Heliyon 10 (2024) e30986

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

IGFBP7 promotes gastric cancer by facilitating epithelial-mesenchymal transition of gastric cells

Jinqing Wang [a], Xinxin Wang [a], Zhaorui Liu [a], Sheng Li [b,c,*], Wenbin Yin [d,**]

[a] Department of Gastrointestinal Surgery, The Second Hospital of Shandong University, Jinan, China
[b] Shandong University Cancer Center, Jinan, China
[c] Shandong First Medical University and Shandong Academy of Medical Sciences, Jinan, China
[d] Department of Geriatric Medicine, Qilu Hospital of Shandong University, Jinan, China

ARTICLE INFO

Keywords:
Gastric cancer
Migration
Invasion
EMT
IGFBP7

ABSTRACT

Gastric cancer (GC) with high morbidity and mortality is one major cause of tumor-related death. Mechanisms underlying GC invasion and metastasis remain unclear. IGFBP7 exerted variable effects in different cancers and its role in GC is controversial. Here, IGFBP7 was found to be upregulated and elevated IGFBP7 expression represented a poorer overall survival in GC using bioinformatics analysis. Moreover, IGFBP7 was up-regulated in human GC specimens and promoted tumor growth in xenograft tumor animals. For GC cell lines, we found that IGFBP7 was also upregulated and facilitated the cell malignant behavior and EMT of GC cells, which may involve NF-κB and ERK signaling pathways. This research may provide new avenues for GC therapy.

1. Introduction

Gastric cancer (GC) is highly heterogeneous malignancy and one leading cause of tumor-related mortality [1]. Due to rapid progression and lack of early clinical signs, about 80 % of GC patients are diagnosed as advanced stage, which seriously affects their prognosis and survival [2]. Although diagnostic techniques and treatment strategies of GC have been greatly improved, advanced patients' prognosis is still poor [3]. Therefore, the identification of specific biomarkers for GC diagnosis and treatment remains an urgent clinical need.

IGFBP7, which belongs to the IGFBP family, is a secreted protein that is a potential tumor suppressor for hepatocellular carcinoma, colorectal, breast and thyroid cancer [4–8]. However, data of IGFBP7 in GC was limited. Recent studies have showed that IGFBP7 has a carcinogenic role in GC, and IGFBP7 upregulation is a poor prognosis predictor for GC patients [9,10]. However, IGFBP7 has also been reported to have a tumor suppressor role in GC [11,12]. Therefore, the confirmation of IGFBP7' biological function in GC needs more evidence.

EMT is a key driver in tumor progression. During EMT process, cancer cells differentiate from epithelial features to stromal features, exhibiting reduced adhesion and highly invasion and metastasis ability [13]. Cellular adhesion proteins such as E-cadherin expression decreased while mesenchymal markers such as N-cadherin and vimentin expression increased [14]. Here, we also identified a novel role of IGFBP7 in GC cell malignant behavior and EMT.

* Corresponding author. Shandong University Cancer Center, Jinan, China.
** Corresponding author.
E-mail addresses: lisheng@sdfmu.edu.cn (S. Li), qiluyinwenbin@email.sdu.edu.cn (W. Yin).

https://doi.org/10.1016/j.heliyon.2024.e30986
Received 21 March 2024; Received in revised form 8 May 2024; Accepted 9 May 2024
Available online 9 May 2024

2. Materials and methods

2.1. Biological analysis

We used biological analysis methods to search for three typical databases related to GC through the GEO online database: GSE13911, GSE19826, and GSE79973 (all three datasets were sourced from the GPL570 platform to eliminate differences between platforms. GEO2R was applied to screen for differentially expressed genes. Draw Venn Diagram was used to create a VENN diagram to identify intersection genes related to GC. The intersection genes were analyzed by STRING (functional protein association networks) online database, and the key gene IGFBP-7 was found. The overall survival rate and disease-free survival rate of key genes were analyzed by cBioportal database, with $P < 0.05$ indicating statistically significance.

2.2. Clinical tissues

GC patients were carefully chosen from our hospital. The patients did not undergo radiotherapy, chemotherapy and other treatments before surgery. The study protocol was approved by the ethics committees of The Second Hospital of Shandong University [No. KYLL-2019(KJ)P-0121]. This study was conducted in accordance with the principles of the Declaration of Helsinki (2013). All patients signed the informed consent.

2.3. Cell culture and transfection

GC cells (SGC-7901, MGC-803, AGS, HGC-27) and human gastric mucosa cells (GES-1) which obtained from GeneChem (Shanghai, China) were cultured as described in a previous study [15]. The lentivirus expressing sh-IGFBP7, sh-NC, ov-IGFBP7 and ov-NC which obtained from GenePharma (Shanghai, China) were transfected into the cells separately by the manufacturer's experimental protocols of Lipofectamine 2000 kit (Invitrogen, USA). Cells were cultured for 48 h.

2.4. Subcutaneous tumorigenicity assay

Male BALB/c nude mice (5 weeks) were obtained from The Model Animal Research Centre of Shandong University. All animal procedures were approved by the Ethics Committee of The Second Hospital of Shandong University [No. KYLL-2019(KJ)A-0166]. After acclimation for one week, animals were randomly divided into mock, sh-IGFBP7 and ov-IGFBP7 group. GC cells (1×10^7 cells) of each group were injected subcutaneously into axilla of the mice. Growth of the subcutaneous tumor was observed daily. After 3 weeks, mice were sacrificed and removed xenograft tumors.

2.5. MTT assay

1×10^4 cells were inoculated in each well of 96-well plates. After adding 10 μl MTT, cells were cultured for 4 h. MTT crystals were dissolved with DMSO. Absorbance at 570 nm was measured using a spectrophotometer to indicate cell viability.

2.6. Flow cytometric analysis

Cells with different treatments were collected and conducted the following procedures of the Annexin V-FITC/PI apoptosis kit and cell cycle staining kit (Multi Sciences, China). Transfected cells were collected and suspended to 1×10^5 cells/ml. 100 μl cell suspension was added into flow tube, then Annexin V/FITC and PI were added. After incubation for 15 min without light, cell apoptosis was measured by flow cytometry and analyzed by ModFit software. The cells of each group were collected, washed and fixed with cold ethanol. DNASE-free RNaseA was added. Then PI was added, mixed well, and incubated at room temperature for 15 min away from light. PI content in cells was detected by flow cytometry. The distribution of cell cycles was analyzed by ModFit software.

2.7. Wound healing assay

Cells were seeded in 6-well plates with 1×10^3 cells/well. When the cells covered 90 % of the space in the plate, a straight scratch was made using a 200 μl sterile pipette tip. After incubation for 48 h, wound closures were visualized under an Olympus microscope. Migration rate was measured using ImageJ and calculated.

2.8. Transwel assay

Upper surface of Transwell chambers were coated with or without 60 μl Matrigel (BD Biosciences, USA). Cells cultured overnight with serum-free medium were added into the upper chambers at 2.5×10^5 cells/well. Low chambers were filled with medium containing 10 % FBS. After incubation for 24 h, cells on the lower surface of membranes were fixed with 4 % paraformaldehyde and stained with crystal violet solution (Solarbio, China), while cells on the upper surface were removed with a cotton swab. Migration and invasion cells were counted under a fluorescence microscope.

2.9. RT-qPCR

Total RNA was extracted with TRIzol reagent, and cDNA was synthesized according to the instructions of reverse transcription kit. IGFBP7 mRNA relative expression was detected by the SYBR Green Premix Ex TaqTM quantitative assay kit. $2^{-\Delta\Delta Ct}$ method was used to quantify the data, with GAPDH as the internal parameter.

2.10. Western blotting

Equal amount of proteins from tissues or cells were separated by SDS-PAGE and were electro-transferred onto a PVDF membrane. After blocking (5 % non-fat milk) at room temperature for 1 h, the membranes were probed with primary antibodies overnight at 4 °C, followed by incubation with HRP-conjugated secondary antibodies. The primary antibodies were against IGFBP7 (Proteintech, 1:1000), E-cadherin, vimentin, snail, N-cadherin, ERK, *p*-ERK, p65, p-p65 and β-actin (Huabio, China, 1:1000). The secondary antibodies were from ZSGB-BIO (China) (1:2000). Bands were visualized using an ECL detection system and analyzed using Image J.

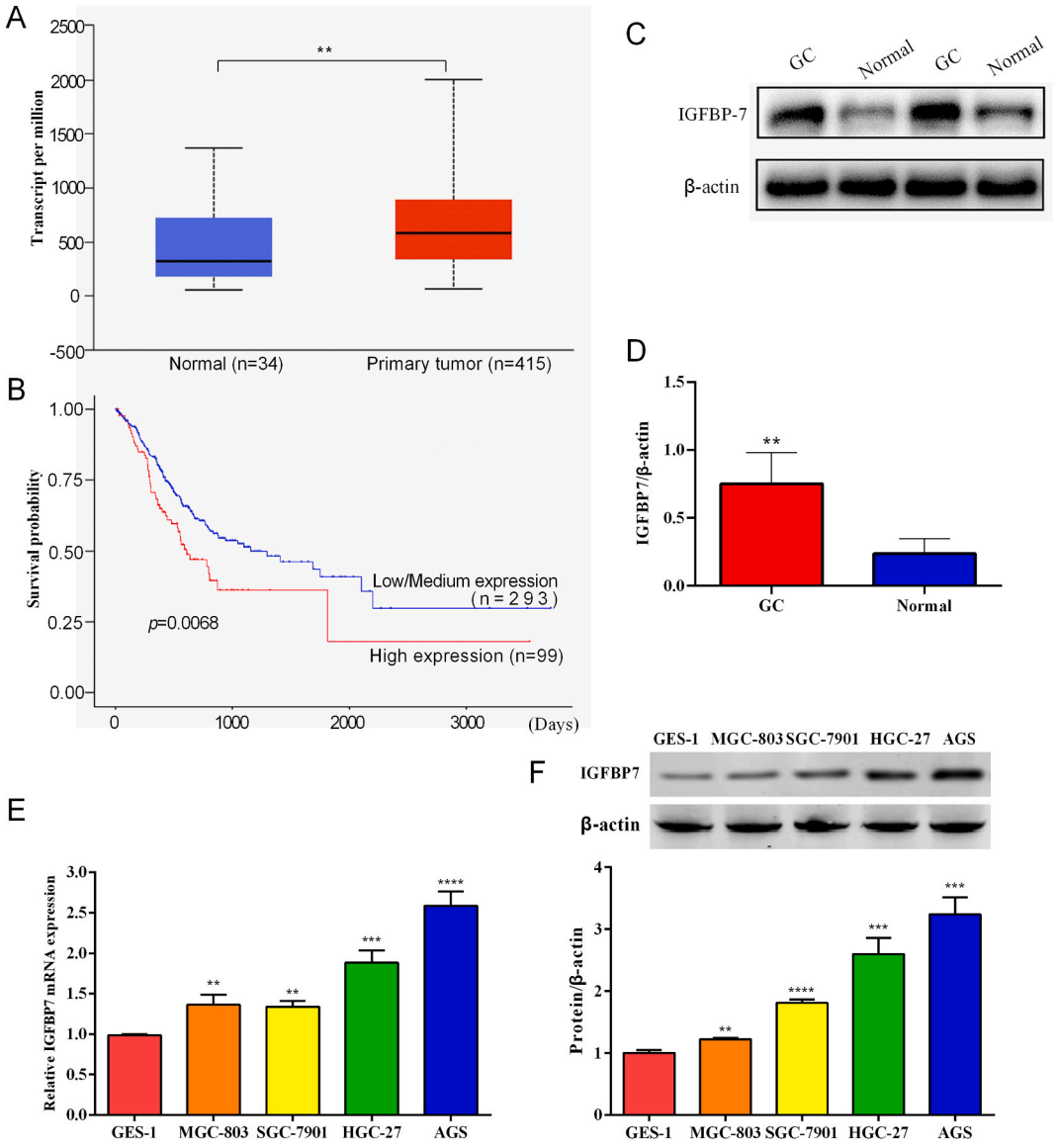

Fig. 1. IGFBP7 is upregulated and indicates poor prognosis in GC. (A) Bioinformatic analysis of IGFBP7 expression and (B) its association with overall survival in GC. (C) Representative western blots of IGFBP7 in human GC specimens (the full, non-adjusted images of western blots was showed in S-Fig. 1C as supplementary material). (D) Quantitative analysis of the IGFBP7/β-actin ratio. (E, F) IGFBP7 expression levels in GC cell lines (the full, non-adjusted images of western blots was showed in S-Fig. 1F as supplementary material). $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

2.11. Statistical analysis

Statistical analysis was performed using SPSS software. Significant differences among groups were evaluated using one-way analysis of variance followed by Tukey's HSD test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. IGFBP7 indicates poor prognosis and is upregulated in GC

The analysis of IGFBP7 expression of 415 STAD (stomach adenocarcinoma, primary tumor) and 34 normal samples (non-tumorous tissue), and the relationship between survival and IGFBP7 expression in 392 STAD samples were conducted through the TCGA database on the University of Alabama at Birmingham Cancer data analysis Portal (https://ualcan.path.uab.edu/). In STAD samples, expression of IGFBP7 series (Transcript per million) was: minimum 59.233, lower quartile 342.563, median 581.831, upper quartile 881.189, maximum 1998.8. High expression >581.381 transcript per million, low/medium expression ≤581.381 transcript per million (https://ualcan.path.uab.edu/). IGFBP7 expression was found to be increased in GC (Fig. 1A). Moreover, high IGFBP7 expression was strongly related to poor survival (Fig. 1B). This indicates a significant clinical correlation between IGFBP-7 and GC, and may relates to clinical diagnosis and treatment of GC. Further research found that IGFBP7 was notably upregulated in GC tissue (Fig. 1C and D). Moreover, IGFBP7 expression was also increased in GC cells (Fig. 1E and F). Among them, AGS has the highest IGFBP7 expression, so AGS was selected for the subsequent experiment. Our data shows that IGFBP7 expression is increased and relates to poor prognosis in GC.

3.2. IGPBP7 promotes xenograft GC tumor growth in mice

The tumorigenic potential of IGFBP7 *in vivo* was assessed by subcutaneous tumorigenicity assay. The size of the xenograft tumors derived from the IGFBP7-overexpression GC cells grew more rapidly, while those from the IGFBP7-knockdown cells grew more slowly than those of mock cells (Fig. 2A and B). Moreover, the weight and size of the xenograft tumors from sh-IGFBP7 group were decreased, while those from ov-IGFBP7 group were notably increased versus mock group (Fig. 2C and D). Thus, IGFBP7 was suggested to exert an oncogenic role in GC.

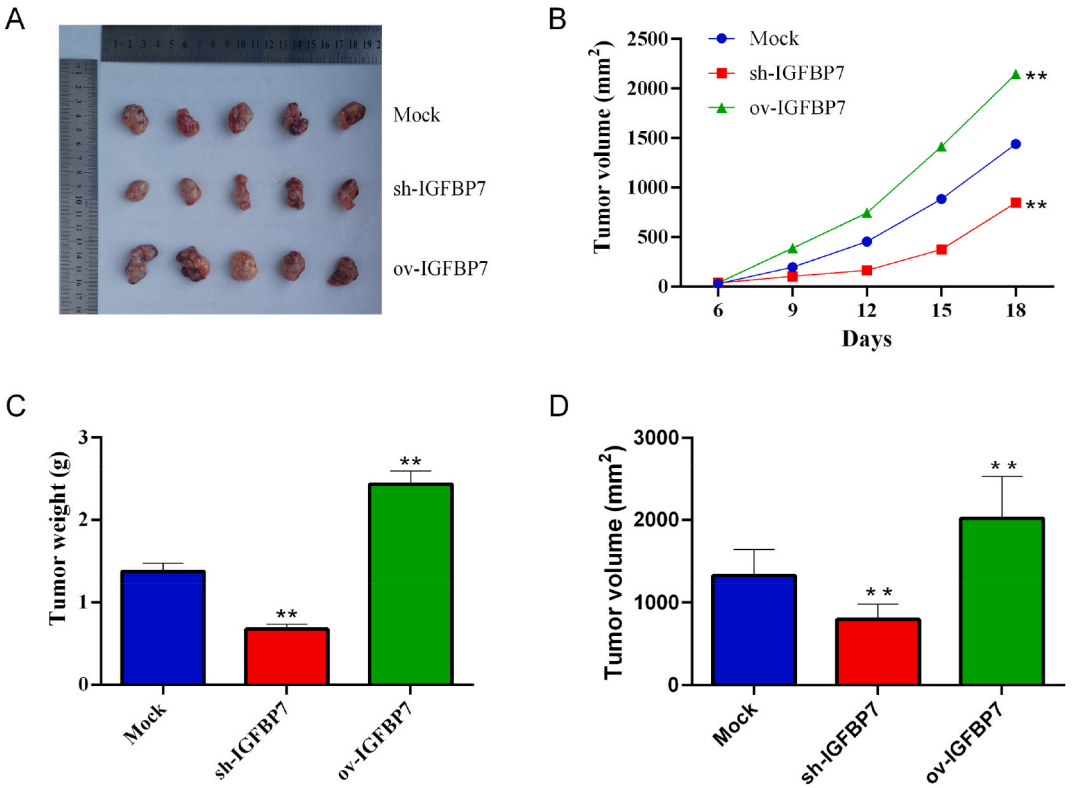

Fig. 2. IGPBP7 promotes xenograft tumor growth in mice. (A) Photographs of the tumors of mock, sh-IGFBP7 and ov-IGFBP7 cells. (B) Growth rate of size of the xenograft tumors. (C, D) Measurements of tumor weight and volume. $^{**}p < 0.01$. Sh-IGFBP7, IGFBP7 knockdown; ov-IGFBP7, IGFBP7 overexpression.

3.3. IGFBP7 promotes proliferation and suppresses apoptosis of GC cells

Cell cycle phase distribution and apoptosis were detected by flow cytometric analysis. IGFBP7 inhibition in GC cells had a dramatically decreased in G2/M phase fraction, while IGFBP7 overexpression induced an increase in G2/M phase fraction (Fig. 3A and C). Compared with NC group, apoptosis of GC cells was obviously facilitated by IGFBP7 knockdown and was largely restrained by IGFBP7 overexpression (Fig. 3B and D). Moreover, IGFBP7 knockdown inhibited cell growth while IGFBP7 overexpression promoted cell growth of GC cells (Fig. 3E). This suggested that IGFBP7 positively regulate proliferation and reduce apoptosis of GC.

3.4. IGFBP7 enhances GC cells migration and invasion

How IGFBP7 affects GC cells migration and invasion was also detected. IGFBP7 knockdown dramatically suppressed and IGFBP7 overexpression largely promoted GC cells migratory abilities (Fig. 4A and C). Accordingly, Transwell assay suggested that both migratory and invaded cells were reduced by IGFBP7 knockdown, while both cells were increased by IGFBP7 overexpression (Fig. 4B, D, 4E). These data together verifies that IGFBP7 contributes to GC cell migration and invasion.

3.5. IGFBP7 promotes EMT and activates NF-κB and ERK1/2 pathways in GC cells

To explore the regulation role of IGFBP7 on EMT of GC cells, EMT-related proteins were detected. IGFBP7 knockdown increased E-

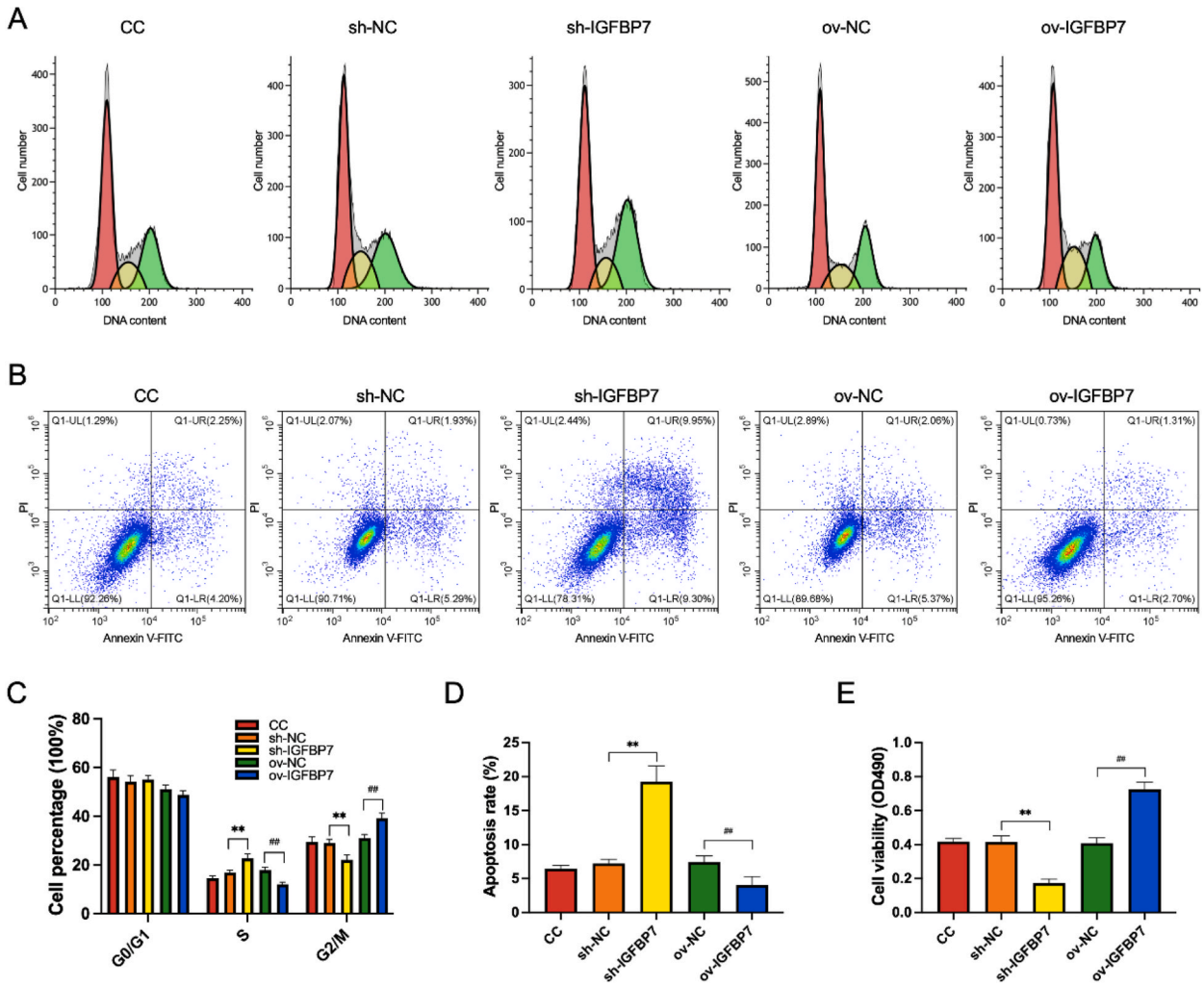

Fig. 3. IGFBP7 suppresses apoptosis and promotes proliferation of GC cells. (A, C) Cell cycle distribution in IGFBP7-knockdown and overexpressed GC cells. (B, D) Flow cytometry and statistic results of apoptosis in IGFBP7-knockdown and overexpressed GC cells. (E) Effects of IGFBP7 knockdown and overexpression on cell viability. $**p < 0.01$. $\#\#p < 0.01$. CC, normal control; sh-NC, negative control for IGFBP7 knockdown; ov-NC, negative control for IGFBP7 overexpression.

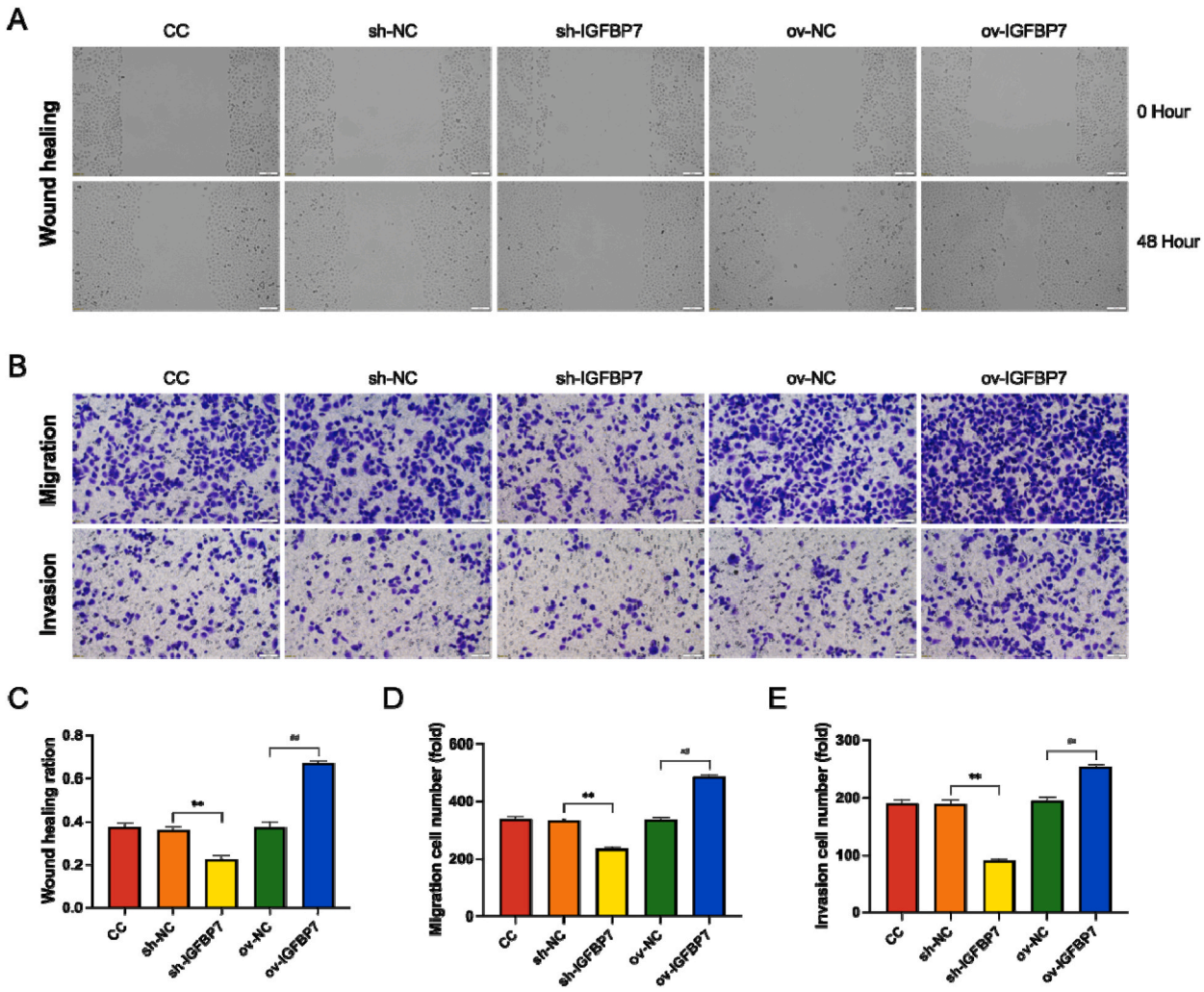

Fig. 4. IGFBP7 enhances GC cell migration and invasion. (A, C) Wound healing assay and quantitative analysis of wound healing ratio. (B, D, E) Transwell assay of migration and invasion cells. $^{**}p < 0.01$. $\#\#p < 0.01$.

cadherin expression and decreased N-cadherin, snail and vimentin expressions, while their expression showed the opposite trend in IGFBP7 overexpression (Fig. 5A).

To further investigate the underlying mechanism, p65, p-p65 and *p*-ERK1/2 expression in GC cells were explored. Results suggested *p*-ERK1/2 and p-p65 were largely decreased by IGFBP7 knockdown, while IGFBP7 overexpression notably increased the expression levels of those proteins (Fig. 5B).

4. Discussion

IGFBPs, an important regulatory factor of insulin-like growth factor (IGF), have altered their expression levels in tumors, thereby affecting the biological activity of IGF and tumor progression [16]. IGFBPs show different expressions in cancers [17], in which IGFBP7 is believed to be upregulated in GC and correlated with pathological stages [18]. However, opinions on IGFBP7 in GC are still not quite definite and even controversial sometimes. Most reports about IGFBP7 on GC are from clinical cohorts data or bioinformatics analysis [9–12], more investigations from human specimens or animal models are still needed to verify the role of IGFBP7 and its potential mechanisms in GC. Results from our bioinformatics analysis found IGFBP7 expression was increased and IGFBP7 overexpression related to poor overall survival in GC. Moreover, IGFBP7 expression was enhanced in human GC specimens and cells. IGFBP7 also promoted tumor growth *in vivo*. Thus, IGFBP7 exhibits carcinogenesis and high IGFBP7 expression represents poor prognosis of GC patients, which was consist with previous studies [9,10].

Metastasis is the most important cause of death in cancer patients [19,20]. EMT is vital in cancer progression due to its invasive and metastatic behaviors [21–24]. However, the association between IGFBP7 and EMT was only investigated in a few studies. IGFBP7 was reported to suppress EMT and tumor metastasis by inhibiting TGF-β-mediated EMT via the Smad signaling cascade in colorectal cancer

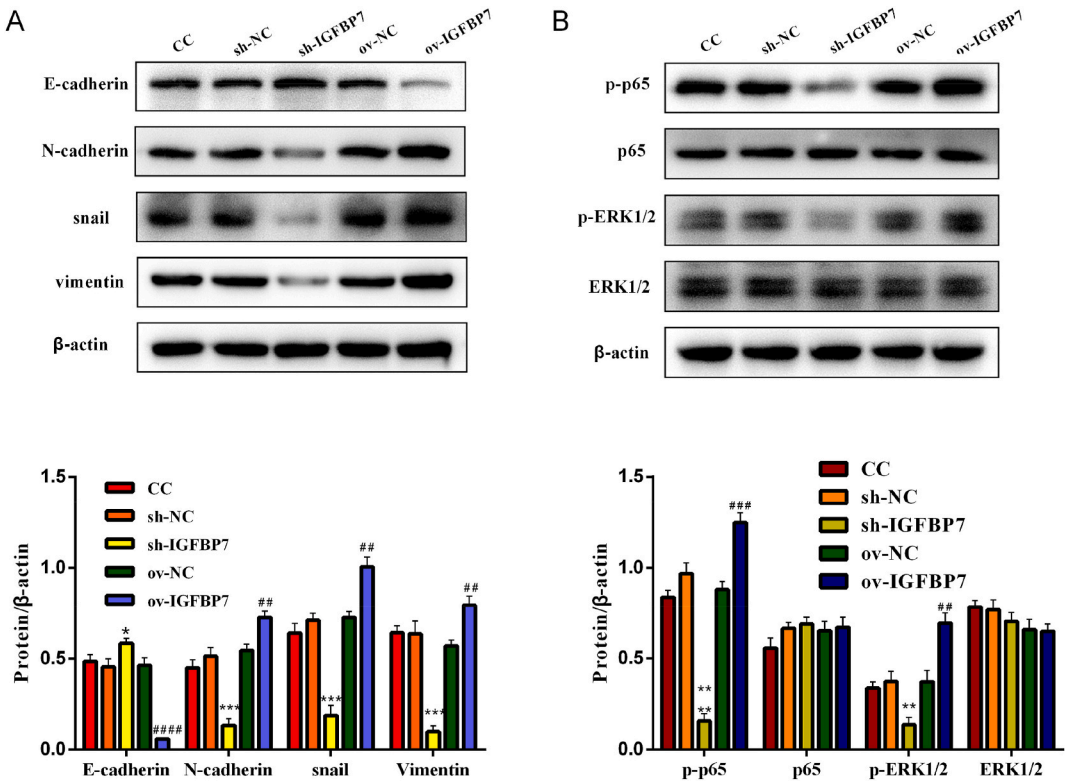

Fig. 5. IGFBP7 promotes EMT and activates NF-κB and ERK1/2 pathways in GC cells. (A) EMT-related proteins were analyzed by western blots. (B) Western blots analysis of p65, ERK1/2, *p*-ERK1/2 and p-p65. The full, non-adjusted images of western blots was showed inS-Fig 5A and S-Fig. 5B as supplementary material. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$. $\#\#p < 0.01$, $\#\#\#p < 0.001$, $\#\#\#\#p < 0.0001$.

[25]. One study also suggested that IGFBP7 downregulation facilitates liver metastasis by regulating EMT in colon cancer [26]. However, IGFBP7 exhibits opposite roles in this study. Our results found that IGFBP7 promoted EMT by increasing N-cadherin, snail and vimentin expressions and decreasing E-cadherin expression of GC cells. Consistent with our study, inhibiting IGFBP7 effectively inhibited the EMT induced by LPS or CLP in HK2 cells or mice [27]. Additionally, IGFBP7 knockdown attenuated radiation-induced EMT-related markers expression in HPAEpic cells [28]. Therefore, IGFBP7 expression is tightly associated with microenvironment and its regulation on EMT varies according to different conditions. Our results suggest that IGFBP7 promotes EMT in GC progression.

NF-κB pathway plays vital role in promoting malignancies development, such as breast, ovarian, pancreatic and gastric cancers [29–32]. NF-κB pathway was also involved in EMT process [33,34]. MAPK/ERK pathways also play important roles in cell proliferation and also participate in metastasis process [35–37]. ERK signaling pathway contributes to radiation-induced EMT via modulating snail, E-cadherin, α-SMA and phosphorylated GSK3β protein levels [28,38,39]. Involvement of NF-κB and ERK pathways with IGFBP7 in GC remains ambiguous. Our results showed that IGFBP7 overexpression activates NF-κB and ERK1/2 pathways while IGFBP7 knockdown attenuates their activation. Therefore, modulation of IGFBP7 in EMT may partially through NF-κB and ERK1/2 pathways. However, several limitations are still exist in this study. For example, only one cell line *in vitro* and *vivo* experiments. Whether IGFBP7 acts directly or indirectly on the NF-κB and ERK pathways to regulate EMT remains unclear. Thus, more investigations are still needed to verify.

In conclusion, IGFBP7 is highly expressed and acts tumorigenic role in GC. IGFBP7 promotes tumor growth by enhancing GC cells proliferation, migration, invasion and EMT, which may involve NF-κB and ERK pathways.

Ethical approval

All animal procedures were approved by the Ethics Committee of The Second Hospital of Shandong University.

Data availability statement

All data generated or analyzed during this study are included in the published article.

Funding

This study was funded by Natural Foundation of Shandong Province (ZR2020QH181), the Academic Promotion Program of Shandong First Medical University (2019QL004).

CRediT authorship contribution statement

Jinqing Wang: Data curation, Conceptualization. **Xinxin Wang:** Formal analysis, Data curation. **Zhaorui Liu:** Methodology, Investigation. **Sheng Li:** Supervision, Software, Resources. **Wenbin Yin:** Writing – original draft, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: This study was funded by Natural Foundation of Shandong Province (ZR2020QH181), the Academic Promotion Program of Shandong First Medical University (2019QL004). Authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30986.

References

[1] R.L. Siegel, K.D. Miller, H.E. Fuchs, A. Jemal, Cancer statistics, 2021, Ca - Cancer J. Clin. 71 (1) (2021) 7–33.
[2] E. Van Cutsem, X. Sagaert, B. Topal, K. Haustermans, H. Prenen, Gastric cancer, Lancet 388 (10060) (2016) 2654–2664.
[3] K.D. Miller, R.L. Siegel, C.C. Lin, A.B. Mariotto, J.L. Kramer, J.H. Rowland, K.D. Stein, R. Alteri, A. Jemal, Cancer treatment and survivorship statistics, 2016, Ca - Cancer J. Clin. 66 (4) (2016) 271–289.
[4] V. Hwa, Y. Oh, Rosenfeld RG: the insulin-like growth factor-binding protein (IGFBP) superfamily, Endocr. Rev. 20 (6) (1999) 761–787.
[5] D. Chen, A. Siddiq, L. Emdad, D. Rajasekaran, R. Gredler, X.N. Shen, P.K. Santhekadur, J. Srivastava, C.L. Robertson, I. Dmitriev, E.A. Kashentseva, D.T. Curiel, P.B. Fisher, D. Sarkar, Insulin-like growth factor-binding protein-7 (IGFBP7): a promising gene therapeutic for hepatocellular carcinoma (HCC), Mol. Ther. 21 (4) (2013) 758–766.
[6] A.M. Burger, X. Zhang, H. Li, J.L. Ostrowski, B. Beatty, M. Venanzoni, T. Papas, A. Seth, Down-regulation of T1A12/mac25, a novel insulin-like growth factor binding protein related gene, is associated with disease progression in breast carcinomas, Oncogene 16 (19) (1998) 2459–2467.
[7] H. Suzuki, S. Igarashi, M. Nojima, R. Maruyama, E. Yamamoto, M. Kai, H. Akashi, Y. Watanabe, H. Yamamoto, Y. Sasaki, F. Itoh, K. Imai, T. Sugai, L. Shen, J. P. Issa, Y. Shinomura, T. Tokino, M. Toyota, IGFBP7 is a p53-responsive gene specifically silenced in colorectal cancer with CpG island methylator phenotype, Carcinogenesis 31 (3) (2010) 342–349.
[8] M.G. Vizioli, M. Sensi, C. Miranda, L. Cleris, F. Formelli, M.C. Anania, M.A. Pierotti, A. Greco, IGFBP7: an oncosuppressor gene in thyroid carcinogenesis, Oncogene 29 (26) (2010) 3835–3844.
[9] D. Li, L. Xia, P. Huang, Z. Wang, Q. Guo, C. Huang, W. Leng, S. Qin, Cancer-associated fibroblast-secreted IGFBP7 promotes gastric cancer by enhancing tumor associated macrophage infiltration via FGF2/FGFR1/PI3K/AKT axis, Cell Death Dis. 9 (1) (2023) 17.
[10] Q. Zhao, R. Zhao, C. Song, H. Wang, J. Rong, F. Wang, L. Yan, Y. Song, Y. Xie, Increased IGFBP7 expression correlates with poor prognosis and immune infiltration in gastric cancer, J. Cancer 12 (5) (2021) 1343–1355.
[11] J. Kim, W.H. Kim, S.J. Byeon, B.L. Lee, M.A. Kim, Epigenetic downregulation and growth inhibition of IGFBP7 in gastric cancer, Asian Pac. J. Cancer Prev. APJCP 19 (3) (2018) 667–675.
[12] L. Liu, Z. Yang, W. Zhang, B. Yan, Q. Gu, J. Jiao, X. Yue, Decreased expression of IGFBP7 was a poor prognosis predictor for gastric cancer patients, Tumour. Biol. 35 (9) (2014) 8875–8881.
[13] J.P. Thiery, H. Acloque, R.Y. Huang, M.A. Nieto, Epithelial-mesenchymal transitions in development and disease, Cell 139 (5) (2009) 871–890.
[14] R. Kalluri, R.A. Weinberg, The basics of epithelial-mesenchymal transition, J. Clin. Invest. 119 (6) (2009) 1420–1428.
[15] Y. Chen, B. Wang, Z. Zhao, M. Li, F. Wang, PRSS2 overexpression relates to poor prognosis and promotes proliferation, migration and invasion in gastric cancer, Tissue Cell (2022) 79101949.
[16] M.K. Kashyap, Role of insulin-like growth factor-binding proteins in the pathophysiology and tumorigenesis of gastroesophageal cancers, Tumour. Biol. 36 (11) (2015) 8247–8257.
[17] Q. Liu, J. Jiang, X. Zhang, M. Zhang, Y. Fu, Comprehensive analysis of IGFBPs as biomarkers in gastric cancer, Front. Oncol. 11723131 (2021).
[18] Y. Sato, M. Inokuchi, Y. Takagi, S. Otsuki, Y. Fujimori, Y. Yanaka, K. Kobayashi, K. Higuchi, K. Kojima, T. Kawano, Relationship between expression of IGFBP7 and clinicopathological variables in gastric cancer, J. Clin. Pathol. 68 (10) (2015) 795–801.
[19] J. Wang, M. Zhang, X. Hu, J. She, R. Sun, S. Qin, D. Li, miRNA-194 predicts favorable prognosis in gastric cancer and inhibits gastric cancer cell growth by targeting CCND1, FEBS Open Bio 11 (7) (2021) 1814–1826.
[20] P. Huang, L. Xia, Q. Guo, C. Huang, Z. Wang, Y. Huang, S. Qin, W. Leng, D. Li, Genome-wide association studies identify miRNA-194 as a prognostic biomarker for gastrointestinal cancer by targeting ATP6V1F, PPP1R14B, BTF3L4 and SLC7A5, Front. Oncol. (2022) 121025594.
[21] L. Li, W. Li, Epithelial-mesenchymal transition in human cancer: comprehensive reprogramming of metabolism, epigenetics, and differentiation, Pharmacol. Ther. (2015) 15033–15046.
[22] T.T. Liao, M.H. Yang, Revisiting epithelial-mesenchymal transition in cancer metastasis: the connection between epithelial plasticity and stemness, Mol. Oncol. 11 (7) (2017) 792–804.
[23] H. Cao, E. Xu, H. Liu, L. Wan, M. Lai, Epithelial-mesenchymal transition in colorectal cancer metastasis: a system review, Pathol. Res. Pract. 211 (8) (2015) 557–569.
[24] S. Heerboth, G. Housman, M. Leary, M. Longacre, S. Byler, K. Lapinska, A. Willbanks, S. Sarkar, EMT and tumor metastasis, Clin. Transl. Med. 46 (2015).
[25] S. Zhu, J. Zhang, F. Xu, E. Xu, W. Ruan, Y. Ma, Q. Huang, M. Lai, IGFBP-rP1 suppresses epithelial-mesenchymal transition and metastasis in colorectal cancer, Cell Death Dis. 6 (3) (2015) e1695.

[26] Y. Li, Y. Xi, G. Zhu, J. Jia, H. Huang, Y. Liu, Y. Guo, L. Liu, Downregulated IGFBP7 facilitates liver metastasis by modulating epithelial-mesenchymal transition in colon cancer, Oncol. Rep. 42 (5) (2019) 1935–1945.
[27] X. Wang, Y. Li, Z. Zhao, Y. Meng, J. Bian, R. Bao, K. Zhu, T. Yang, IGFBP7 regulates sepsis-induced epithelial-mesenchymal transition through ERK1/2 signaling, Acta Biochim. Biophys. Sin. 51 (8) (2019) 799–806.
[28] Y. Zhong, Z. Lin, X. Lin, J. Lu, N. Wang, S. Huang, Y. Wang, Y. Zhu, Y. Shen, J. Jiang, S. Lin, IGFBP7 contributes to epithelial-mesenchymal transition of HPAEpiC cells in response to radiation, J. Cell. Biochem. 120 (8) (2019) 12500–12507.
[29] B. Zheng, L. Geng, L. Zeng, F. Liu, Q. Huang, AKT2 contributes to increase ovarian cancer cell migration and invasion through the AKT2-PKM2-STAT3/NF-kappaB axis, Cell. Signal. (2018) 45122–45131.
[30] P. Ekambaram, J.L. Lee, N.E. Hubel, D. Hu, S. Yerneni, P.G. Campbell, N. Pollock, L.R. Klei, V.J. Concel, P.C. Delekta, A.M. Chinnaiyan, S.A. Tomlins, D. R. Rhodes, N. Priedigkeit, A.V. Lee, S. Oesterreich, L.M. McAllister-Lucas, Lucas PC: the CARMA3-bcl10-MALT1 signalosome drives NFkappaB activation and promotes aggressiveness in angiotensin II receptor-positive breast cancer, Cancer Res. 78 (5) (2018) 1225–1240.
[31] K. Saito, Y. Matsuo, H. Imafuji, T. Okubo, Y. Maeda, T. Sato, T. Shamoto, K. Tsuboi, M. Morimoto, H. Takahashi, H. Ishiguro, S. Takiguchi, Xanthohumol inhibits angiogenesis by suppressing nuclear factor-kappaB activation in pancreatic cancer, Cancer Sci. 109 (1) (2018) 132–140.
[32] H. Yang, S. Huang, Y. Wei, S. Cao, C. Pi, T. Feng, J. Liang, L. Zhao, G. Ren, Curcumin enhances the anticancer effect of 5-fluorouracil against gastric cancer through down-regulation of COX-2 and NF- kappaB signaling pathways, J. Cancer 8 (18) (2017) 3697–3706.
[33] X. Long, Y. Hu, S. Duan, X. Liu, W. Huang, X. Liu, Q. Xu, W. Song, J. Zhou, MRGBP promotes colorectal cancer metastasis via DKK1/Wnt/beta-catenin and NF-kB/p65 pathways mediated EMT, Exp. Cell Res. 421 (1) (2022) 113375.
[34] X. Lin, N. Yoshikawa, W. Liu, T. Matsukawa, K. Nakamura, M. Yoshihara, Y. Koya, M. Sugiyama, S. Tamauchi, Y. Ikeda, A. Yokoi, Y. Shimizu, H. Kajiyama, DDIT4 facilitates lymph node metastasis via the activation of NF-kappaB pathway and epithelial-mesenchymal transition, Reprod. Sci. 30 (9) (2023) 2829–2841.
[35] C. Dong, R.J. Davis, R.A. Flavell, MAP kinases in the immune response, Annu. Rev. Immunol. (2002) 2055–2072.
[36] W. Yang, R.E. Redpath, C. Zhang, N. Ning, Long non-coding RNA H19 promotes the migration and invasion of colon cancer cells via MAPK signaling pathway, Oncol. Lett. 16 (3) (2018) 3365–3372.
[37] T. Xiang, R. Fei, Z. Wang, Z. Shen, J. Qian, W. Chen, Nicotine enhances invasion and metastasis of human colorectal cancer cells through the nicotinic acetylcholine receptor downstream p38 MAPK signaling pathway, Oncol. Rep. 35 (1) (2016) 205–210.
[38] D. Nagarajan, T. Melo, Z. Deng, C. Almeida, W. Zhao, ERK/GSK3beta/Snail signaling mediates radiation-induced alveolar epithelial-to-mesenchymal transition, Free Radic. Biol. Med. 52 (6) (2012) 983–992.
[39] N.H. Ding, J.J. Li, L.Q. Sun, Molecular mechanisms and treatment of radiation-induced lung fibrosis, Curr. Drug Targets 14 (11) (2013) 1347–1356.