TGFβ1-Induced LncRNA UCA1 Upregulation Promotes Gastric Cancer Invasion and Migration

Zhong-Kun Zuo, Yi Gong, Xiang-Heng Chen, Fei Ye, Zheng-Ming Yin, Qian-Ni Gong, and Jiang-Sheng Huang

According to recent studies, long noncoding RNA urothelial carcinoma associated 1 (UCA1) is involved in the development and progression of many malignant tumors, including gastric cancer (GC). We validated the detailed role of UCA1 in human GC cell lines and GC tissues so as to determine its exact function and the underlying mechanism of GC invasion and migration. In our research, lncRNA-UCA1 was specifically upregulated in GC tissues and cell lines, and augmented GC cell proliferation, and invasive and migratory capabilities. High UCA1 expression in GC was related with poorer prognosis (poorer invasion depth, lymph node metastasis, advanced TNM [T is for the original (primary) tumor, N for nearby (regional) lymph nodes that are involved, and M for distant metastasis] stage, and shorter overall survival). Epithelial mesenchymal transition (EMT), associated with malignancy of cancers, was reported to be responsible for invasion and migration of cancer cells. Transforming growth factor β1 (TGFβ1)-induced EMT was well evaluated. UCA1 silence reduced the protein levels of EMT-related factors, vimentin and snail, while promoted E-cadherin and zonula occludens-1 protein levels in GC cells; the effect of UCA1 could be partly restored by TGFβ1 treatment. Taken together, UCA1 might regulate the tumor proliferation, invasion, and metastasis under TGFβ1 induction. Taken together, UCA1 might present a potential oncogenic factor by promoting GC cell proliferation, invasion, and migration. UCA1 could serve as a novel biomarker for prognosis and a novel therapeutic target of GC treatment.

Keywords: TGFβ1, UCA1, gastric cancer, invasion, migration

Introduction

Gastric cancer (GC) has long been regarded as the most common malignant tumors of the digestive system. In 2008, 0.989 million GC cases newly occurred; 0.738 million GC deaths occur every year worldwide (Ferlay et al., 2010; Xu et al., 2013). Although the pathogenic factors of GC include environmental factors, diet, infections, and host genes, the disorders in gene expression exert major functions in the pathogenesis of GC (Ueda et al., 2010; Guo and Yan, 2015). With study on the underlying mechanism of GC initiation, progress and transiting, targeted drug therapy for GC attracted more concern in clinical trials (Takahashi et al., 2013).

Human genome sequence data indicates that more than 90% of the DNA sequences actively transcribe but only 2% of it encodes protein, thus the majority of transcripts are referred to as non-coding RNAs (ncRNAs) (Djebali et al., 2012; Martens-Uzunova et al., 2014). Small non-coding RNAs, long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) included, have been studied widely and their roles in biological process regulation and cell function have been elucidated in numerous cancers (Martens-Uzunova et al., 2014).

Recently, several reports revealed that lncRNAs exert crucial functions in both normal biological processes and disease processes including malignant tumors and cancers (Ponting et al., 2009). Emerging evidence indicated that some lncRNAs function as oncogenes, tumor-inhibiting factor genes or both, depending on the circumstance (Zhou et al., 2014). According to recent studies, urothelial carcinoma associated 1 (UCA1), one of the novel-found long non-coding RNA, might be associated with the development of many types of malignant tumors, including breast cancer, GC, and bladder cancer (Pan et al., 2016; Shang et al., 2016; Xiao et al., 2016). By far, the detailed roles and the underlying mechanisms of UCA1 in GC invasion and migration remain unclear yet.

According to the previous studies, epithelial mesenchymal transition (EMT) is frequently related with cancer cell migration and tumor metastasis (Catalano et al., 2015; Wrighton, 2015). EMT in GC is a key factor for GC cell metastasis and also is the primary cause of GC death (Yoo et al., 2011; Zhang et al., 2016b). Zong et al. (2016) demonstrated that transforming growth factor β1 (TGFβ1) induces EMT in GC cells, as well as the cell invasion and migration. Given the key role of UCA1 in modulating
cancer cell proliferation, invasion, and migration, we speculated that UCA1 might be involved in TGFβ1-induced EMT in GC, and then influence GC process.

In this study, GC tissues and cell lines were obtained and UCA1 expression was determined. The detailed function of UCA1 in GC cell proliferation, and invasive and migratory capabilities were detected. In addition, protein levels of EMT-related factors in si-UCA1-transfected GC cells were monitored under TGFβ1 treatment, as well as the cell proliferation, invasion, and migration. Taken together, we report a significant higher UCA1 expression in GC tissues and cell lines; UCA1 silence could inhibit TGFβ1-induced EMT in GC, as well as the cell proliferation, and invasive and migratory capabilities. Our findings provide a novel understanding of the role of UCA1 in GC metastasis and the mechanism involved.

Materials and Methods

Tissue specimens, cell lines, and cell transfection

We collected 37 paired GC tissues and the corresponding adjacent normal tissues. All clinic specimens were collected from patients who received a surgical operation at the Second Xiangya Hospital, Central South University (Changsha, China). The clinic specimens were later stored in liquid nitrogen at −80°C. The project of the present study is under the approval of the Ethics Committee of the Second Xiangya Hospital, Central South University.

GES-1, human gastric mucosa epithelial cell, and human GC cell lines, including HGC27, MGC803, NCI-N87, BGC-823, and SGC7901 cells were purchased from the ATCC, cultured in RPMI-1640 medium (Invitrogen, CA) adding 10% FBS (Gibco, CA) as the supplement, and incubated at 37°C in a humidified atmosphere with 5% CO2. A si-UCA1 vector was used to achieve knockdown of UCA1 (GeneCopoeia, Guangzhou, China). After being plated in 96-well or 6-well plates, cells were then transfected with indicated siRNAs, cultured for 24 h, and consequently used for the next experiments.

RNA extraction and real-time PCR assays

We extracted total RNA from target cells by using TRIzol reagent (Invitrogen). UCA1, E-cadherin, vimentin, snail, and zonula occludens-1 (ZO-1) expressions were monitored using SYBR Green real-time quantitative PCR assay. RNU6B expression was used as an endogenous normalization. Data were analyzed by using 2^(-ΔΔCT) method.

Cell Counting Kit-8 assay

The proliferation rates of GC cells were monitored by using the Cell Counting Kit-8 (CCK-8) (Sigma-Aldrich, MO). We seeded 0.5 × 10^4 target cells in each well of the 96-well plate for 24 h. The target cells were then transfected with the indicated si-NC/si-UCA1, and further incubated cells for 1–5 days, respectively. One hour before the endpoint, 10 μL CCK-8 reagent was added into each well. A microplate reader was used to determine OD_{490nm} value in each well.

5-Bromo-2-deoxyuridine cell proliferation assay

By measuring 5-Bromo-2-deoxyuridine (BrdU) incorporation, the DNA synthesis in proliferating cells was determined. BrdU assays were conducted at 48 h after GC cells were transfected with the indicated vectors. Cells were seeded in 96-well culture plates at a density of 2 × 10^3 cells/well, cultured for 48 h, then incubated with a final concentration of 10 μM BrdU (BD Pharnmgen, San Diego, CA) for 2 h. When the incubation period ended, the medium was removed, the cells were fixed for 30 min at RT, incubated with peroxidase-coupled anti-BrdU antibody (Sigma-Aldrich) for 60 min at RT, washed three times with PBS, incubated with peroxidase substrate (tetramethylbenzidine) for 30 min, and the 450 nm absorbance values were measured for each well. Background BrdU immunofluorescence was determined in cells not exposed to BrdU, but stained with the BrdU antibody.

Western blot analysis

E-cadherin, vimentin, snail, and ZO-1 expressions in GC cells were detected by performing immunoblotting. We lysed the target cells in RIPA buffer supplemented with 1% phenylmethanesulfonyl fluoride, loaded the target protein onto a 15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis mini gel, and transferred the target protein onto the PVDF membrane. The blots were then probed with 1:1000 diluted rabbit polyclonal E-cadherin, vimentin, snail, and ZO-1 antibody (Abcam, MA) at 4°C overnight, then incubated with 1:5000 HRP-conjugated secondary antibody. Enhanced chemiluminescence substrate (Millipore, MA) was used to visualize signals. PageRuler™ Plus Prestained Protein Ladder (Thermo, MA) was used to calculate sample molecular weights. The molecular weights of the indicated proteins are as follows: E-cadherin: 110 kDa, vimentin: 57 kDa, snail: 68 kDa, ZO-1: 195 kDa, tubulin: 50 kDa. The relative protein expression was normalized to tubulin.

TGFβ1 induction

The SGC-7901 cells were seeded into appropriate culture plates with 5 × 10^3/cm². The cells growing to subconfluence were washed three times with nonserum RPMI-1640 and serum starved for 24 h at 37°C. Then the cells were washed with nonserum RPMI-1640 for a second time. Cells were induced by TGF-β1 (Sigma Company) with a concentration of 10 ng/mL for 48 h. BSA (Sigma Company) with a concentration of 10 ng/mL was used as the control (Vehicle group). The processed cells were washed with PBS twice.

Statistical analysis

Data from at least three independent experiments were exhibited as mean ± standard deviation, analyzed by SPSS 17.0 statistical software (SPSS, Chicago). One-way ANOVA was used to analyze the differences between groups in the proliferation, and invasion and migration assays. A p value of <0.05 was considered statistically significant.
**Results**

*High UCA1 expression in GC was related with poorer clinicopathological parameters and shorter overall survival*

To validate the exact function of UCA1 in GC development, the expression of UCA1 was first monitored in GC tissues, as well as in GC cell lines. In 37 paired GC tissues and the corresponding adjacent tissues, the expression of UCA1 was significantly increased in GC tissues, compared with the corresponding normal tissues (Fig. 1A). Besides, the expression of UCA1 was at a significantly higher level in the five human GC cell lines, HGC27, MGC803, NCI-N87, BGC-823, and SGC7901, compared with normal human gastric mucosa epithelial cell, GES-1 (Fig. 1B). Among the five cell lines, UCA1 expressed at higher levels in NCI-N87 and SGC7901 cell lines. To validate this result, we performed quantitative real-time PCR in 37 cases of GC tissues and adjacent normal tissues in training cohort. Compared with the corresponding normal tissues, UCA1 showed to be significantly upregulated (more than 1.4-fold [i.e., \( \log_2 (\text{fold change}) > 0.5 \)]) in 19 GC cases (>51.35%). Thirty-seven cases of GC tissues were divided into two groups: a high UCA1 expression group (above the median UCA1 expression, \( n = 18 \)) and a low UCA1 expression group (below the median UCA1 expression, \( n = 19 \)). High expression of UCA1 in GC showed to be related with poorer invasion depth (\( p = 0.005 \)), lymph node metastasis (\( p = 0.013 \)), and advanced TNM stage (\( p = 0.002 \)) as exhibited in Table 1. To determine the potential relationship between UCA1 expression and the patient’s prognosis, Kaplan–Meier analysis and log-rank test were used to evaluate the effects of UCA1 expression on overall survival (OS). The results indicated that patients with higher UCA1 expression had a significantly poorer prognosis compared with patients with lower UCA1 expression (\( p = 0.0447 \)) (Fig. 1D). A COX risk proportional regression model was further used to analyze the survival and pathological characteristics of 37 patients. The results of univariate analysis showed that tumor size, invasion depth, TNM stage, and UCA1 expression caused significant difference in survival time; the results of multivariate analysis showed that UCA1 expression that caused differences in survival time were statistically significant (hazard ratio [HR] = 2.917; 95% CI: 1.069–7.962) (Table 2). The above data suggested that UCA1 expression in GC tissues and cell lines, and its high expression is related with GC invasion depth, lymph node metastasis, advanced TNM stage, and a shorter OS. High UCA1 expression in GC is related with poorer prognosis; however, the detailed role of UCA1 in GC progression still remains unclear.

**FIG. 1.** UCA1 expression in GC and relation with invasion, migration, and staging. (A) In a large panel of 37 cases of primary GC tissues and the adjacent normal tissues the expression of UCA1 was determined. (B) The expression levels of UCA1 in five GC cell lines, U373, A172, T98G, SHG44, and U251, and a normal cell line, NHA, were determined using real-time PCR. Data are presented as mean ± SD of three independent experiments. **\( p < 0.01 \). (C) Expression of UCA1 in 37 pairs of GC tissues and their corresponding adjacent nontumorous tissues (ANTs) in a training cohort. Expression level of UCA1 was determined by real-time PCR and normalized to U6. Fold change was analyzed using the formula 2-(\( \Delta \Delta CT \) [GC/ANT]). Red line indicates fold change of UCA1 equal to 1.4. (D) Kaplan–Meier overall survival curves for 37 patients with GC classified according to relative UCA1 expression level. GC, gastric cancer; UCA1, urothelial carcinoma-associated 1. SD, standard deviation. Color images available online at www.liebertpub.com/dna
The role of UCA1 in GC cell proliferation, invasion and migration

To validate experimentally the detailed role of UCA1 expression in GC cell proliferation, invasive and migratory capabilities, and UCA1 expression was knocked down by si-UCA1 transfection in NCI-N87 and SGC7901 cells (Fig. 2A). The cell proliferation of NCI-N87 and SGC7901 cells was monitored. As shown by BrdU cell proliferation assays and CCK-8 assays, the cell proliferation of both NCI-N87 and SGC7901 cells was reduced by UCA1 knockdown (Fig. 2B–D). For a better understanding of the detailed role of UCA1 in GC, Transwell assays were performed. As exhibited in Figure 2E, cell invasive capability of NCI-N87 and SGC7901 cells was significantly suppressed after UCA1 knockdown. In addition to the invasive capability, the migration capability of NCI-N87 and SGC7901 cells was also downregulated in response to UCA1 knockdown (Fig. 2F). Taken together, UCA1 could promote GC cell proliferation, and invasion and migration.

The effect of TGFβ1 on the expressions of UCA1, E-cadherin, vimentin, snail, and ZO-1

Given the important role of EMT in cancer metastasis, we investigated whether TGFβ1 could induce EMT in GC, and if UCA1 was involved in this progress. UCA1 expression in SGC7901 cells was monitored using real-time PCR under TGFβ1 treatment on the time point of 0, 12, 24, 48, and 72 h after treatment. As exhibited in Figure 3A, UCA1 expression increased along with time under TGFβ1 treatment. The expressions of EMT-related proteins, E-cadherin, vimentin, snail, and ZO-1, were then determined using real-time PCR under TGFβ1 treatment. E-cadherin and ZO-1 expressions were significantly downregulated by TGFβ1 treatment, whereas vimentin and snail expressions were upregulated, suggesting that TGFβ1 treatment could induce EMT in GC cells (Fig. 3B). To confirm this, western blot assays were performed to determine the protein expression of E-cadherin, vimentin, snail, and ZO-1. As exhibited in Figure 3C, D, the relative expression of E-cadherin and ZO-1 protein was significantly downregulated by TGFβ1 treatment, whereas that of vimentin and snail was upregulated. Taken together, TGFβ1 treatment could promote UCA1 expression and EMT in GC.

TGFβ1 regulates GC cell proliferation, and invasive and migratory capabilities through UCA1

Since we demonstrated the effect of UCA1 on GC cell proliferation, invasion, and migration, and that TGFβ1 treatment promoted UCA1 expression, we then investigated the detailed function of TGFβ1 treatment on GC cells. UCA1 expression in si-NC/si-UCA1-transfected SGC7901 cells with or without TGFβ1 treatment was determined using real-time PCR first. Results showed that UCA1 expression could be inhibited by si-UCA1 while promoted by TGFβ1; the inhibitory effect of si-UCA1 on UCA1 expression could be partly restored by TGFβ1; the inhibitory effect of si-UCA1 on UCA1 expression could be partly restored by TGFβ1; the inhibitory effect of si-UCA1 on UCA1 expression could be partly restored by TGFβ1. As exhibited by the BrdU and CCK-8 assays, the proliferation and migration of SGC7901 cells were monitored. As exhibited in Figure 4A, the cell proliferation and migration capability of SGC7901 cells was significantly suppressed after UCA1 knockdown. In addition to the invasive capability, the migration capability of SGC7901 cells was also downregulated in response to UCA1 knockdown (Fig. 4F). Taken together, UCA1 could promote GC cell proliferation, and invasion and migration.

### Table 1. Relative Expression Levels of UCA1 According to Clinicopathological Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total</th>
<th>High expression</th>
<th>Low expression</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45</td>
<td>23</td>
<td>10</td>
<td>13</td>
<td>0.420</td>
</tr>
<tr>
<td>≥45</td>
<td>14</td>
<td>8</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Female</td>
<td>18</td>
<td>8</td>
<td>10</td>
<td>0.618</td>
</tr>
<tr>
<td>Male</td>
<td>19</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>17</td>
<td>10</td>
<td>7</td>
<td>0.254</td>
</tr>
<tr>
<td>Well and moderate</td>
<td>20</td>
<td>8</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 cm</td>
<td>17</td>
<td>6</td>
<td>11</td>
<td>0.134</td>
</tr>
<tr>
<td>≥5 cm</td>
<td>20</td>
<td>12</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Invasion depth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2–T4</td>
<td>18</td>
<td>13</td>
<td>5</td>
<td>0.005**</td>
</tr>
<tr>
<td>Tis and T1</td>
<td>19</td>
<td>5</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>18</td>
<td>5</td>
<td>13</td>
<td>0.013*</td>
</tr>
<tr>
<td>Positive</td>
<td>19</td>
<td>13</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>TNM stage</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I+II</td>
<td>18</td>
<td>4</td>
<td>14</td>
<td>0.002**</td>
</tr>
<tr>
<td>III+IV</td>
<td>19</td>
<td>14</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

UCA1, urothelial carcinoma associated 1.

### Table 2. Univariate and Multivariate Analysis for Factors Related to Overall Survival Using the COX Proportional Hazard Model

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Univariate analysis</th>
<th></th>
<th>Multivariate analysis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p</td>
<td>HR</td>
<td>95% CI</td>
<td>p</td>
</tr>
<tr>
<td>Age &lt;45 vs. ≥45</td>
<td>0.169</td>
<td>0.557</td>
<td>0.242–1.283</td>
<td>NA</td>
</tr>
<tr>
<td>Gender Female vs. male</td>
<td>0.652</td>
<td>0.827</td>
<td>0.362–1.890</td>
<td>NA</td>
</tr>
<tr>
<td>Differentiation Poor vs. well and moderate</td>
<td>0.636</td>
<td>1.220</td>
<td>0.535–2.782</td>
<td>NA</td>
</tr>
<tr>
<td>Tumor size &lt;5 cm vs. ≥5 cm</td>
<td>0.018</td>
<td>0.351</td>
<td>0.147–0.835</td>
<td>0.087</td>
</tr>
<tr>
<td>Invasion depth T2–T4 vs. Tis and T1</td>
<td>0.014</td>
<td>2.910</td>
<td>1.245–6.798</td>
<td>0.248</td>
</tr>
<tr>
<td>Lymph node metastasis Negative vs. positive</td>
<td>0.913</td>
<td>0.955</td>
<td>0.419–2.178</td>
<td>NA</td>
</tr>
<tr>
<td>TNM stage I+II vs. III+IV</td>
<td>0.011</td>
<td>0.314</td>
<td>0.129–0.767</td>
<td>0.414</td>
</tr>
<tr>
<td>UCA1 expression High vs. low</td>
<td>0.003</td>
<td>3.909</td>
<td>1.592–9.599</td>
<td>0.037</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval; NA, not adopted.
of SGC7901 cells was suppressed by UCA1 knockdown, promoted by TGFβ1 treatment; the inhibitory effect of si-UCA1 on GC cell proliferation could be partly restored by TGFβ1 treatment (Fig. 4B, C). Similarly, as exhibited by Transwell assays, the invasive ability of GC cells was suppressed by UCA1 knockdown, promoted by TGFβ1 treatment; the inhibitory effect of si-UCA1 on GC cell invasive ability could be partly restored by TGFβ1 treatment (Fig. 4D). In addition, the migratory capability of GC cells was also inhibited by UCA1 knockdown, promoted by TGFβ1 treatment, and the inhibitory effect of si-UCA1 on GC cell migration could be partly restored by TGFβ1 treatment (Fig. 4E). The above data suggested that TGFβ1 could regulate GC cell proliferation, invasion, and migration through UCA1.

The effect of TGFβ1/UCA1 on EMT in GC

To validate the effect of TGFβ1/UCA1 on EMT, mRNA expression of E-cadherin, vimentin, snail, and ZO-1 in
FIG. 3. The effect of TGFβ1 on the expressions of UCA1, E-cadherin, vimentin, snail, and ZO-1. (A) UCA1 expression was monitored in response to TGFβ1 at five time points: 0, 12, 24, 48, and 72 h after TGFβ1 treatment. (B) The mRNA expression of EMT-related protein, E-cadherin, ZO-1, vimentin and snail was monitored in response to TGFβ1 treatment using real-time PCR. (C, D) The protein expression of E-cadherin, ZO-1, vimentin and snail was monitored in response to TGFβ1 treatment using western blot. Data are presented as mean ± SD of three independent experiments. *p < 0.05, **p < 0.01. EMT, epithelial mesenchymal transition; TGFβ1, transforming growth factor β1; ZO-1, zonula occludens-1. Color images available online at www.liebertpub.com/dna

FIG. 4. TGFβ1 regulates GC cell proliferation, invasion, and migration through UCA1. (A) UCA1 expression was monitored in si-NC/si-UCA1-transfected SGC7901 cells, with or without TGFβ1 treatment using real-time PCR. (B) SGC7901 cells were divided into four groups: vehicle + si-NC, vehicle + si-UCA1, TGFβ1 + si-NC, and TGFβ1 + si-UCA1; the cell proliferation was monitored in each group using BrdU cell proliferation assays. (C) SGC7901 cells were divided into four groups: vehicle + si-NC, vehicle + si-UCA1, TGFβ1 + si-NC, and TGFβ1 + si-UCA1; the cell proliferation was monitored in each group using CCK-8 assays. (D) The cell invasive abilities of the four groups were monitored in each group using Transwell assays. (E) The cell migration abilities of the four groups were monitored in each group using Transwell assays. Data are presented as mean ± SD of three independent experiments. *p < 0.05, **p < 0.01. Color images available online at www.liebertpub.com/dna
si-NC/si-UCA1-transfected SGC7901 cells, with or without TGFβ1 treatment, was determined using real-time PCR. As exhibited in Figure 5A, TGFβ1 promoted vimentin and snail mRNA expressions, while inhibited E-cadherin and ZO-1 mRNA expressions; si-UCA1 inhibited vimentin and snail mRNA expressions, whereas promoted E-cadherin and ZO-1 mRNA expressions; the effect of TGFβ1 on EMT-related protein expression could be partly restored by si-UCA1 transfection. Similar results were observed from western blot assays: TGFβ1 promoted vimentin and snail protein expressions, whereas inhibited E-cadherin and ZO-1 protein expressions; si-UCA1 inhibited vimentin and snail protein expressions, whereas promoted E-cadherin and ZO-1 protein expressions; the effect of TGFβ1 on EMT-related protein expression could be partly restored by si-UCA1 transfection (Fig. 5B). Taken together, TGFβ1 promoted EMT in GC, whereas UCA1 knockdown by si-UCA1 transfection could partly restore the effect of TGFβ1 on EMT in GC.

Discussion

The noncoding portion of the genome accounts for greater than 90% of the total mammalian genome. Studies have demonstrated that among these ncRNAs, ~18% of lncRNAs are associated with human tumors, compared with only 9% of human protein-coding genes (Khachane and Harrison, 2010), suggesting that lncRNAs could act as major contributors to carcinogenesis and cancer progression. The roles of dysregulated lncRNAs in the proliferation, invasion, and migration of many kinds of cancers have garnered increased scientific interest in recent years (Liu et al., 2016a; Yue et al., 2016; Zou et al., 2016).

LncRNA UCA1 has been reported to be upregulated in many cancers (Nie et al., 2016; Zhang et al., 2016a). Recent study reported that high expression level of UCA1 is associated with poor clinical outcome. UCA1 serves as a novel biomarker for prognosis and might be a potential predictive factor for clinicopathological characteristics in various cancers (Wang et al., 2015; Liu et al., 2016b). According to a study by Lu et al., UCA1 is regarded as a poor prognosis factor in endometrial cancer, and UCA1 silence inhibited the cancer cell migration (Lu et al., 2016); UCA1 serves as a diagnostic biomarker and upregulation of UCA1 expression contributes to poor prognosis in patients with GC; silence of UCA1 inhibits malignant proliferation and chemotherapy resistance to adriamycin in GC (Gao et al., 2015; Shang et al., 2016). In this study, we initially monitored UCA1 mRNA expression in a large panel of 37 paired GC tissues and adjacent normal tissues. A significant higher expression level of UCA1 in GC tissues was observed compared with the adjacent normal tissues. Consistently, the expression level of UCA1 was obviously higher in GC cell lines compared with that of the normal cell lines. According to the clinical information in 37 cases of patients with GC, we found that UCA1 expression was correlated with poor clinicopathological characteristics and was one of independent risk factors of OS (HR = 2.917; 95% CI: 1.069–7.962). Moreover, UCA1 might accelerate the GC progress through promoting the proliferation, invasion, and migration of GC cells, which is consistent with previous study (Zheng et al., 2015). However, the mechanism by which UCA1 affects the progression of GC still remains to be validated.

Emerging evidence revealed the close association of EMT progress and cancer metastasis (Gu et al., 2015; Chen et al., 2016). Among a sum of factors, TGFβ1 obviously promotes
EMT of cancer cells and their invasion abilities (Kasai et al., 2005; Borthwick et al., 2012). Pang et al. revealed that TGFβ1-induced EMT promotes targeted migration of breast cancer cells (Pang et al., 2016). In the present study, by examining the expression of epithelial and mesenchymal markers, E-cadherin, ZO-1, vimentin and snail in GC cells, we demonstrated that TGFβ1 treatment promotes EMT in GC; in addition, the expression of UCA1 could be promoted by TGFβ1 treatment.

Given this, we further investigated the effect of combined conduction of TGFβ1 treatment and UCA1 on GC cells. By determining the cell proliferation, and invasive and migratory capabilities, we proved that TGFβ1 treatment promotes GC cell proliferation, and invasive and migratory capabilities, whereas si-UCA1-induced UCA1 silence exerts an opposite effect on GC cells; the effect of TGFβ1 on GC cells could be partly restored by UCA1 silence. As we demonstrated the function of TGFβ1 on EMT in GC, we then monitored the mRNA and protein expression of EMT-related proteins under the combined conduction of TGFβ1 treatment and UCA1. As exhibited by the mRNA and protein expressions of E-cadherin, ZO-1, vimentin and snail, UCA1 silence inhibited EMT progress, and could partly reverse the promotive effect of TGFβ1 on EMT in GC, suggesting that TGFβ1 regulates EMT progress in GC through UCA1 to modulate GC cell proliferation, and invasive and migratory capabilities.

In summary, it was identified that through regulating UCA1 and consequently the EMT progress, GC cell proliferation, and invasive and migratory capabilities are promoted by TGFβ1; these effects of TGFβ1 on GC cells and EMT progress could be partly restored by UCA1 silence. TGFβ1-induced UCA1 upregulation and acceleration of EMT may be a key role in regulating GC cell proliferation, invasion, and migration, and may provide a potential therapeutic target for GC treatment.

Conclusion

In the present study, we revealed that TGFβ1-induced UCA1 upregulation and acceleration of EMT may be a key role in regulating GC cell proliferation, invasion, and migration, and may provide a potential therapeutic target for GC treatment.

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