ZBTB20 promotes cell migration and invasion of gastric cancer by inhibiting IκBα to induce NF-κB activation

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ABSTRACT
Zinc finger and BTB domain containing 20 (ZBTB20), a sequence-specific transcriptional repressor, has been found to be involved in tumorigenesis. However, its role(s) in gastric cancer and the molecular mechanisms involved are poorly investigated. Here, our data demonstrated that ZBTB20 expression was markedly upregulated in gastric cancer cell lines infected with Helicobacter pylori (H. pylori) and in gastric cancer tumor samples. Loss- and gain-of-function studies showed that ZBTB20 promoted cell proliferation, invasion and migration of gastric cancer cell lines. Mechanistically, the phosphorylation of NF-κBp65 and expression and activity of MMP-2 and MMP-9 were increased, while IκBα expression was decreased by ZBTB20 in gastric cancer cells. We further revealed that IκBα overexpression significantly inhibited NF-κB signaling as well as cell migration, invasion and proliferation in gastric cancer cell lines induced by ZBTB20 overexpression. Therefore, our findings emphasize an important role for ZBTB20 in controlling gastric cancer development, which is helpful to identify potential therapeutic targets for its treatment.

Introduction
Gastric cancer is one of the most common malignancies worldwide with an overall 5-year survival rate lower than 25% [1]. Despite efforts to improve diagnostic technology and patient management, the prognosis of gastric cancer patients has not been improved significantly [2]. The formation of gastric cancer is a complex process, which eventually develops into gastric cancer from precancerous lesions such as superficial and atrophic gastritis, intestinal metaplasia, and dysplasia. Helicobacter pylori (H. pylori) infection is a common chronic infection that is one of the most important risk factors for gastric cancer compared with others such as genetic factors, high-salt diets, smoked foods, smoking, and alcohol abuse [3]. H. pylori infection is necessary but not sufficient for the development of gastric adenocarcinoma. Globally, the infection rate of H. pylori is about 40%–80%, but only 1% of H. pylori infections progress to gastric cancer [4]. It is suggested that individuals with different genetic backgrounds have different susceptibility to gastric cancer when exposed to the same environment. Given that different DNA, mRNA and protein expressions modulated by H. pylori infection could regulate gastric cancer cell proliferation, apoptosis, migration and invasion, they could serve as therapeutic targets in gastric cancer [5,6].

Zinc finger and BTB domain containing 20 (ZBTB20) has been implicated in developmental neurogenesis [7], glucose and lipid homoeostasis, and the tumorigenesis of glioblastoma [8], liver [9] and lung cancer [10]. Polymorphisms of ZBTB20 appeared to be associated with gastric cancer susceptibility in allelic, homozygous, dominant and recessive models, suggesting its role in gastric cancer [11]. NF-κB is activated in a variety of cancers as detected by phosphorylation and degradation of IκBα proteins, phosphorylation of RelA/p65, and elevated expression of NF-κB target genes. ZBTB20 enhances the innate immune responses induced by Toll-like receptor (TLR) through promoting NF-κB activation by repressing IκBα gene transcription [12]. Moreover, the activation of NF-κB was significantly attenuated by ZBTB20 deficiency after partial hepatectomy and was associated with hepatocyte proliferation in mouse liver regeneration [13]. However, whether and how IκBα/NF-κB signaling pathway involved in the ZBTB20-associated carcinogenesis in gastric cancer is still unknown.

Here, we determine the effect of ZBTB20 on the regulation of cell migration, invasion and proliferation of gastric cancer and explore the possible molecular mechanism involved in NF-κB signaling. Our findings reveal that ZBTB20 promotes cell migration and invasion of gastric cancer by inhibiting IκBα to induce NF-κB activation.
Materials and methods

Clinical samples

In total, the primary tumor samples were collected from 37 patients at Xuzhou Central Hospital who underwent surgery for gastric cancer between 2014 and 2017. None of the patients received preoperative radiotherapy or chemotherapy. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of Xuzhou Central Hospital and with the 1964 Helsinki declaration. This study protocol was approved by the Ethics Committee of Xuzhou Central Hospital. Written informed consents were provided prior to enrollment of patients.

Immunohistochemistry

Tissues were fixed in 4% paraformaldehyde, dehydrated and embedded in paraffin. Five-micrometer-thick slices were incubated in 3% H₂O₂ in methanol and 5% normal horse serum to minimize nonspecific staining. Sections were subsequently incubated overnight with the primary anti-ZBTB20 antibody (1:500; ab127702; Abcam, USA) at 4 °C, and then incubated with horseradish peroxidase (HRP)-labeled IgG secondary antibody (Beyotime Institute of Biotechnology, Shanghai, China) for half an hour at 25 °C. The section was then stained with diaminobenzidine (DAB) and hematoxylin for 3 min and washed in water for 10 min. Fields from each slide were examined and photographed under a light microscopy (×200). Quantitative data of positive area for immunocytochemistry (IHC) was analyzed using Image-pro Plus 6.0 software (Media Cybernetic, Silver Springs, MD, USA).

Cell culture and H. pylori infection

Human gastric cancer cell lines (SGC-7901, MKN-28, MKN-45 and BGC-823) andGES-1 human gastric epithelial mucosa cell line were obtained from the Shanghai Cell Bank (Shanghai, China) and cultured in RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 0.1 mg/ml streptomycin in an incubator at 37 °C with 5% CO₂ atmosphere. BGC-823 and MKN-45 cells were infected with H. pylori (strain 43504, ATCC, Wesel, Germany) at a multiplicity of infection (MOI) of 25, 50 and 100 for 24 h.

Luciferase reporter gene assay

BGC-823 and MKN-45 cells were transfected with the pGL3-ZBTB20 promoter using Lipofectamine 2000 (Invitrogen Life Technologies) following the manufacturer’s protocol. After 12 h transfection, cells were infected with H. pylori. Then, the cells were collected, and Luciferase activity was determined using a Luciferase assay kit (Promega).

Cell transfection

The RNAi (RNA interference) sequences targeting position 1161–1181 (siRNA-1; 5‘-CGAGAUGGACAGACAGGGCUU-3‘), position 1612–1630 (siRNA-2; 5‘-CCAAACAGAACUCGUAAUU-3‘) or 1687–1705 (siRNA-3; 5‘-CUUCUCUUUAAGGAUAAUU-3‘) of the human ZBTB20 gene were transfected into MKN-45 and BGC-823 cells using Lipofectamine 2000 in accordance with the manufacturer’s protocol. ZBTB20 and IκBα overexpression was constructed by integrating the coding sequence (CDS) of ZBTB20 and IκBα into the mammalian expression vector pCDNA3.1(+) ZBTB20 and/or pCDNA3.1(+)IκBα at 37 °C for 5 h using Lipofectamine 2000 in accordance with the instruction of the manufacturer. 48 h after transfection recombinant lentivirus were collected and used for infecting BGC-823 and MKN-45 cells. Cells transfected with scramble siRNA (siNC) or infected with blank pCDNA3.1(+) vector (blank vector) were used as negative controls.

CCK-8 assay

To assess cell proliferation, a Cell Counting Kit (CCK)-8 (Signalway Antibody LLC., College Park, MD, USA) assay was performed. Briefly, BGC-823 and MKN-45 cells (3 × 10⁵ cells/well) were cultured in a 96-well plate and transfected with pCDNA3.1(+)ZBTB20 and/or pCDNA3.1(+)IκBα at 37 °C for 24 h in BGC-823 and MKN-45 cells transfected with ZBTB20-siRNA or infected with pCDNA3.1(+)ZBTB20 and/or pCDNA3.1(+)IκBα. The number of viable cells was determined at 450 nm cell absorbance readings using a DNM-9602 microplate reader (Pulang New Technology, Beijing, China).

Transwell assay

BGC-823 and MKN-45 cells (3 × 10⁵ cells/well) were grown in a 6-well plate and maintained at 37 °C. After 24 h incubation, BGC-823 and MKN-45 cells transfected with ZBTB20-siRNA or infected with pCDNA3.1(+)ZBTB20 and/or pCDNA3.1(+)IκBα were serum-starved in basic medium for 24 h. Then, 300 μl cell suspension adjusted to 6 × 10⁴ cells was filled in the upper Transwell chamber (8.0-μm with Size 24 Cluster Platelet; Costar, USA) without or with 30 ml of 1 mg/ml Matrigel (BD Bioscience, San Jose, CA, USA), and 700 μl complete medium containing 10% FBS was added into the lower chamber. The cells were then cultured for 24 h (migration assay) or 48 h (invasion assay) at 37 °C, after which cells were fixed with 1 ml 4% methanol for 10 min and stained with 1 ml 0.5% crystal violet for 30 min. The BGC-823 and MKN-45 cells that migrated to the lower chamber and attached to the membrane were counted under a microscope (Shanghai CaiKang Optical Instrument Factory, Shanghai, China).
**Wound healing assay**

BGC-823 and MKN-45 cells were seeded in 35 mm² tissue culture dishes at a density of $8 \times 10^4$ and further seeded until reached 100% confluence. Then confluence cultures were scratched using a pipette tip. After scratching, gently wash the well twice with medium to remove the detached cells. Scratched cultures were photographed under a microscope at 0 and 24 h. Migration of cells was established by measuring the width of the scratched area at each time point in the scratched area.

**Gelatin zymography gel assay**

To investigate the MMP-2 and MMP-9 activities in gastric cancer cell lines, proteins were separated on a 10% SDS-PAGE containing 1% gelatin (Sigma-Aldrich, München, Germany). The gels were incubated at 37°C overnight, stained with Coomassie Blue, de-stained, and then scanned. Signal intensity was quantified by densitometry using Bio-1D software version 15.01 (Vilber Lourmat, Eberhardzell, Germany).

**Real-time PCR assay**

Total RNA from BGC-823 and MKN-45 cells was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Then Reverse Transcription System Kit (Takara Biomedical Technology (Beijing) Co., Ltd, Beijing, China) was used to synthesize the first-strand from 1 μg total RNA according to the manufacturer’s protocols. Quantitative real-time PCR using Maxima SYBR Green/ROX qPCR Master Mix (Thermo scientific, Pittsburgh, PA, USA) was performed using an ABI Prism 7500 sequence detection PCR system (Applied Biosystems, Shanghai, China). The specific primers used in real-time PCR were shown as below: ZBTB20, forward primer: 5’-GCTTGATAGGCACAGCATC-3’ and reverse primer: 5’-GAAACATCAGGCCCCAGTTC-3’; IκBα, forward primer: 5’-CTCCGAGACTTTCGAGGAAATAC-3’ and reverse primer: 5’-GCCATTGTAGTTGGTGAGCCTTCA-3’; GAPDH, forward primer: 5’-ATTCCTACCATCACCACCTTC-3’ and reverse primer: 5’-AGGCTGTGGTCGATACCT-3’. Expression levels are given as ratios to GAPDH, and each assay was performed in triplicate. The relative fold changes in messenger RNA expression were calculated using the $2^{-\Delta \Delta C_t}$ method.

**Western blotting**

BGC-823 and MKN-45 cells were lysed by RIPA lysis buffer (Solarbio). After centrifugation, the supernatants were collected and BCA reagent was used to determine the concentration of protein. Next, 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed to isolate the proteins. After being transferred to polyvinylidene fluoride membranes, the protein was blocked with nonfat dry milk, added antibodies, and detected with supersignal west picochemiluminescent substrate. Primary antibodies include anti-ZBTB20 (1:1000; Abcam), anti-MMP-2 (1:1000; Abcam), anti-MMP-9 (1:1000; Abcam) and anti-GAPDH (1:2000; Cell Signaling Technology, Danvers, MA, USA), anti-p-NF-κBp65 (1:1000; Cell Signaling Technology), anti-MMP-2 (1:1000; Abcam), anti-MMP-9 (1:1000; Abcam) and anti-GAPDH (1:2000; Cell Signaling Technology) antibody. Secondary antibodies include HRP-labeled Goat Anti-Mouse or Goat Anti-Rabbit IgG antibody (1:1000; Beyotime Institute of Biotechnology).

**Statistical analysis**

All data were representative of experiments done in triplicate and were expressed as the mean±SD. Samples were evaluated by the SPSS/Win11.0 software (SPSS, Inc., Chicago, IL, USA) using the Student’s t test for two groups and analysis of variance (ANOVA) for multiple groups. Overall survival of gastric cancer patients from Kaplan-Meier plotter database was analyzed by the Kaplan-Meier survival curves and log-rank nonparametric test. When $p<.05$, the difference between groups was statistically significant.

**Results**

**ZBTB20 expression is increased in human gastric cancer tumors and correlated with overall survival**

Analysis of tissues collected from our independent hospital demonstrated that ZBTB20 mRNA expression levels in human gastric cancer tumor samples ($n=37$) were higher than those in the paired adjacent normal gastric tissue samples ($n=37$) (Figure 1(A)). Similarly, ZBTB20 protein expression measured by immunohistochemistry was also upregulated in human gastric cancer tumor samples compared with the paired adjacent normal gastric tissue samples (Figure 1(B)). Moreover, ZBTB20 mRNA expression levels were also increased in patients with *H. pylori* infection or metastasis (Figure 1(C,D)).

According to the Kaplan Meier-plotter database, higher ZBTB20 expression also correlated with a poor overall survival of gastric cancer patients (Figure 1(E)).

**ZBTB20 expression is increased by *H. pylori* infection in gastric cancer cell lines**

To assess the role of ZBTB20 in gastric cancer development, the expression of ZBTB20 in gastric cancer cell lines was measured. Our data showed that ZBTB20 expression was increased in gastric cancer cell lines compared with GES-1 cells, with the highest expression detected in BGC-823 and MKN-45 cells (Figure 2(A)). These two cell lines were therefore used for subsequent experiments. Given that *H. pylori* infection is considered a major risk factor for gastric cancer, the effect of *H. pylori* infection on the expression of ZBTB20 was further examined in BGC-823 and MKN-45 gastric cancer cell lines. As shown in Figure 2(B,C), *H. pylori* infection significantly increased the mRNA and protein expression of ZBTB20 in BGC-823 and MKN-45 cells in a dose-dependent manner. Moreover, the transcriptional activation of ZBTB20 upon *H. pylori* infection was measured by the luciferase reporter gene assay (Figure 2(D,E)). To investigate the role of IκBα/NF-κB signaling pathway in gastric...
cancer, the effect of *H. pylori* infection on the expression of \( I_{\beta}B \alpha \), p-NF-\( \beta \)B and NF-\( \beta \)B was also measured. We found that *H. pylori* infection significantly decreased \( I_{\beta}B \alpha \) expression, increased p-NF-\( \beta \)B levels, but had no effect on NF-\( \beta \)B expression in BGC-823 and MKN-45 cells (Figure 2(F,G)). Taken together, these data support the notion that ZBTB20 and \( I_{\beta}B \alpha \)/NF-\( \beta \)B signaling pathway may participate in the occurrence and development of gastric cancer.

**ZBTB20 silencing represses cell proliferation of gastric cancer**

To examine the effect of ZBTB20 on the tumorigenesis of gastric cancer *in vitro*, BGC-823 and MKN-45 cells were transfected with three siRNAs targeting human ZBTB20 or a scramble siRNA (siNC). Silence of ZBTB20 significantly decreased \( I_{\beta}B \alpha \) expression, increased p-NF-\( \beta \)B levels, but had no effect on NF-\( \beta \)B expression in BGC-823 and MKN-45 cells (Figure 3(A,B)). Taken together, these data support the notion that ZBTB20 and \( I_{\beta}B \alpha \)/NF-\( \beta \)B signaling pathway may participate in the occurrence and development of gastric cancer.

**ZBTB20 silencing represses cell invasion and migration of gastric cancer**

Furthermore, siRNA-1 and siRNA-2 also significantly inhibited the migration of BGC-823 cells by 48.2% and 57.2% and the invasion of BGC-823 cells by 57.4% and 58.3%, respectively, compared with siNC (Figure 4(A)). siRNA-1 and siRNA-2 also significantly inhibited the migration of MKN-45 cells by 41.8% and 50.5% and the invasion of MKN-45 cells by 53.3% and 56.1%, respectively, compared with siNC (Figure 4(B)). Compared with siNC, the scratch wound was wider with fewer migrating cells at 24 h after transfected with siRNA-1 and siRNA-2 (Figure 4(C,D)). Moreover, the expression of \( I_{\beta}B \alpha \), p-NF-\( \beta \)p65 and NF-\( \beta \)p65 as well as the expression and activity of MMP-2 and MMP-9 were measured by western blotting and Gelatin zymography gel assay. It showed that siRNA-1 and siRNA-2 transfection in BGC-823 and MKN-45 cells significantly reduced the phosphorylation of NF-\( \beta \)p65 and the expression and activity of MMP-2 and MMP-9, but induced \( I_{\beta}B \alpha \) expression compared with siNC (Figure 4(E-H)).
Figure 2. Effect of H. pylori infection on the expression of IxBα, p-NF-κBp65 and NF-κBp65 in gastric cancer cells. (A) ZBTB20 expression levels in gastric cancer cell lines and GES-1 human gastric epithelial mucosa cell line were determined by western blot. ZBTB20 expression levels (B, C), transcriptional activation of ZBTB20 (D, E), and the protein expression of IxBα, p-NF-κBp65 and NF-κBp65 (F, G) upon H. pylori infection in BGC-823 and MKN-45 gastric cancer cell lines were determined by real-time PCR, western blot or luciferase reporter gene assay. **p < .01, ***p < .001 compared with control group.

Figure 3. Effect of ZBTB20 silencing on the proliferation of gastric cancer cell lines. BGC-823 (A, B) and MKN-45 cells (C, D) were transfected with three siRNA targeting human ZBTB20, and the expression levels of ZBTB20 were determined by real-time PCR and western blot analysis. (E, F) BGC-823 and MKN-45 cells were transfected with siRNA-1 and siRNA-2 targeting human ZBTB20, and cell proliferation was determined by CCK-8. ***p < .001 compared with siNC.
ZBTB20 overexpression promotes cell proliferation, invasion and migration of gastric cancer

In addition, recombinant pCDNA3.1(+)–ZBTB20 or blank pCDNA3.1(+) as a negative control was also transduced into BGC-823 and MKN-45 cells. Overexpression of ZBTB20 markedly increased the mRNA and protein expression of ZBTB20 by 3.98-fold and 1.10-fold in BGC-823 cells and by 7.12-fold and 1.61-fold in MKN-45 cells, respectively, compared with the blank vector (Figure 5(A–D)).

We found that pCDNA3.1(+)–ZBTB20 infection significantly promoted the proliferation of BGC-823 cells by 10.5%, 20.7% and 29.3% and that of MKN-45 cells by 10.4%, 20.6% and 29.3% compared with the blank vector (Figure 5(A–D)).

**Figure 4.** Effect of ZBTB20 silencing on migration and invasion of gastric cancer cell lines. BGC-823 and MKN-45 cells were transfected with siRNA-1 and siRNA-2 targeting human ZBTB20. Cell migration and invasion were determined by Transwell (A, B) and Wound healing assay (C, D); the protein expression of IκBα, p-NF-κBp65, NF-κBp65, MMP-2 and MMP-9 was determined by western blotting (E, G); and the activity of MMP-2 and MMP-9 was determined by Gelatin zymography gel assay (F, H). ***p < .001 compared with siNC.
32.3% at 24, 48 and 72 h, respectively, compared with blank vector (Figure 5(E,F)). Moreover, pCDNA3.1(+) -ZBTB20 infection significantly promoted the migration and invasion of BGC-823 cells by 26.0% and 62.2% and that of MKN-45 cells by 23.7% and 49.2%, respectively, compared with blank vector (Figure 6(A,B)). Compared with blank vector, the scratch wound was closer with more migrating cells at 24 h after infected with pCDNA3.1(+) -ZBTB20 (Figure 6(C,D)). As shown in Figure 6(E–H), pCDNA3.1(+) -ZBTB20 infection in BGC-823 and MKN-45 cells significantly increased the phosphorylation of NF-κBp65 and the expression and activity of MMP-2 and MMP-9, but reduced IκBα expression compared with the blank vector.

The involvement of IκBα/NF-κB signaling in the function of ZBTB20

Give the role of ZBTB20 in the regulation of expression of IκBα, NF-κBp65, MMP-9 and MMP-2, we speculate that inhibition of IκBα expression to induce NF-κB activation may involve in the function of ZBTB20 in gastric cancer. Therefore, IκBα expressing vector (pCDNA3.1(+) -IκBα) was constructed in BGC-823 and MKN-45 cells and its effect on NF-κB activation, cell proliferation, migration and invasion was also measured. As shown in Figure 7(A–D), overexpression of IκBα markedly increased the mRNA and protein expression of IκBα by 9.43-fold and 7.19-fold in BGC-823 cells and by 9.78-fold and 0.60-fold in MKN-45 cells, respectively, compared with the blank vector. Moreover, our data also demonstrated that IκBα overexpression significantly inhibited the proliferation, invasion and migration of MKN-45 and BGC-823 cells induced by ZBTB20 overexpression (Figures 7(E,F) and 8(A–D)). We further checked up the phosphorylation of NF-κBp65 as well as the expression of IκBα, NF-κBp65, MMP-2 and MMP-9. As shown in Figure 8(E–H), IκBα overexpression significantly inhibited the phosphorylation of NF-κBp65 and the expression and activity of MMP-2 and MMP-9 in BGC-823 and MKN-45 cells induced by ZBTB20 overexpression, suggesting the inactivation of NF-κB signaling. These data indicate that ZBTB20 promotes invasion, migration and proliferation of gastric cancer cell lines by inhibiting IκBα expression to induce NF-κB activation.

Discussion

Although increasing studies have focused on ZBTB20 and cancer [8–10], the underlying mechanisms of ZBTB20 in cancer initiation remain largely unknown. In this study, we found that ZBTB20 expression is markedly upregulated in gastric cancer tissues and in gastric cancer cell lines infected with H. pylori. ZBTB20 knockdown significantly inhibits cell proliferation, invasion and migration as well as induces IκBα expression to inactivate NF-κB signaling in gastric cancer, while ZBTB20 overexpression induced the inverse effects were inhibited by IκBα overexpression.

Gastric cancer (GC) is the third leading cause of cancer-related deaths worldwide, and H. pylori infection is an important factor in the pathogenesis of gastric cancer. The major mechanisms of H. pylori-associated gastric cancer
include perturbed multiple signaling pathways, induced mutations, and accumulated aberrant DNA methylation [14]. In this study, we presented that ZBTB20 expression was significantly upregulated in gastric cancer tissues and in gastric cancer cell lines infected with *H. pylori*, which suggest that ZBTB20 may involve in the initiation and progression of gastric cancer. Our study evaluated for the first time correlations between *H. pylori* infection and ZBTB20 expression in gastric...
cancer cell lines. Similar to our findings, previous studies have demonstrated that ZBTB20 is significantly up-regulated in liver and lung cancer tissues compared with adjacent normal tissues [9,10].

ZBTB20 suppressed apoptosis and induced cell invasion, migration and proliferation of glioblastoma [8]. ZBTB20 promoted cell cycle progression and cell proliferation in hepatocellular carcinoma and non-small cell lung cancer [9,10]. Gastric cancer is characterized by poor prognosis, strong invasion, and early metastasis. Identify new and specific markers for gastric cancer that contributes to this neoplasm at an early stage will improve the poor prognosis. A study showed that the rs9841504 polymorphism in ZBTB20 was statistically susceptible to gastric cancer [15]. Consistently, our data showed that ZBTB20 knockdown in gastric cancer cell lines markedly suppressed cell proliferation, invasion and migration while ZBTB20 overexpression significantly promoted those. However, Shi et al., found a significant association between the rs9841504 polymorphism in ZBTB20 and decreased gastric cancer susceptibility, suggested that the ZBTB20 rs9841504 polymorphism is a protective factor for gastric cancer [11]. Meanwhile, no statistical difference was found between the rs9841504 polymorphism in ZBTB20 and gastric cancer risk in other previous studies [16–18]. It is likely that the contradictory results were due to the relatively small sample sizes and different genetic backgrounds. Therefore, the role of ZBTB20 in regulating gastric cancer process needs to be further investigated.

In cancer, NF-κB is proposed to contribute to oncogenesis through the induction of genes encoding proteins involved in promoting invasion and migration and suppressing apoptosis. Suppression of NF-κB activity through the inhibition of IκBα degradation contributes to the decreased cell migration in triple negative breast cancer [19]. Activating the NF-κB signaling pathway also promoted cell invasion, migration and proliferation in gastric cancer [20]. The NF-κB protein dimer is complexed with IκB protein under resting conditions. After stimulation with cytokines or lipopolysaccharide, IκBα is phosphorylated by upstream IKK (IκB kinases) and degradation, finally resulting in the activation of NF-κB [21]. Consistently, our loss- and gain- of-function studies showed that ZBTB20 knockdown significantly induced IκBα expression, and then resulted in the decrease in phosphorylation of NF-κBp65 in gastric cancer cell lines. Consistent with our findings, and mechanistically, ZBTB20 promotes NF-κB activation through inhibiting IκBα gene transcription and governing IκBα protein expression [12]. Moreover, ZBTB20 promotes hepatocyte proliferation and activation of NF-κB in regenerating liver after partial hepatectomy [13].

In this study, we also found that the expression and activity of MMP-9 and MMP-2 were markedly downregulated in ZBTB20 knockdown cells but upregulated in ZBTB20 ectopic cells. Matrix metalloproteinases (MMPs) are of great importance in regulating cell migration and proliferation. Dysregulation of MMPs is correlated with many diseases.
including cancer [22,23]. NF-κB has been shown to directly regulate the transcription of MMP-2 and MMP-9 [24]. The upregulation of MMP-2 and MMP-9 expression by activating NF-κB signaling pathway could promote metastasis in hepatocellular carcinoma [25], induce cell proliferation, invasion and migration in gastric cancer in vitro, and promote tumor growth and metastasis in gastric cancer in vivo [23]. Thus, it is indicates that ZBTB20 regulates cell invasion,
migration and proliferation in gastric cancer through IκBα/ NF-κB signaling pathway.

**Conclusion**

In summary, we have identified upregulated ZBTB20 expression in gastric cancer cell lines infected with *H. pylori* and in human gastric cancer tumor samples. ZBTB20 promotes cell invasion, migration and proliferation of gastric cancer through inhibiting IκBα to induce NF-κB activation. These findings demonstrated that ZBTB20 may be an appropriate target for gastric cancer prevention and treatment.

**Disclosure statement**

There are no competing financial interests in relation to this work.

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