI3K). (B) The statistical column chart represents the relative expression of EMT-related transcription factors ZEB1 and Snail. n = 3, * $p < 0.05$ vs. control or $^{\#}p < 0.05$ vs. shRNA2.

Snail and ZEB1, which further altered the expression of EMT-related proteins.

4. Discussion

Gastric carcinoma is considered to be a highly malignant tumor with complicated pathogenesis. Notably, the average five-year survival rate for gastric carcinoma is extremely poor and tumor metastasis is considered to be the major cause of gastric carcinoma-related deaths [5,16]. Thus, in order to get a better therapeutic effect and to improve the prognosis of this malignant tumor, the development of more efficacious targets is strongly required for improvement of clinical management.

Metastasis involved a multi-step cellular biological process, while EMT was identified as the essential initiation step for cancer metastasis [18,20]. When intracellular EMT occurred, loss of E-cadherin was considered as a fundamental event, which was accompanied with up-regulation of important markers of EMT, such as **N**-cadherin and vimentin [8,15]. Accumulating evidences have demonstrated that EMT played a key role in the process of tumorous progression and metastasis. Therefore, to reduce the capacity of gastric cancer metastasis, the potential molecular mechanisms regarding the pathway of EMT are highly valued.

In the previous researches, the functions of inflammation and immune regulator TNFAIP3 are well studied in inflammation diseases and immune responses [1,2], while it remains unknown whether it contributes to gastric tumorigenesis, invasion and metastasis. Although there was no direct evidence showing that TNFAIP3 is involved in gastric tumor metastasis, the TNFAIP3 interacting proteins, including transcription factors ZEB1 and Snail, have been demonstrated to be associated with gastric tumor invasion and migration [4,14,19], which prompted us to investigate the potential relationship between TNFAIP3 and gastric cancer metastasis.

In the present study, we identified that TNFAIP3 was frequently overexpressed in gastric cancer tissues and cell lines, and TNFAIP3 silencing statistically inhibited the proliferation, migration and invasion in vitro. The underlying mechanism may be ascribed to the pivotal role of TNFAIP3 in the ubiquitination degradation of EMT- related transcription factors Snail and ZEB1, which further altered the expression of EMT-related proteins and eventually contributing to the tumorous metastasis.

However, there are some limitations in our present research. Tumor cells possess high specificity and complexity, we only investigated the function of TNFAIP3 in MGC-803 cells which expressing the highest TNFAIP3 in a panel of 5 gastric cancer cell lines (SGC-7901, MGC-803, HGC-27, MK**N**-45, AGS) and a normal human gastric mucosal cell (GES-1). There is still lack of in vivo evidence in our study to further confirm the role of TNFAIP3 in gastric cancer. Besides, based on our current data, we conclude that TNFAIP3 plays a multi-faceted role in the development of gastric cancer. However, if knocking out TNFAIP3 gene in normal cells, will it affect the proliferation and apoptosis of normal cells? This requires further experimental evidence to prove.

In conclusion, the evidences in our study strongly propose a pathway linking the TNFAIP3 to the EMT-mediated metastatic process in gastric cancer cells, indicating that TNFAIP3 may serve as a clinically potential prognostic biomarker or effective therapeutic target for gastric cancer treatment.

Conflict of interest disclosures

The authors declare no actual or potential conflicts of interest in the present research.

Availability of data and materials

The data and graphs used in the present study are available from the corresponding authors on reasonable request.

Acknowledgements

Not applicable.

References

[1] Y.C. Chang, Y.S. Chang, C.C. Chang, T.C. Liu, Y.C. Ko, C.C. Lee, S.J. Chang, J.G. Chang, Development of a high-resolution melting method for the screening of TNFAIP3 gene mutations, Oncol. Rep. 35 (2016) 2936–2942.
[2] Y. Chu, V. Soberon, L. Glockner, R. Beyaert, R. Massoumi, G. van Loo, D. Krappmann, M. Schmidt-Supprian, A20 and CYLD do not share significant overlapping functions during B cell development and activation, J. Immunol. 189 (2012) 4437–4443.
[3] C. Da, Y. Liu, Y. Zhan, K. Liu, R. Wang, Nobiletin inhibits epithelial-mesenchymal transition of human non-small cell lung cancer cells by antagonizing the TGF-beta1/Smad3 signaling pathway, Oncol. Rep. 35 (2016) 2767–2774.
[4] J.H. Lee, S.M. Jung, K.M. Yang, E. Bae, S.G. Ahn, J.S. Park, D. Seo, M. Kim, J. Ha, J. Lee, J.H. Kim, A. Ooshima, J. Park, D. Shin, Y.S. Lee, S. Lee, G. van Loo, J. Jeong, S.J. Kim, S.H. Park, A20 promotes metastasis of aggressive basal-like breast cancers through multi-monoubiquitylation of Snail1, Nat. Cell Biol. 19 (2017) 1260–1273.
[5] Z. Li, J. Liu, L. Li, S. Shao, J. Wu, L. Bian, Y. He, Epithelial mesenchymal transition induced by the CXCL9/CXCR3 axis through AKT activation promotes invasion and metastasis in tongue squamous cell carcinoma, Oncol. Rep. (2017).
[6] S. McGuire, World cancer report 2014, World Health Organization, International Agency for Research on Cancer, WHO Press, Geneva, Switzerland, 2015, pp. 418–419. Adv Nutr 7 (2016).
[7] M. Orditura, G. Galizia, V. Sforza, V. Gambardella, A. Fabozzi, M.M. Laterza, F. Andreozzi, J. Ventriglia, B. Savastano, A. Mabilia, E. Lieto, F. Ciardiello, F. De Vita, Treatment of gastric cancer, World J. Gastroenterol. 20 (2014) 1635–1649.
[8] H. Peinado, D. Olmeda, A. Cano, Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? Nat. Rev. Cancer 7 (2007) 415–428.
[9] P.P. Shah, W.W. Lockwood, K. Saurabh, Z. Kurlawala, S.P. Shannon, S. Waigel, W. Zacharias, L.J. Beverly, Ubiquilin1 represses migration and epithelial-to-mesenchymal transition of human non-small cell lung cancer cells, Oncogene 34 (2015) 1709–1717.
[10] G.V. Shrikhande, S.T. Scali, C.G. da Silva, S.M. Damrauer, E. Csizmadia, P. Putheti, M. Matthey, R. Arjoon, R. Patel, J.J. Siracuse, E.R. Maccariello, N.D. Andersen, T. Monahan, C. Peterson, S. Essayagh, P. Studer, R.P. Guedes, O. Kocher, A. Usheva, A. Veves, E. Kaczmarek, C. Ferran, O-glycosylation regulates ubiquitination and degradation of the anti-inflammatory protein A20 to accelerate atherosclerosis in diabetic ApoE-null mice, PLoS One 5 (2010) e14240.
[11] S.L. Tang, Y.L. Gao, H. Wen-Zhong, Knockdown of TRIM37 suppresses the proliferation, migration and invasion of glioma cells through the inactivation of PI3K/Akt signaling pathway, Biomed. Pharmacother. 99 (2018) 59–64.
[12] L. Verstrepen, K. Verhelst, G. van Loo, I. Carpentier, S.C. Ley, R. Beyaert, Expression, biological activities and mechanisms of action of A20 (TNFAIP3), Biochem. Pharmacol. 80 (2010) 2009–2020.
[13] X. Wang, B. Liu, F. Wen, Y. Song, MicroRNA-454 inhibits the malignant biological behaviours of gastric cancer cells by directly targeting mitogen-activated protein kinase 1, Oncol. Rep. (2017).
[14] Y. Wang, M. Wen, Y. Kwon, Y. Xu, Y. Liu, P. Zhang, X. He, Q. Wang, Y. Huang, K.Y. Jen, M.A. LaBarge, L. You, S.C. Kogan, J.W. Gray, J.H. Mao, G. Wei, CUL4A induces epithelial-mesenchymal transition and promotes cancer metastasis by regulating ZEB1 expression, Cancer Res. 74 (2014) 520–531.
[15] J. Yang, R.A. Weinberg, Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis, Dev. Cell 14 (2008) 818–829.
[16] S. Yuan, X. Dong, X. Tao, L. Xu, J. Ruan, J. Peng, A. Xu, Emergence of the A20/ABIN-mediated inhibition of NF-kappaB signaling via modifying the ubiquitinated proteins in a basal chordate, Proc. Natl. Acad. Sci. U. S. A 111 (2014) 6720–6725.
[17] Z. Yuan, X. Yu, B. Ni, D. Chen, Z. Yang, J. Huang, J. Wang, L. Wang, Overexpression of long non-coding RNA-CTD903 inhibits colorectal cancer invasion and migration by repressing Wnt/beta-catenin signaling and predicts favorable prognosis, Int. J. Oncol. 48 (2016) 2675–2685.
[18] J. Zhao, X. Huang, Z. Xu, J. Dai, H. He, Y. Zhu, H. Wang, LDHA promotes tumor metastasis by facilitating epithelialmesenchymal transition in renal cell carcinoma, Mol. Med. Rep. 16 (2017) 8335–8344.
[19] S. Zou, C. Ma, F. Yang, X. Xu, J. Jia, Z. Liu, FBXO31 suppresses gastric Cancer emt by targeting Snail1 for proteasomal degradation, Mol. Cancer Res. (2017).
[20] L.L. Zuo, J. Zhang, L.Z. Liu, Q. Zhou, S.J. Du, S.Y. Xin, Z.P. Ning, J. Yang, H.B. Yu, W.X. Yue, J. Wang, F.X. Zhu, G.Y. Li, J.H. Lu, Cadherin 6 is activated by Epstein-Barr virus LMP1 to mediate EMT and metastasis as an interplay node of multiple pathways in nasopharyngeal carcinoma, Oncogenesis 6 (2017) 402.