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Original Article

XBP1 induces MMP-9 expression to promote proliferation and invasion in human esophageal squamous cell carcinoma

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Abstract: X-box binding protein 1 (XBP1) was found to be overexpressed in glioma and breast cancers, suggesting that XBP1 might act as a potent oncogenic protein. However, the clinical significance and biological role of XBP1 in esophageal squamous cell carcinoma (ESCC) remain unknown. In this study, we report that XBP1 is markedly overexpressed in ESCC cell lines and clinical samples. XBP1 overexpression was significantly correlated with ESCC tumor stage, lymph node metastasis and poor outcome. A functional study demonstrated that XBP1 promoted cell growth and cell invasion both in *vitro* and in *vivo*. Further study found that the XBP1-mediated invasion and proliferation of cancer cells requires the up-regulation of matrix metalloproteinase-9 (MMP-9). Importantly, a significant correlation between XBP1 and MMP-9 levels was observed in ESCC clinical samples. Our findings demonstrate that XBP1 is an oncogene that plays an important role in the development of ESCC by activating MMP-9 expression.

Keywords: ESCC, XBP1, MMP-9, invasion, proliferation

Introduction

Esophageal cancer is ranked as the sixth leading cause of cancer deaths in the world. Esophageal squamous cell carcinoma (ESCC) is the major histologic form of esophageal cancer occurring in the Chinese population [1]. Despite advances in diagnostic and treatment modalities, the 5-year survival rate for patients with ESCC remains poor [2]. Hence, comprehensive understanding of the molecular mechanisms underlying ESCC development may allow the identification of effective therapeutic targets for ESCC.

It is well-established that the invasion of tumor cells is associated with the degradation of the extracellular matrix, which can be induced by matrix metalloproteinases (MMPs) [3]. There are at least 28 members in the MMP family, and numerous studies had shown that the overexpression of MMPs promotes tumor cell invasion, resulting in poor clinical outcomes [4]. MMP-9, a member of the MMP family, was reported to participate in the development of ESCC, and high levels of this enzyme correlated with poor prognosis [5]. The expression of MMP-9 in ESCC is significantly associated with the tumor invasion depth and lymph node metastasis [6]. Therefore, elucidation of the mechanisms that regulate MMPs may allow the development of targeted cancer therapies.

Human X-box binding protein 1 (XBP1), is an important transcription factor that is comprehensively overexpressed in cancer cells under microenvironment stress [7]. Under conditions of endoplasmic reticulum (ER) stress, XBP1 induces the upregulation of a variety of target genes [8]. Loss of XBP1 significantly inhibits glioma growth and promotes apoptosis [9]. Although the overexpression of XBP1 has been observed in certain cancers, the function of XBP1 in ESCC is unknown. In the present study, we report that XBP1 overexpression is associated with ESCC malignancy and that MMP-9 is up-regulated by XBP1.

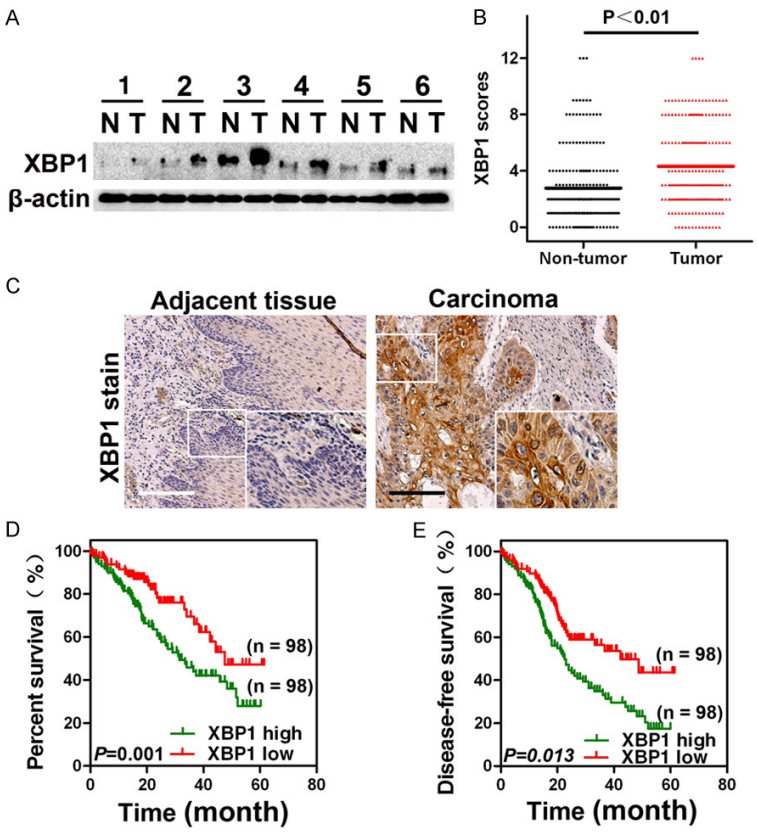


Figure 1. XBP1 is overexpressed in primary human ESCC cells. A. Western blot analysis of XBP1 expression in 6 paired human ESCC tissues (T) and the matched adjacent non-tumor tissues (N) from the same patient. B, C. Relative expression levels of XBP1 detected by IHC in 196 pairs of ESCC tissues. D, E. Kaplan-Meier overall survival curves for patients with ESCC indicating the correlation of XBP1 overexpression with worse overall survival and disease-free survival rates. Scale bar, 25 μm.

Materials and methods

ESCC samples and cell lines

A total of 196 paired specimens (adjacent non-tumor tissues and tumor tissue) were collected immediately following surgical resection of patients with ESCC at the Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China). All patients signed informed consent forms for sample collection and had not received treatment prior to surgery. The samples used in this study were approved by the Committee for Ethical Review of Research at Huazhong University of Science and Technology. The ESCC cell lines EC18, Eca109, KYSE150 and KYSE180 were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China).

Plasmid constructs and transfection

Full-length human XBP1 complementary DNA (cDNA) was amplified with PCR and cloned into the pcDNA3.1(+) expression vector (Invitrogen) before being transfected into Eca109 and KYSE150 cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Cells transfected with empty vector were used as controls. Lentiviruses containing shRNAs targeting XBP1 and MMP-9 were purchased from Shanghai GeneChem (GeneChem, China). The shXBP1 target sequence was 5'-GAACAGCAAGTGGTAGATTTA-3', and the shMMP-9 target sequence was 5'-CATTCAGGGAGACGCCCATTT-3'. Cells transfected with scrambled shRNA were used as controls.

Quantitative real-time PCR

Total RNA was extracted from clinical samples using TRIzol reagent (Invitrogen) and was reverse transcribed with the SuperRT cDNA Synthesis Kit (CWBIO, China). Real-time qPCR was carried out using a SYBR® Premix Ex Taq™ Kit (Takara, Japan) to detect the levels of XBP1, MMP-9 and GAPDH. The sequences of the XBP1 primers were as follows: forward, 5'-CCCTCCAGAACATCTCCCCAT-3'; reverse, 5'-ACATGACTGGGTCCAAGTTGT-3'. The sequences of the MMP-9 primers were as follows: forward, 5'-GGGACGCAGACATCGTCATC-3'; reverse, 5'-TCGTCATCGTCGAAATGGGC-3'. The relative mRNA expression level was normalized as previously described [10]. XBP1 levels in tumors that were higher than the median value (50th) were classified as 'high', and levels equal to and/or lower than the median value were classified as 'low'.

MTT assay

For the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, cells were

Table 1. The association between clinical parameters with XBP1 mRNA

Features	*No.*	XBP1		*P-value*
		Low	*High*	
Gender				
Male	110	63	47	0.03
Female	86	35	51	
Age				
≤ 60	107	54	53	0.99
> 60	89	44	45	
Smoking status				
Nonsmokers	72	41	31	0.18
Smokers	124	57	67	
Differentiation				0.11
Well	41	26	15	
Moderate	121	54	67	
Poor	34	18	16	
Tumor invasion				
T1/T2	109	62	47	0.04
T3/T4	87	36	51	
Lymph node metastasis				0.02
N0	130	73	57	
N1	66	25	41	
Stage				
I/IIA	55	34	21	0.06
IIB/III	141	64	77	

plated at a density of 4 × 10^3/well in 96-well plates. After treatment with MTT (20 μl, Sigma) and DMSO (200 μl, Sigma), the absorbance was determined in a microplate reader at 490 nm.

Cell invasion assays

Cells were seeded on a Matrigel (50 μl, BD, USA)-coated transwell apparatus (Costar, UK). RPMI 1640 containing 20% fetal bovine serum (FBS, Invitrogen) was added to the lower chamber. After incubation for 30 h, the cells on the top surface of the membrane were removed. The remaining cells were stained with crystal violet and counted in three random fields at × 100 magnification.

Wound-healing assay

Cells were seeded into 35-mm dishes and grown until confluence before scrape wounds were made in each sample. The Eca109 and KYSE150 cells were photographed at 0 and 24 h.

Antibodies and western blotting

The western blot assay was performed as previously described [11] using anti-XBP1 (ab-37152, Abcam) and anti-MMP-9 antibodies (ab38898, Abcam).

Xenografted tumor model

Experimental procedures were approved by the Institutional Animal Care and Use Committee of Huazhong University of Science and Technology. BALB/c nude mice were randomly divided into four groups (n = 8 per group). One group of mice was injected subcutaneously with Eca109/sh-XBP1 cells in the right dorsal flank and with Eca109/sh-control cells in the left dorsal flank. Another group was injected subcutaneously with Eca109/vector cells in the left dorsal flank and with Eca109/XBP1 cells (6 × 10^6 cells) in the right dorsal flank. Tumor volumes were calculated weekly. Mice were killed 21 days after injection.

For the tumor metastasis assay, 1 × 10^6 cells transduced with control vector, XBP1 plasmid or shMMP-9 lentivirus were injected into the tail vein of BALB/c nude mice. Mice were killed 49 days after injection. The metastasis nodules in the lung tissues were stained with hematoxylin and eosin.

Immunohistochemistry

IHC analysis was performed to study XBP1 and MMP-9 expression using anti-XBP1 and anti-MMP-9 antibodies in 196 paraffin-embedded human ESCC samples. The procedure was performed in a similar manner to a previously described procedure [12]. Quantification of the immunostaining of ESCC specimens was scored separately by two independent pathologists. IHC staining intensity scores were defined according to the extent of stained cells (0% positive tumor cells = 0, 1-24% positive tumor cells = 1, 25-49% positive tumor cells = 2, 50-74% positive tumor cells = 3, 75-100% positive tumor cells = 4) multiplied by the cell staining intensity (0 = negative; 1 = weak; 2 = moderate; and 3 = strong), leading to scores from 0-12.

Statistical analysis

The statistical tests used for data analysis included the χ^2 test and Student's two-tailed

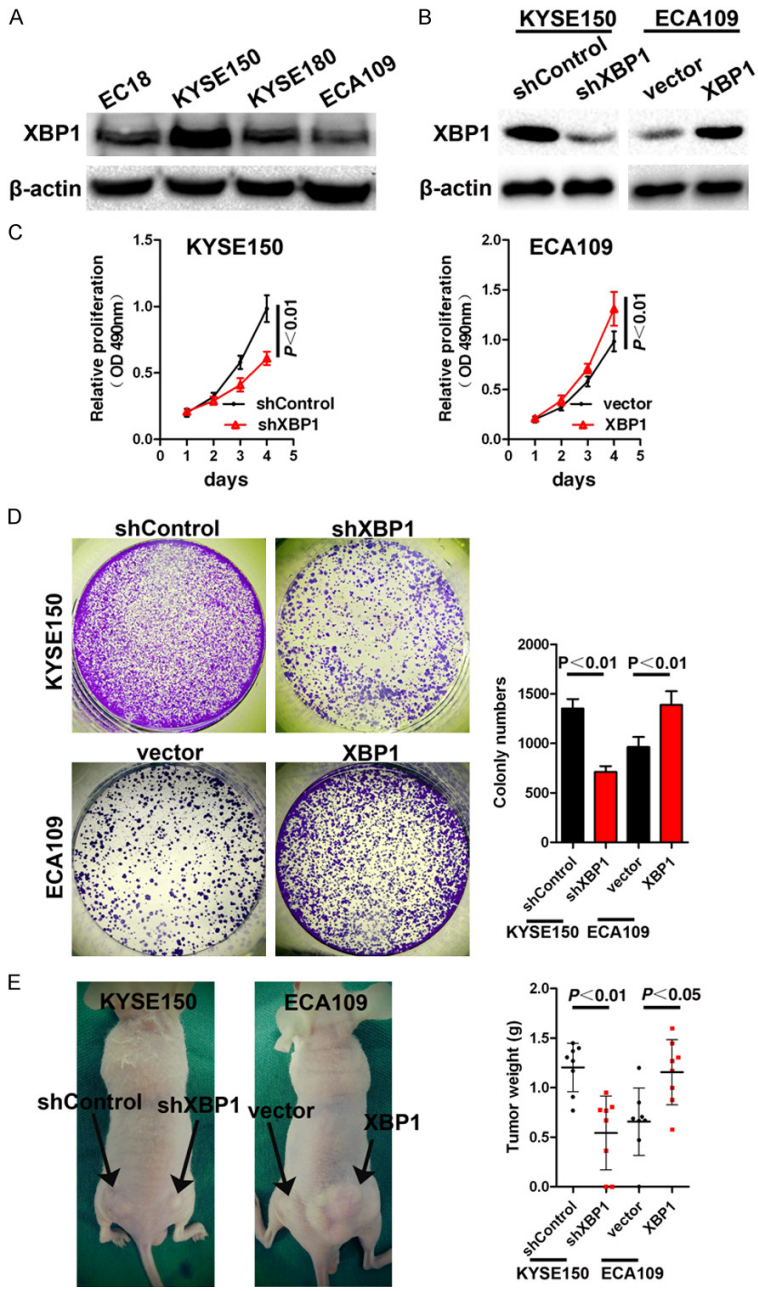


Figure 2. XBP1 promotes ESCC proliferation *in vitro* and in *vivo*. A. Western blot analysis showing that in ESCC cell lines, the highest expression of XBP1 occurs in KYSE150 cells and the lowest expression occurs in ECA109 cells. B. Western blot analysis of XBP1 expression in KYSE150 (left panel) and ECA109 (right panel) ESCC cells stably expressing XBP1-short hairpin RNA(s) or XBP1-cDNA. C. MTT analysis of cell proliferation rate in transfected KYSE150 (left panel) and ECA109 (right panel) ESCC cells. D. Evaluation of the effects of XBP1 shRNA or XBP1-complementary DNA on the growth of KYSE150 and ECA109 cells using a colony formation assay. E. Images of the mice subcutaneously injected with XBP1-shRNA or XBP1-cDNA transfected cells. The weights of the xenograft tumors are summarized in the right panel.

t-test. Survival curves were assessed using the Kaplan-Meier method and log-rank tests. Multivariate statistical analysis was performed using a Cox proportional hazards regression model. *P* values < 0.05 were considered statistically significant. SPSS 12.0 statistical software was used for all statistical analyses.

Results

Clinical significance of XBP1 overexpression in ESCC

To confirm the expression of XBP1 in ESCC, we analyzed 196 ESCC samples with their matched normal tissues using western blotting. The results showed that XBP1 was frequently overexpressed in ESCC tissues (**Figure 1A**). We performed IHC analyses to examine the expression of XBP1 in 196 ESCC specimens. As shown in **Figure 1B** and **1C**, the immunostaining intensity of XBP1 was significantly stronger in esophageal squamous cell carcinoma tissues than in matched adjacent tissues.

Subsequently, the association between XBP1 overexpression and clinicopathological features was statistically analyzed in 196 ESCC samples with IHC scores. The results indicated that overexpression of XBP1 was significantly associated with tumor stage (P = 0.04) and lymph node metastasis (*P* = 0.02, **Table 1**). Furthermore, Kaplan-Meier analysis revealed that XBP1 overexpression was significantly associated with worse disease-free survival (DFS) rates (*P* = 0.013) and the overall survival (OS) rate (*P* = 0.001) of patients with ESCC (**Figure 1D** and **1E**). Multivariate Cox regression analysis showed that the hazard ratios (HR) for DFS (HR 2.19,

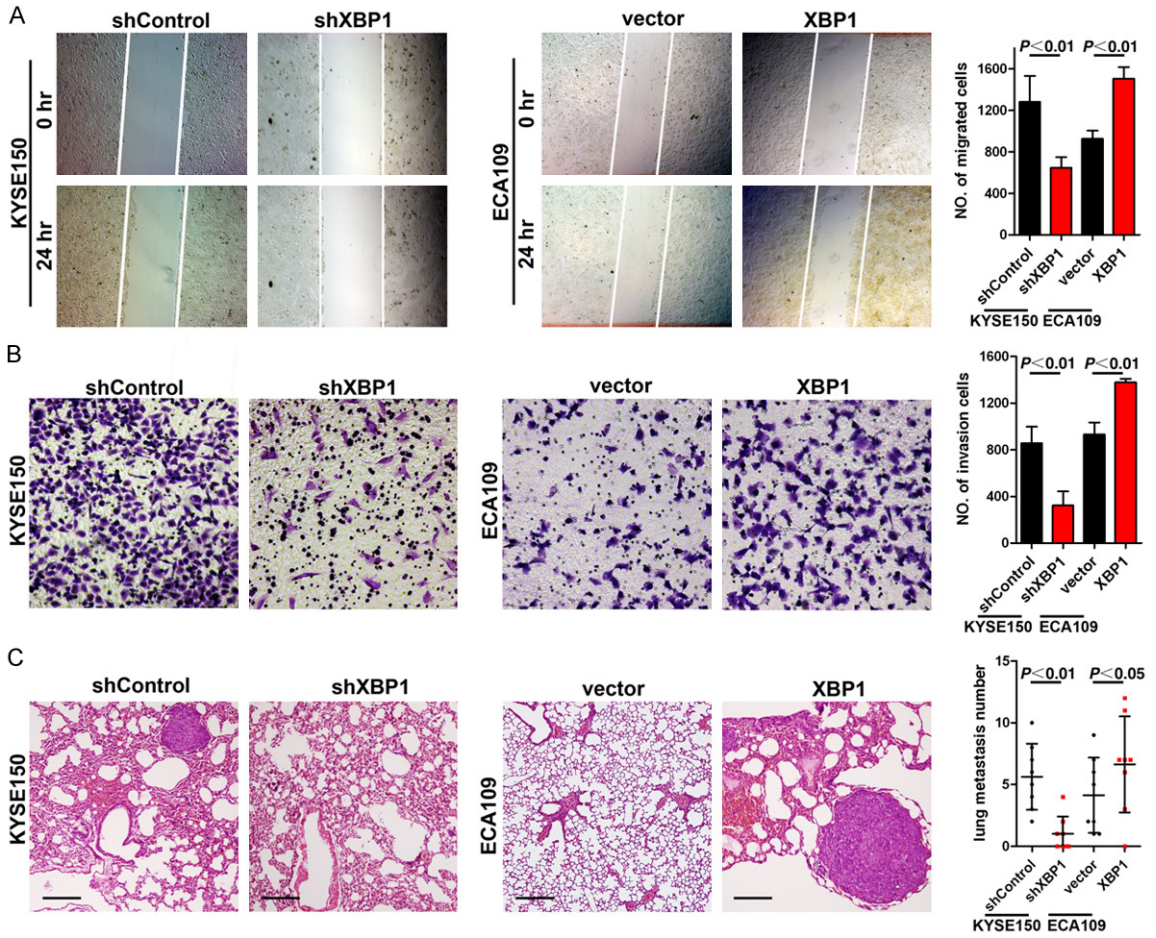


Figure 3. XBP1 is involved in the invasion and metastasis of esophageal cancer cells. A. Representative photos of wound-healing assays in control, XBP1-cDNA KYSE150 cells (left panel) and XBP1-shRNA ECA109 (right panel) cells. B. Transwell invasion assay showing that XBP1 promoted cell invasion. C. Representative images of H&E-stained sections derived from lung metastatic nodules. Sections of lung derived from mice injected with empty vector-transfected KYSE150 cells or shControl-transfected ECA109 cells were used as controls (original magnification, 100 ×). Scale bar, 100 μm.

95% CI 1.42-3.62, $P = 0.01$) and OS (HR 2.23, 95% CI 1.51-3.44, $P = 0.005$) were higher for tumors with high XBP1 expression than for tumors with low XBP1 expression.

XBP1 promotes ESCC proliferation in vitro and in vivo

To investigate the role of XBP1 in esophageal cancer, we determined the protein level of XBP1 in ESCC cell lines and found the lowest expression of XBP1 in ECA109 cells and the highest expression in KYSE150 cells (**Figure 2A**). XBP1 shRNA or XBP1 cDNA were used for downregulating or upregulating XBP1 expression. After transfection, XBP1 shRNA effectively suppressed the expression of XBP1 in KYSE150 cells, and XBP1 cDNA could promote the expression of XBP1 in ECA109 cells (**Figure 2B**). Compared with control cells, *in vitro* and *in vivo* assays found that the ectopic expression of XBP1 effectively inhibited the tumorigenic properties of transfected cells by inhibiting the rate of cell proliferation (**Figure 2C**), reducing the frequency of focus formation (**Figure 2D**), and inhibiting the formation of tumors (**Figure 2E**). In agreement with these observations, depletion of XBP1 substantially suppressed cellular growth, colony formation and tumor formation (**Figure 2C**, **2D**).

XBP1 is involved in the invasion and metastasis of esophageal cancer cells

Because XBP1 overexpression was significantly associated with ESCC tumor stage and lymph

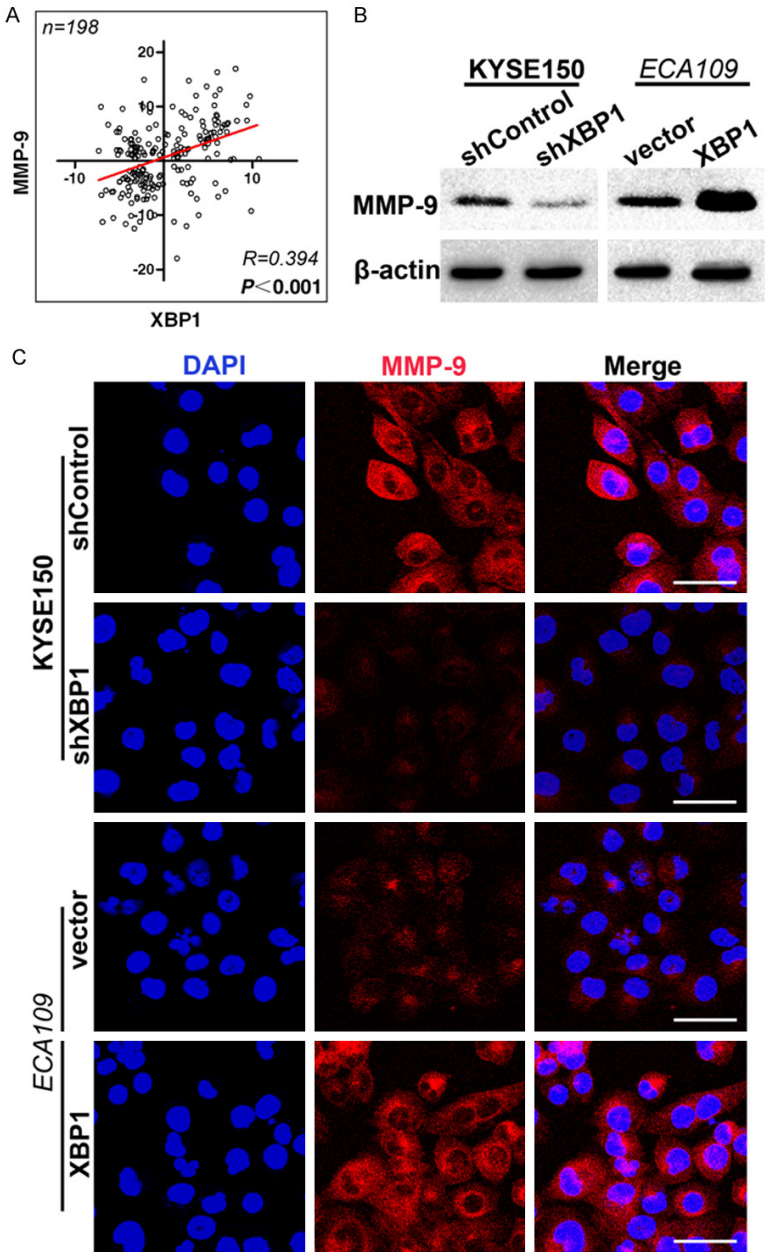


Figure 4. XBP1 upregulates MMP-9 expression. A. Analysis showing linear regressions and significant Pearson correlations of XBP1 with MMP-9 (n = 196) in ESCC tumor and nontumor samples. B. Expression of MMP-9 in XBP1-expressing or XBP1-silenced cells as detected by western blotting. C. Representative immunofluorescence staining images showing increased expression of MMP-9 in XBP1-expressing KYSE150 cells and decreased expression of MMP-9 in XBP1-silenced ECA109 cells. Scale bar, 25 μm.

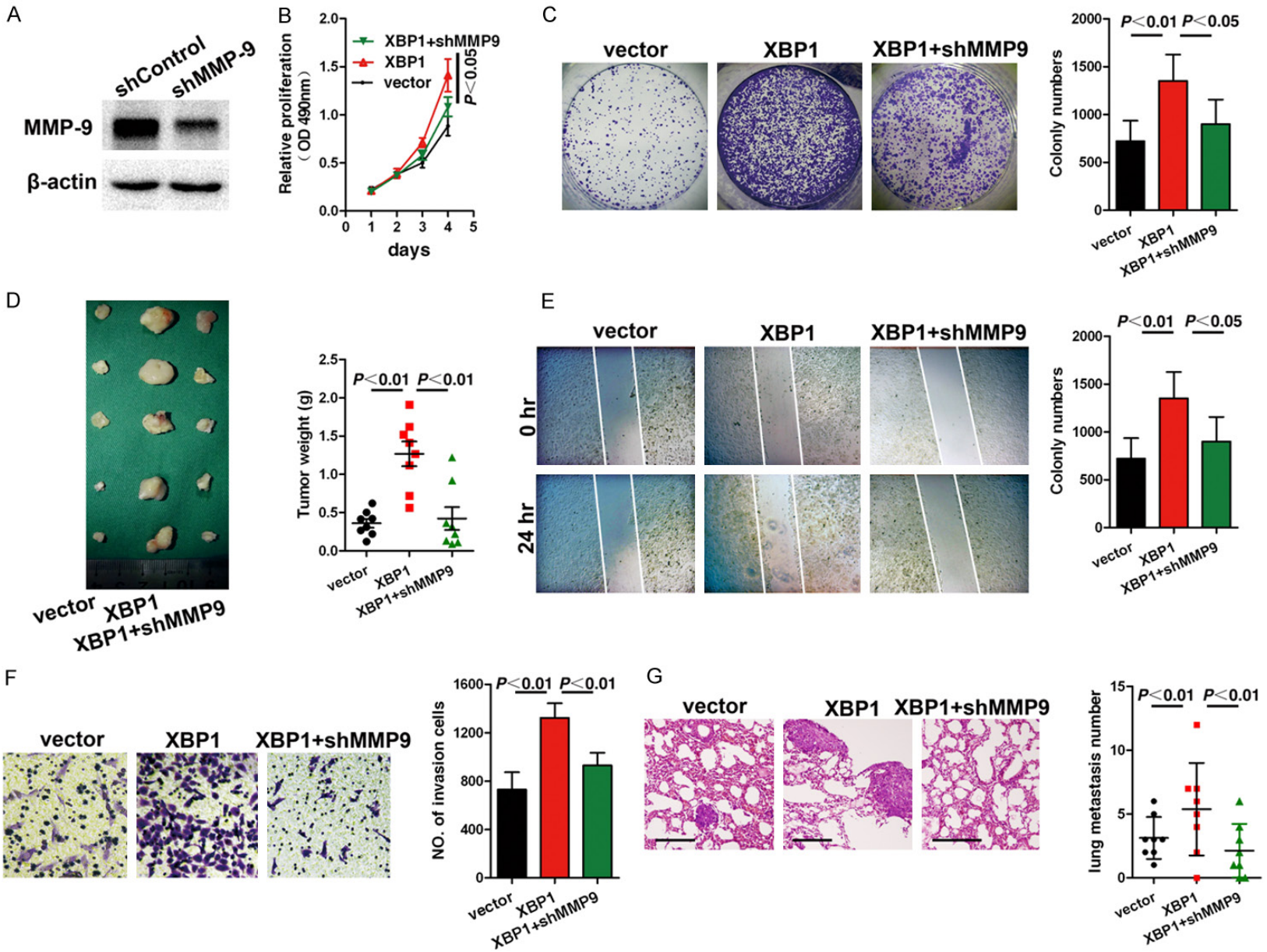
node metastasis, the role of XBP1 in tumor cell migration, invasion and metastasis was investigated. The wound-healing assay showed that XBP1-transfected cells obtained quicker closure of the "wound" (**Figure 3A**). The transwell invasion assay (**Figure 3B**) and metastasis assay (**Figure 3C**) further revealed significantly increased cell invasion and metastasis with XBP1 expression. In agreement with these observations, depletion of XBP1 substantially suppressed cellular migration, invasion and metastasis (**Figure 3A-C**).

XBP1 upregulates MMP-9 expression

Cellular invasion and metastasis often requires the expression of matrix metalloproteinases (MMPs), which degrade the extracellular matrix [13]. To test whether XBP1 might alter MMP-9 expression, we performed qRT-PCR analysis to investigate the correlation between XBP1 and MMP-9 levels in the cohort of ESCC samples. The results confirmed that MMP-9 expression was significantly correlated with XBP1 expression ($R = 0.394$, $P < 0.001$, **Figure 4A**). Western blot analysis further demonstrated that the levels of MMP-9 increased dramatically in XBP1-expressing cells but decreased in XBP1-silenced cells (**Figure 4B**). Immunofluorescence staining confirmed that increased MMP-9 expression was observed in XBP1-expressing KYSE150 cells and decreased MMP-9 expression was observed in XBP1-silenced ECA109 cells (**Figure 4C**).

MMP-9 is involved in XBP1-regulated ESCC cell proliferation and invasion

To test whether MMP-9 is involved in XBP1-regulated ESCC cell proliferation and invasion, we inhibited MMP-9 expression by transfecting MMP-9-shRNA into ECA109 cells (**Figure 5A**). MMP-9-shRNA abolished the stimulatory effects of XBP1 on ECA109 cell growth (**Figure 5B-D**), migration (**Figure 5E**), invasion (**Figure**


A
shControl
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MMP-9
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B
Relative proliferation (OD 490nm)
XBP1+shMMP9
XBP1
vector
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C
vector
XBP1
XBP1+shMMP9
Colonly numbers
P<0.01 P<0.05
D
vector
XBP1
XBP1+shMMP9
Tumor weight (g)
P<0.01 P<0.01
E
0 hr
24 hr
F
NO. of invasion cells
G
lung metastasis number

Figure 5. MMP-9 is involved in XBP1-regulated ESCC cell proliferation and invasion. A. Expression of MMP-9 in MMP-9-silenced ECA109 cells as detected by western blotting. B. MTT assay showing that MMP-9 shRNA inhibited XBP1-induced cell proliferation. C. Evaluation of the effects of MMP-9 shRNA on the XBP1-induced proliferation of ECA109 cells using a colony formation assay. D. Tumor formation in mice indicates that MMP-9 shRNA abolished the effects of XBP1 on promoting *in vivo* tumorigenicity in ECA109 cells. E. Wound-healing assays showing that MMP-9 shRNA inhibited XBP1-induced cell migration. F. Transwell invasion assay showing that MMP-9 shRNA abolished the effects of XBP1 on promoting cell invasion. G. Representative images of H&E-stained sections derived from lung metastatic nodules (original magnification, 100 ×). Scale bar, 100 μm.

5F), and metastasis (**Figure 5G**). These results demonstrate that the promotion of tumor growth and invasion by XBP1 is mediated by MMP-9.

Discussion

Distant metastasis and extensive local invasion are the major cause of death in patients with esophageal squamous cell carcinoma [14, 15], but the molecular mechanisms that drive these processes remain unclear. In this study, we found that XBP1 enhances ESCC cell proliferation and invasion by positively regulating MMP-9.

XBP1 was initially discovered as a transcription factor in the early 1990s [16]. XBP1 was first identified as a key regulator of major histocompatibility complex class II gene expression in B cells and is critical for cell fate determination in response to endoplasmic reticulum stress [17]. In recent years, multiple studies have shown that XBP1 is frequently upregulated in breast cancer [18, 19]; this upregulation is essential for tumor growth. It is unknown whether XBP1 has an oncogenic function in ESCC. In our work, XBP1 protein levels were upregulated in ESCC tumors compared with adjacent normal tissues. Furthermore, high XBP1 expression was associated with increased tumor invasion, lymph node metastasis and poor patient outcome.

We characterized key cellular aspects of XBP1 function, finding that MAP4 induced cell proliferation and invasion *in vitro* and tumor growth and metastasis *in vivo*. Then, we explored the molecular mechanisms through which XBP1 promoted the malignant phenotypes of ESCC cells, observing that the expression of MMP-9 was significantly increased in XBP1-expressing cells compared with control cells. We further demonstrated that MMP-9 knockdown can abolish the effects of XBP1 on promoting tumorigenicity and malignant phenotypes in ESCC cells.

The role of MMP-9 in cancer biology is emerging as an area of importance. MMP-9 degrades the major component of the extracellular matrix (ECM) and basement membrane, and this event appears to be crucial in tumor cell growth and invasion [20, 21]. High MMP-9 expression is associated with poor prognosis in ESCC [22]. In the present study, higher levels of MMP-9 mRNA were detected in ESCC cells expressing XBP1. Our results also revealed increased expression of MMP-9 in XBP1-expressing cells and decreased expression of MMP-9 in XBP1-silenced cells. These data indicate that XBP1 functions by up-regulating MMP-9 signaling. However, further studies are required to determine how XBP1 positively regulates MMP-9 expression.

In summary, our current work reveals a novel mechanism of tumor cell proliferation and invasion in ESCC that involves the activation of the XBP1- MMP-9 pathway. We show that XBP1 is of prognostic and therapeutic relevance. As an important regulator of MMP-9, XBP1 may serve as a candidate molecular target for ESCC therapy.

Acknowledgements

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Disclosure of conflict of interest

None.

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References

[1] Enzinger PC, Mayer RJ. Esophageal cancer. N Engl J Med 2003; 349: 2241-2252.

[2] Sato T, Iizuka N, Hamamoto Y, Yoshino S, Abe T, Takeda S, Uchimura S, Miyamoto T, Sei F, Hamada K, Yamada-Okabe H, Oka M. Esophageal squamous cell carcinomas with distinct invasive depth show different gene expression profiles associated with lymph node metastasis. Int J Oncol 2006; 28: 1043-1055.

[3] Gu ZD, Li JY, Li M, Gu J, Shi XT, Ke Y, Chen KN. Matrix metalloproteinases expression correlates with survival in patients with esophageal squamous cell carcinoma. Am J Gastroenterol 2005; 100: 1835-1843.

[4] Zeng R, Duan L, Kong Y, Liang Y, Wu X, Wei X, Yang K. Clinicopathological and prognostic role of MMP-9 in esophageal squamous cell carcinoma: a meta-analysis. Chin J Cancer Res 2013; 25: 637-645.

[5] Tanioka Y, Yoshida T, Yagawa T, Saiki Y, Takeo S, Harada T, Okazawa T, Yanai H, Okita K. Matrix metalloproteinase-7 and matrix metalloproteinase-9 are associated with unfavourable prognosis in superficial oesophageal cancer. Br J Cancer 2003; 89: 2116-2121.

[6] Li Y, Ma J, Guo Q, Duan F, Tang F, Zheng P, Zhao Z, Lu G. Overexpression of MMP-2 and MMP-9 in esophageal squamous cell carcinoma. Dis Esophagus 2009; 22: 664-667.

[7] Wang M, Kaufman RJ. The impact of the endoplasmic reticulum protein-folding environment on cancer development. Nat Rev Cancer 2014; 14: 581-597.

[8] Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. Nat Rev Mol Cell Biol 2012; 13: 89-102.

[9] Romero-Ramirez L, Cao H, Nelson D, Hammond E, Lee AH, Yoshida H, Mori K, Glimcher LH, Denko NC, Giaccia AJ, Le QT, Koong AC. XBP1 is essential for survival under hypoxic conditions and is required for tumor growth. Cancer Res 2004; 64: 5943-5947.

[10] Liu L, Dai Y, Chen J, Zeng T, Li Y, Chen L, Zhu YH, Li J, Li Y, Ma S, Xie D, Yuan YF, Guan XY. Maelstrom promotes hepatocellular carcinoma metastasis by inducing epithelial-mesenchymal transition by way of Akt/GSK-3beta/Snail signaling. Hepatology 2014; 59: 531-543.

[11] Tong S, Xiong N, Shen J. RNA interference suppression of Nogo-66 receptor prevents Nogo-66-mediated inhibition of invasion and adhesion and simultaneously increases cell apoptosis in C6 cells. Oncol Rep 2013; 30: 2171-2178.

[12] Lin C, Song L, Liu A, Gong H, Lin X, Wu J, Li M, Li J. Overexpression of AKIP1 promotes angiogenesis and lymphangiogenesis in human esophageal squamous cell carcinoma. Oncogene 2015; 34: 384-393.

[13] Rorth P. Collective cell migration. Annu Rev Cell Dev Biol 2009; 25: 407-429.

[14] Jiang YY, Shang L, Shi ZZ, Zhang TT, Ma S, Lu CC, Zhang Y, Hao JJ, Shi C, Shi F, Xu X, Cai Y, Jia XM, Zhan QM, Wang MR. Microtubule-associated protein 4 is an important regulator of cell invasion/migration and a potential therapeutic target in esophageal squamous cell carcinoma. Oncogene 2016; [Epub ahead of print].

[15] Zhang W, Lei C, Fan J, Wang J. miR-18a promotes cell proliferation of esophageal squamous cell carcinoma cells by increasing cylin D1 via regulating PTEN-PI3K-AKT-mTOR signaling axis. Biochem Biophys Res Commun 2016; 477: 144-149.

[16] Liou HC, Boothby MR, Finn PW, Davidon R, Nabavi N, Zeleznik-Le NJ, Ting JP, Glimcher LH. A new member of the leucine zipper class of proteins that binds to the HLA DR alpha promoter. Science 1990; 247: 1581-1584.

[17] He Y, Sun S, Sha H, Liu Z, Yang L, Xue Z, Chen H, Qi L. Emerging roles for XBP1, a sUPeR transcription factor. Gene Expr 2010; 15: 13-25.

[18] Chen X, Iliopoulos D, Zhang Q, Tang Q, Greenblatt MB, Hatziapostolou M, Lim E, Tam WL, Ni M, Chen Y, Mai J, Shen H, Hu DZ, Adoro S, Hu B, Song M, Tan C, Landis MD, Ferrari M, Shin SJ, Brown M, Chang JC, Liu XS, Glimcher LH. XBP1 promotes triple-negative breast cancer by controlling the HIF1alpha pathway. Nature 2014; 508: 103-107.

[19] Li H, Chen X, Gao Y, Wu J, Zeng F, Song F. XBP1 induces snail expression to promote epithelial-to-mesenchymal transition and invasion of breast cancer cells. Cell Signal 2015; 27: 82-89.

[20] Kang L, Hao X, Tang Y, Wei X, Gong Y. RABEX-5 overexpression in gastric cancer is correlated with elevated MMP-9 level. Am J Transl Res 2016; 8: 2365-2374.

[21] Fu X, Zeng L, Liu Z, Ke X, Lei L, Li G. MicroRNA-206 regulates the secretion of inflammatory cytokines and MMP9 expression

by targeting TIMP3 in Mycobacterium tuberculosis-infected THP-1 human macrophages. Biochem Biophys Res Commun 2016; 477: 167-173.

[22] Yamamoto H, Vinitketkumnuen A, Adachi Y, Taniguchi H, Hirata T, Miyamoto N, Nosho K, Imsumran A, Fujita M, Hosokawa M, Hinoda Y, Imai K. Association of matrilysin-2 (MMP-26) expression with tumor progression and activation of MMP-9 in esophageal squamous cell carcinoma. Carcinogenesis 2004; 25: 2353-2360.