

**LAMA4 expression is activated by zinc finger E-box-binding homebox 1 and independently predicts poor overall survival in gastric cancer**

XIANGJUN WANG¹, QINGE HOU² and XINLING ZHOU³

¹Clinical Laboratory, Linyi Central Hospital, Linyi, Shandong 276400;
²Nursing Department, Traditional Chinese Medicine Hospital of Yangxin, Binzhou, Shandong 251800;
³Digestive Endoscopy Room, Weifang People's Hospital, Weifang, Shandong 261041, P.R. China

Received January 1, 2018; Accepted July 2, 2018

DOI: 10.3892/or.2018.6564

Correspondence to: Dr Xinling Zhou, Digestive Endoscopy Room, Weifang People's Hospital, 151 Guangwen Street, Weifang, Shandong 261041, P.R. China
E-mail: xlingzhou@foxmail.com

Abbreviations: TCGA, The Cancer Genome Atlas; STAD, stomach adenocarcinoma; OS, overall survival; LAMA4, laminin subunit α4; ZEB1, zinc finger E-box-binding homebox 1; EMT, epithelial-mesenchymal transition; ECM, extracellular matrix

**Key words:** laminin subunit α4, zinc finger E-box-binding homebox 1, overall survival, gastric cancer

---

**Abstract.** The present study aimed to investigate the association between the expression of ZEB1 and LAMA4 in gastric cancer and the possible underlying mechanisms of this. In addition, the present study also investigated the prognostic value of LAMA4 in gastric cancer. LAMA4, MMP2, MMP9 and ZEB1 expression and their associations were analyzed by data mining in The Cancer Genome Atlas-stomach adenocarcinoma (TCGA-STAD). Overall survival (OS) curves of patients with gastric cancer were generated using data from TCGA and Kaplan-Meier plotting. Gastric cancer HGC-27 and SGC-7901 cell lines were used as in vitro cell models to assess the effect of LAMA4 on cell migration and invasion and to study the regulatory effect of ZEB1 on LAMA4 expression. The results of the present study indicated that LAMA4 upregulation was associated with higher grade tumors. LAMA4-knockdown significantly reduced MMP2 expression in gastric cancer cells and impaired the speed of wound healing and the invasive capability of the cancer cells. ZEB1 was strongly co-expressed with LAMA4 in TCGA-STAD (Pearson's r=0.85). Induced ZEB1 expression significantly increased LAMA4 expression at the mRNA and protein level in HGC-27 and SGC-7901 cells. A dual-luciferase assay confirmed that ZEB1 directly binded to the promoter of LAMA4. High LAMA4 expression independently predicted a poor OS (HR, 1.614; 95% CI, 1.155-2.256; P=0.005) in patients with primary gastric cancer. These results indicated that ZEB1 was able to epigenetically activate LAMA4 expression via binding to its promoter in gastric cancer cells. High LAMA4 expression was an independent indicator of a poor OS in patients with gastric cancer.

**Introduction**

Laminins are a family of extracellular matrix (ECM) glycoproteins, which are the major non-collagenous constituent of basement membranes and serve an important role in cell differentiation, migration and adhesion (1,2). In gastric cancer, certain laminin family members are dysregulated and are associated with malignant phenotypes. For example, laminin γ2 upregulation may constitute an adaptive stimulus that allows E-cadherin-defective cells to survive and invade, which contributes toward the subsequent cancer progression (3). Co-expression of laminin β3 and γ2 is significantly correlated with the depth of invasion and advanced tumor stage (4). Epigenetic silencing of laminin β3 chain may reduce cancer cell invasion (4).

The a4 subunit[ laminin a4 (LAMA4)] is a component of laminin-8 and laminin-9, which is present in tissues of mesenchymal origin, in endothelial basement membranes and in certain epithelial basement membranes (5). Recent studies have reported that aberrant LAMA4 expression is associated with enhanced cell migration and metastasis of certain types of cancer, including hepatocellular (6) and breast cancer (7), and renal carcinoma (8). However, the effect of LAMA4 dysregulation on gastric cancer is poorly understood.

Zinc finger E-box-binding homebox (ZEB) 1 is an E-box binding transcription factor and is one of the key epithelial-mesenchymal transition (EMT)-inducible genes in multiple types of cancer (9-11). Previous studies have demonstrated that ZEB1 is an independent factor for peritoneal dissemination in patients with gastric cancer (12,13). Knockdown of ZEB1 can significantly reduce Vimentin expression and increase E-cadherin expression in gastric cancer cells (14), and can also decrease the invasive potential of the cancer cells (15,16). As a transcriptional factor, ZEB1 can act as a transcriptional activator (via binding to CtBP co-repressors) and repressor (via binding to chromatin
remodeling ATPase BRG1, histone acetyl-transferase TIP60 and histone deacetylase SIRT1) (17), depending on specific genes and cells (18,19). In gastric cancer, its downstream regulation remains to be fully elucidated.

The present study investigated the prognostic value and functional role of LAMA4 in gastric cancer and further investigated the association between the expression of ZEB1 and LAMA4.

Materials and methods

Bioinformatic analysis. The clinicopathological data of patients with primary gastric cancer, the mRNA expression of LAMA4, MMP2, MMP9 and ZEB1, and their associations in The Cancer Genome Atlas-stomach adenocarcinoma (TCGA-STAD) were analyzed using UCSC Xena Browser (http://xena.ucsc.edu/). The genes co-upregulated with LAMA4 in TCGA-STAD were also identified using the UCSC browser. LAMA4 protein expression in gastric cancer tissues and in normal gastric tissues was reviewed using immunohistochemistry (IHC) images from the Human Protein Atlas (http://www.proteinatlas.org) (20), via www.protein-nohistochemistry (IHC) images from the Human Protein browser.

LAMA4 shRNA particles or negative controls. A shRNA LAMA4 shRNA lentiviral (SHCLNV-NM) and the corresponding negative control (empty pLOK.1-CMV-tGFP) were purchased from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany); ZEB1 lentiviral particles and the corresponding negative controls were purchased from GeneCopoeia (Rockville, MD, USA). The total transducing units needed (TU) for infection was calculated by (total number of cells per well x3). The cancer cells were infected with the lentiviral particles in the presence of Polybrene (8 µg/ml; Sigma-Aldrich; Merck KGaA) according to the manufacturer's protocols and were subjected to analysis 48 h later.

Western blot analysis. Conventional western blotting was performed to detect protein band signals. Cells were lysed using a radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology, Haimen, China) for protein extraction. Protein concentration was determined using a bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology, Haimen, China). The proteins (25 µg protein/lane) were separated by 10% SDS-PAGE and transferred onto polyvinylidene difluoride membranes. The membranes were blocked with 5% skimmed milk for 1 h at room temperature and then incubated with primary antibodies overnight at 4°C. The primary antibodies used were as follows: Anti-LAMA4 (1:1,000; cat. no. ab209675; Abcam, Cambridge, UK), anti-MMP2 (1:1,000; cat. no. ab37150; Abcam), anti-MMP9 (1:1,000; cat. no. ab38898; Abcam), anti-ZEB1 (1:2,000; cat. no. ab180905; Abcam) and anti-β-actin (1:2,000; cat. no. ab3280; Abcam). Following incubation with horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG H&L (1:5,000; cat. no. ab205719; Abcam) or HRP-conjugated goat anti-rabbit IgG H&L (1:10,000; cat. no. ab205718; Abcam) secondary antibody for 1 h in TBST with 5% skimmed milk at room temperature, protein band signals were developed using the enhanced chemiluminescence Plus kit (Amersham, Piscataway, NJ, USA). Band densitometry was performed using ImageJ software (v2.1.4.6; National Institutes of Health, Bethesda, MD, USA).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from cell samples using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and were used as the template for reverse transcription with the ProtoScript First Strand cDNA Synthesis kit (New England Biolabs, Ipswich, MA, USA). In brief, RNA was denatured for 5 min at 70°C. Next, cDNA synthesis reaction was conducted at 42°C for 1 h. Finally, the enzyme was inactivated at 80°C for 5 min. Subsequently, qPCR was performed to detect the expression of LAMA4 mRNA using the SYBR® Select Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) in an ABI 7900HT Fast Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The primer sequences used were as follows: LAMA4 forward, 5'-GGAAATAAAGCCAGAGCCACC-3' and reverse, 5'-AGCCACAGAGGAGAACC-3' and GAPDH forward, 5'-GTCTCCTCTGACCTCAACGCG-3' and reverse, 5'-ACCACCTGTGCTGGAGGCA-3'. The relative expression of LAMA4 mRNA was calculated using the 2^ΔΔCq method (22).

Wound healing assay. In brief, HGC-27 and SGC-7901 cells were cultured in 6-well plates and were infected with lentiviral LAMA4 shRNA particles or negative controls. A total of 24 h later, confluent cell monolayers were manually wounded by scraping the cells with a 200 µl pipette tip. Wound images were taken at 0 and 24 h after the scratch under an inverted microscope (IX73; Olympus Corporation, Tokyo, Japan), at a magnification of x10. The wound areas were measured using ImageJ software (v2.1.4.7; n=3).
A Transwell assay was conducted using a Matrigel invasion chamber (BD Biosciences, San Jose, CA, USA) in a 24-well cell culture plate according to the manufacturer's protocols. Briefly, 3x10⁴ HGC-27 and SGC-7901 cells infected with LAMA4 shRNA particles or the negative controls were seeded into the upper chamber inserts containing an 8-µm pore size membrane with a thin layer Matrigel matrix, with 500 µl serum-free DMEM. The lower chamber of the well was filled with 700 µl DMEM with 20% FBS. A total of 48 h later, cells that had invaded the lower surface of the membrane were fixed with 70% methanol at room temperature for 10 min while the non-invading cells on the upper surface were removed. The invaded cells were stained with 0.1% crystal violet for 30 min at room temperature, and the number was then determined for 3 independent fields under an inverted microscope (IX73; Olympus Corporation), at a magnification of x100.

**Dual-luciferase reporter assay.** The promoter sequence of LAMA4 was obtained from GeneCopoeia (>HPRM34295 NM_001105206). The possible ZEB1 binding sites in the LAMA4 promoter region were predicted using the JASPAR database (http://jaspar.genereg.net/). LAMA4 promoter fragments (-1,351 to +219 and -700 to +219) were PCR amplified from the promoter clone (>HPRM34295). The fragments were then inserted into the sites between XhoI-HindIII of pGL3-basic plasmids (Promega Corporation, Madison, WI, USA). 293 cells cultured in 12-well plates were initially infected with lentiviral ZEB1 expression particles or the empty control. A total of 24 h later, the cells were co-transfected with 1.5 µg luciferase construct plasmids (Promega Corporation) or the empty reporter vector DNA and 0.05 µg phRL-TK (Promega Corporation) using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). A total of 24 h after transfection, the cells were lysed. The luciferase activity of the lysate was measured using the dual-luciferase reporter assay system with a luminometer and was normalized to that of Renilla luciferase activity (Promega Corporation, Madison, WI, USA).

**Statistical analysis.** Statistical analysis was performed using GraphPad Prism 6.0 software (GraphPad Software, Inc., La Jolla, CA, USA). All assays were performed in triplicate and data are reported as the mean ± standard deviation. The group difference was examined by two-tailed Student's t-tests or one-way analysis of variance with Student-Newman-Keuls test as a post hoc test. The association between LAMA4 RNA expression and the clinicopathological features was assessed using χ² tests. Receiver operating characteristic (ROC) curves for mortality were constructed and the optimal cut-off value of LAMA4 expression was determined based on the Youden index. Log-rank tests were performed to assess the difference between the survival curves. Prognostic values were analyzed by univariate and multivariate Cox regression models. P<0.05 was considered to indicate a statistically significant difference.

**Results**

LAMA4 upregulation is associated with higher grade tumors in gastric cancer. Using RNA-seq data in TCGA-STAD, it was revealed that LAMA4 RNA expression was not altered in gastric cancer tissues compared with normal stomach tissues (Fig. 1A). However, in the cancer cases, the grade 3 tumors had significantly higher LAMA4 expression than the
grade 1/2 tumors (P=0.0018; Fig. 1B). By reviewing LAMA4 IHC images in Human Protein Atlas, it was revealed that the LAMA4 staining was usually low in the glandular cells in normal tissues (Fig. 1C). However, the intensity of LAMA4 staining varied significantly in different gastric cancer cases (Fig. 1D). Among 11 gastric cancer tissues, 3 cases had moderate/high LAMA4 staining, while 3 cases had low LAMA4 staining (Fig. 1D).

Knockdown of LAMA4 impaired the migration and invasion of gastric cancer cells. One previous study reported that LAMA4 could promote trophoblast cell invasion and migration via upregulating MMP2 and MMP9, two enzymes facilitating invasion by degrading the ECM (5). By data mining in TCGA-STAD, we characterized the co-expression trend between LAMA4 and MMP2 or MMP9 (Fig. 2A). Heat-map and subsequent regression analysis demonstrated that LAMA4 was significantly co-upregulated with MMP2 (Pearson’s r=0.70), but not with MMP9 (Pearson’s r=0.18) among the 415 patients with gastric cancer (Fig. 1D). Among 11 gastric cancer tissues, 3 cases had moderate/high LAMA4 staining, while 3 cases had low LAMA4 staining (Fig. 1D).

ZEB1 directly increases LAMA4 expression via binding to its promoter. By screening the genes co-upregulated with LAMA4 in TCGA-STAD, it was revealed that ZEB1 was correlated with LAMA4 in gastric cancer (Pearson’s r=0.85; Fig. 3A). In fact, ZEB1 upregulation has well-characterized oncogenic effects on gastric cancer (13,23). By using the UCSC Xena browser (Fig. 3A and B) and the cBioPortal for Cancer Genomics (Fig. 3C), two online tools to analyze data in TCGA-STAD, a strong correlation between the expression of ZEB1 and that of LAMA4 was confirmed (Fig. 3A-C). To further investigate the effect of ZEB1 on LAMA4 expression, HGC-27 and SGC-7901 cells were infected with lentiviral LAMA4 expression particles for overexpression (Fig. 3D). Enforced ZEB1 expression significantly elevated LAMA4 expression at the mRNA and protein levels (Fig. 3D and E). By promoter scanning, two possible and close ZEB1 binding sites were identified in the promoter of LAMA4 (Fig. 3F). pGL3-basic-based luciferase reporter plasmids carrying the intact LAMA4 promoter sequence or truncated sequence

<table>
<thead>
<tr>
<th>Parameter</th>
<th>High (n=204)</th>
<th>Low (n=184)</th>
<th>χ²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD</td>
<td>65.30±10.68</td>
<td>65.30±10.62</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>67</td>
<td>69</td>
<td>0.92</td>
<td>0.34</td>
</tr>
<tr>
<td>Male</td>
<td>137</td>
<td>115</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histological grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1/G2</td>
<td>65</td>
<td>82</td>
<td>6.62</td>
<td>0.01</td>
</tr>
<tr>
<td>G3</td>
<td>134</td>
<td>98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GX</td>
<td>5</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodal status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>56</td>
<td>60</td>
<td>0.99</td>
<td>0.32</td>
</tr>
<tr>
<td>N+</td>
<td>141</td>
<td>121</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>7</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastasis status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>181</td>
<td>166</td>
<td>0.14</td>
<td>0.71</td>
</tr>
<tr>
<td>M1</td>
<td>14</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MX</td>
<td>9</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I/II</td>
<td>81</td>
<td>91</td>
<td>3.01</td>
<td>0.080</td>
</tr>
<tr>
<td>III/IV</td>
<td>114</td>
<td>89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discrepancy + Null</td>
<td>9</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>106</td>
<td>125</td>
<td>10.25</td>
<td>0.0014</td>
</tr>
<tr>
<td>Deceased</td>
<td>98</td>
<td>59</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GX, grade could not be assessed; MX, the presence of distant metastasis could not be assessed; null, no data.
Figure 2. Knockdown of LAMA4 impaired the migration and invasion of gastric cancer cells. (A) Heat-map of LAMA4, MMP2 and MMP9 expression in TCGA-STAD (top) and the results of regression analysis (bottom) between LAMA4 and MMP2 or MMP9. (B) Western blot analysis of LAMA4 protein expression in HGC-27 and SGC-7901 cells 36 h after infection with LAMA4 lentiviral shRNA. (C) Western blot analysis of MMP2 and MMP9 expression in HGC-27 and SGC-7901 cells 48 h after infection with LAMA4 lentiviral shRNA. (D) Representative images and (E) quantitation results of wound healing assay. (F) Representative images and (G) quantitation results of Transwell assay, which were conducted 48 h after infection of HGC-27 and SGC-7901 cells with LAMA4 lentiviral shRNA. (D and E) The relative wound areas at 24 h compared with 0 h after scratching were calculated to reflect the speed of wound healing. (F and G) The relative proportion of invaded cells in LAMA4 shRNA groups compared with shNC groups were calculated to reflect the capability of cell invasion. LAMA4, laminin subunit α4; MMP, matrix metalloproteinase; TCGA-STAD, The Cancer Genome Atlas-stomach adenocarcinoma; shRNA, short hairpin RNA; NC, negative control.
Figure 3. ZEB1 directly increases LAMA4 expression via binding to its promoter. (A) Heat-map and (B) regression analysis of the correlation between ZEB1 and LAMA4. Data analysis was performed by using the UCSC Xena browser. (C) Regression analysis of the correlation between ZEB1 and LAMA4. Data analysis was performed using the cBioPortal for Cancer Genomics. (D) Western blot analysis of ZEB1 and LAMA4 expression in HGC-27 and SGC-7901 cells 48 h after infection of ZEB1 lentiviral expression particles or empty controls. (E) qRT-PCR analysis of LAMA4 mRNA expression in HGC-27 and SGC-7901 cells 48 h after infection of ZEB1 lentiviral expression particles or empty controls. (F) Predicted ZEB1 binding sites in the LAMA4 promoter. (G) Design of reconstructed pGL3-basic plasmids carrying intact or truncated LAMA4 promoter fragments. (H) The luciferase reporter constructs carrying intact or truncated LAMA4 promoter sequences were introduced into 293 cells pre-infected with lentiviral ZEB1 expression particles or the empty control. Luciferase activity was measured 24 h post-transfection. ZEB1, zinc finger E-box-binding homeobox 1; LAMA4, laminin subunit α4; TCGA, The Cancer Genome Atlas.
were generated (Fig. 3G). A luciferase assay revealed that ZEB1 overexpression significantly increased the luciferase activity of the reporter with the intact LAMA4 promoter sequence (Fig. 3H). By comparison, ZEB1 overexpression had little influence on the luciferase activity of the reporter with the truncated LAMA4 promoter sequence (Fig. 3H).

High LAMA4 expression independently predicts a poor OS in patients with primary gastric cancer. In order to investigate the prognostic value of LAMA4 in gastric cancer, the association between LAMA4 expression and OS was further assessed based on data in TCGA-STAD and by data mining in Kaplan-Meier plotter. The associations between LAMA4 expression and the clinicopathological parameters in patients with primary gastric cancer in TCGA were summarized in Table I. The high LAMA4 expression group had significantly higher ratios of grade 3 (G3) tumors (134/199, 67.3%) and mortality (98/204, 48.0%) than the low LAMA4 expression group (G3, 98/180, 54.4%; mortality, 59/184, 32.1%; Table I). Kaplan-Meier curves demonstrated that the high LAMA4 expression group (n=204) had significantly poorer OS rates than the low LAMA4 expression group (n=184; P=0.0022; Fig. 4A). Data mining in Kaplan-Meier plotter also confirmed this association (HR, 1.4; 95% CI, 1.17-1.67; P<0.001; Fig. 4B).

In univariate analysis, it was revealed that high age (>65), high grade (G3/G4), nodal invasion, metastasis, advanced disease stage (III/IV) and high LAMA4 expression were associated with significantly shorter OS times (Table II). Multivariate

Table II. Univariate and multivariate analyses of overall survival in patients with primary gastric cancer in TCGA-STAD.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Univariate analysis</th>
<th></th>
<th></th>
<th></th>
<th>Multivariate analysis</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-value</td>
<td>HR</td>
<td>95% CI</td>
<td>HR</td>
<td>95% CI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &gt;65 vs. ≤65</td>
<td>0.006</td>
<td>1.572</td>
<td>1.139</td>
<td>2.171</td>
<td>0.002</td>
<td>2.001</td>
<td>1.421</td>
</tr>
<tr>
<td>Sex Female vs. male</td>
<td>0.296</td>
<td>0.835</td>
<td>0.596</td>
<td>1.171</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade G3/G4 vs. G1/G2</td>
<td>0.022</td>
<td>1.479</td>
<td>1.057</td>
<td>2.069</td>
<td>0.039</td>
<td>1.463</td>
<td>1.020</td>
</tr>
<tr>
<td>Nodal status N1+ vs. N0</td>
<td>0.001</td>
<td>2.042</td>
<td>1.362</td>
<td>3.061</td>
<td>0.136</td>
<td>1.526</td>
<td>0.876</td>
</tr>
<tr>
<td>Metastasis status M1 vs. M0</td>
<td>0.002</td>
<td>2.334</td>
<td>1.367</td>
<td>3.986</td>
<td>0.002</td>
<td>2.429</td>
<td>1.371</td>
</tr>
<tr>
<td>Clinical stage III/IV vs. I/II</td>
<td>&lt;0.001</td>
<td>2.063</td>
<td>1.460</td>
<td>2.916</td>
<td>0.173</td>
<td>1.398</td>
<td>0.863</td>
</tr>
<tr>
<td>LAMA4 expression High vs. low</td>
<td>0.002</td>
<td>1.664</td>
<td>1.205</td>
<td>2.300</td>
<td>0.005</td>
<td>1.614</td>
<td>1.155</td>
</tr>
</tbody>
</table>

Figure 4. LAMA4 upregulation is associated with unfavorable OS in patients with gastric cancer. Kaplan-Meier curves of OS in patients with gastric cancer grouped by high and low LAMA4 expression. Survival curves were generated by (A) using data from TCGA-STAD or (B) by data mining in Kaplan-Meier plotter.
analysis revealed that the high LAMA4 expression could independently predict a poor OS (HR, 1.614; 95% CI, 1.155-2.256; P=0.005; Table II).

Discussion

In the present study, the results of bioinformatic analysis indicated that LAMA4 upregulation was associated with higher grades of gastric cancer. LAMA4 upregulation is associated with enhanced invasion and metastasis of cancer cells. In hepatocellular carcinoma, LAMA4 has specific in vivo distribution in the tumor basement membrane and its upregulation is correlated with tumor invasion and metastasis (6). In renal cell carcinoma, LAMA4 is upregulated in locally advanced tumors and in primary tumor and secondary metastases (8). LAMA4 upregulation may also predict poor survival in patients with renal cell carcinoma (8). One recent study reported that LAMA4 could promote trophoblast cell invasion and migration via upregulating MMP2 and MMP9 (5). MMP2 and MMP9 are two critical enzymes degrading ECM, thereby supporting cancer cell migration and invasion (24,25). In fact, trophoblast research over the past decades revealed that placental cells have high levels of similarities in proliferative, migratory and invasive properties to those of cancer cells (26). As LAMA4 dysregulation may be associated with tumor grade in gastric cancer, the present study investigated its regulative effect on the migration and invasion of gastric cancer cells. In HGC-27 and SGC-7901 cells, LAMA4-knockdown significantly reduced MMP2 expression, but had little influence on MMP9 expression. Functional assays revealed that LAMA4 inhibition impaired the speed of wound healing and also reduced the invasive capability of the cancer cells.

To investigate the mechanism of LAMA4 dysregulation in gastric cancer, we identified the genes significantly co-expressed with LAMA4 in TCGA-STAD and observed that ZEB1 is correlated with LAMA4 expression. The oncogenic effects of aberrant ZEB1 expression in gastric cancer have been widely reported (13,15,27). As a transcription factor, ZEB1 can modulate the expression of a series of genes in different types of cancer. In breast cancer, ZEB1 can upregulate VEGF expression and promote angiogenesis (28). Additionally, ZEB1 can reduce NGN3 transcription via forming a ZEB1/DNA methyltransferase (DNMT)3B/histone deacetylase 1 (HDAC1) complex on the NGN3 promoter (29). In tongue cancer cells, ZEB1 can bind to the CA9 promoter and positively regulate its expression, thereby leading to enhanced chemoresistance (30). In gallbladder cancer cells, ZEB1 can repress T-cadherin expression via binding to the promoter, thereby increasing their invasive capability (31). These results suggested that ZEB1 can be either an epigenetic activator or repressor, depending on specific gene and cancer types. However, the regulative effect of ZEB1 in gastric cancer is not yet fully understood. The present study revealed that ZEB1 can directly increase LAMA4 expression via binding to its promoter in gastric cancer cells. This finding revealed a novel regulative effect of ZEB1 in gastric cancer.

Based on data mining in two large databases, including TCGA-STAD and Kaplan-Meier plotter, it was revealed that LAMA4 upregulation is associated with unfavorable OS rates in patients with gastric cancer. Univariate and multivariate analysis demonstrated that the high LAMA4 expression could independently predict a poor OS rate (HR, 1.614; 95% CI, 1.155-2.256; P=0.005), suggesting that LAMA4 expression may be a valuable biomarker in gastric cancer.

Based on the aforementioned results, we hypothesized that ZEB1 could epigenetically activate LAMA4 expression via binding to its promoter in gastric cancer cells, while high LAMA4 expression was an independent indicator for a poor OS in patients with gastric cancer.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

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

Authors' contributions

XW and QH performed cellular studies and conducted data analysis and interpretation. XW and XZ collected and analyzed data from databases. All authors participated in the manuscript preparation and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References


