miR-301-3p directly regulates *Cx43* to mediate the development of gastric cancer

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Abstract

Objective: Identifying novel biomarkers involved in the development of gastric cancer (GC) can provide potential therapeutic strategies and improve clinical prognosis. miR-301-3p and *Cx43* are reportedly dysregulated in GC. miR-301-3p and *Cx43* interaction, and their functions in GC progression, are still poorly understood.

Methods: The expression levels of miR-301-3p and *Cx43* in GC tissues and cell lines with various differentiation degrees were evaluated by RT-qPCR. The interaction between miR-301-3p and *Cx43* was assessed by dual-luciferase reporter assays. CCK8 and Transwell assays were employed to assess the effects of the miR-301-3p-*Cx43* axis on GC cell proliferation, migration, and invasion.

Results: *Cx43* was significantly downregulated in GC tissues and cell lines, while miR-301-3p expression was negatively correlated with *Cx43* mRNA levels. The expression levels of *Cx43* and miR-301-3p were closely associated with the differentiation, TNM stage, vascular invasion, and lymph node metastasis status of GC patients. *Cx43* overexpression could suppress the proliferation, migration, and invasion of GC cells. *Cx43* mRNA is a direct target of miR-301-3p, and transfection of an miR-301-3p mimic could reverse the inhibitory effects of *Cx43*.

Conclusion: The miR-301-3p-*Cx43* axis is involved in the development and progression of GC by affecting the proliferation, migration, and invasion of GC cells.

Keywords

Gastric cancer, *Cx43*, miR-301-3p, differentiation, development, miRNA, patient

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Introduction

Cancer has become a major public health problem and its increasing incidence and mortality have made it a leading cause of death worldwide.[1] Gastric cancer is a common digestive tract malignancy. Although the incidence of gastric cancer has decreased in the past decades, it remains a major cause of cancer-related death.[2] The degree of cancer differentiation is an important factor in the development of gastric cancer.[3] Identifying effective biomarkers associated with the differentiation of gastric cancer can help physicians adjust the therapy used to treat this disease according to the development of an individual patient's condition.

Connexins are part of a group of proteins that can form gap junction channels, which can benefit the intercellular communication between cells and facilitate the transfer of signaling molecules.[4] Connexin 43 (*Cx43*) is a common connexin protein isoform that is widely expressed in epithelial tissues.[5] The clinical significance of *Cx43* has been reported in various human diseases, such as hepatocellular cancer, breast cancer, and ovarian cancer.[6–8] *Cx43* expression was found to be downregulated in gastric cancer and implicated in the occurrence, development, and metastasis of this disease. It can also enhance the chemosensitivity of gastric tumors.[9–11] The expression and function of *Cx43* in various differentiation levels of gastric cancer could possibly provide a clinical reference for risk prediction of this malignancy.

MicroRNAs (miRNAs) are part of a group of small non-coding RNAs that regulate gene expression. They are involved in tumorigenesis and control a variety of cellular pathways that affect proliferation, differentiation, migration, and invasion. miR-301-3p reportedly binds to the 3′ untranslated region (UTR) of *Cx43* mRNA and participate in the pathogenesis of bacterial meningitis.[12] miR-301-3p also promotes the proliferation and cell cycle progression of hepatocellular carcinoma cells, while inhibiting apoptosis.[13] Additionally, miR-301-3p regulates various pathways in gastric cancer cells and promotes the development of this disease.[14] However, the interaction between miR-301-3p and *Cx43* and its effects on the development of gastric cancer are not fully clear. Hence, this study focused on the expression of *Cx43* in gastric cancer tissues of different differentiation levels and its interaction with miR-301-3p to identify novel biomarkers that affect the differentiation and progression of gastric cancer.

Materials and methods

Patients and tissue samples

Sixty gastric cancer patients with various differentiation degrees that underwent surgery at the Affiliated Hospital of Chengde Medical University from 2017 to 2019 were included in this study. The following criteria were required for inclusion: a) patients diagnosed with gastric adenocarcinoma of no special type (NST) and received surgical resection; b) tumors were M0 without any other malignancy; c) patients were not diagnosed with any other malignant tumor; d) patients had never received any type of chemotherapy or radiotherapy before surgery; e) patients had complete clinical data. Normal gastric tissues (5 cm from the tumor site) and gastric cancer tissues were collected by surgical resection and frozen in liquid nitrogen for further analyses. The collected tissues were diagnosed by at least two pathologists who reached an agreement. All patients never received any antitumor therapies or had a history of gastric cancer. This study obtained approval from the Ethics Committee of Affiliated Hospital of Chengde Medical University (Approval No. 2016135). Written informed consent

was obtained from each participant. The reporting of this study confirms to the STROBE statement.[15]

Cell lines and transfections

Various differentiated gastric cancer cell lines, including AGS (well-differentiated) and MKN-45 (poorly differentiated) cells, and a normal gastric epithelial cell line GES-1, were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Sigma Aldrich, St. Louis, MO, USA) and penicillin (100 IU/mL) at 37°C in a 5% CO_2 environment.

MKN-45 cells were transfected with 100 nM miR-301-3p mimic (Guangzhou RiboBio Co., Guangzhou, China) to over-express miR-301-3p. *Cx43* was overexpressed by transfecting cells with pcDNA 3.1-*Cx43*. pcDNA 3.1 (empty vector) was used as the negative control. Transfections were performed with Lipofectamine 2000 reagent (Invitrogen, Waltham, MA, USA) following the suggested protocols.

Immunohistochemistry (IHC)

Collected tissues were fixed with 4% paraformaldehyde solution, dehydrated, and embedded in paraffin. Cx43 protein expression was detected by IHC after the embedded tissues were cut into 4-μm sections. The affinity-purified rabbit polyclonal antiserum raised against the carboxy terminal 362-382 peptide segment of Cx43 was used to immunolabel Cx43 (Abcam, Cambridge, UK). The tissues were first incubated with the Cx43 primary antibody at 4°C overnight, then washed in PBS three times. Then, the secondary antibody was added and incubated at 37°C for 1 hour, followed by three more PBS washes. DAB, which

yields brown reaction products, was then used as the chromogen, followed by hematoxylin counter staining. The stained tissues were observed under a microscope and analyzed with ImageJ software (https://imagej.nih.gov/ij/). The immunohistochemical scores were based on the percentage of positive cells in the observed field.

Quantitative reverse transcription PCR (RT-qPCR)

The expression levels of miR-301-3p and *Cx43* mRNA were evaluated by RT-qPCR using the SYBR Green I Master Mix Kit (Invitrogen). Briefly, total RNA was isolated using Trizol Reagent (Invitrogen), then cDNA was generated using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA, USA) for *Cx43* and the TaqMan Reverse Transcription Kit (Applied Biosystems) for miR-301-3p. The relative expression levels of miR-310-3p and *Cx43* were normalized to U6 and *β*-actin mRNA, respectively, and calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences used were: miR-301-3p: forward, 5′-TCCGACGAAACTGTTATAGTA-3′ and reverse, 5′-GTGCAGGGTCCGAGGT-3′; *Cx43*: forward, 5′-TGGGACAGGAAGAGTTTGCAC-3′ and reverse, 5′-CACCTGGTGCACTTTCTACAGCAC-3′; U6: forward, 5′-CGCAAGGATGACACGCAAATTCC-3′ and reverse, 5′-GTGCAGGGTCCGAGGT-3′; *β*-actin: forward, 5′-AGCGGGAAATCGTGCGTG-3′ and reverse, 5′-CAGGGTACATGGTGGGTGCC-3′.

Cell proliferation assay

MKN-45 and AGS cells were seeded at a density of 1×10^5 cells/well in 96-well plates and cultured in DMEM with 10% FBS. The plates were incubated at 37°C with 5% CO_2 for 0, 24, 48, or 72 hours, then a CCK8 Kit (Dojindo, Tokyo, Japan) was

used to evaluate cell proliferation rates according to the manufacturer's instructions. The absorbance values at 450 nm were detected using a microplate reader (Bio-Rad, Hercules, CA, USA).

Cell migration and invasion assays

MKN-45 and AGS cells were seeded at a density of 1×10^5 cells/well in the upper chambers of a 24-well Transwell chamber with a pore size of 8 μm (Thermo Fisher Scientific, Waltham, MA, USA) and cultured in serum-free DMEM. DMEM with 10% FBS was placed in the bottom chamber as a chemoattractant. The upper chambers were pre-coated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) for the invasion assays. After incubating for 24 hours, the upper chamber and membrane were disposed. Migrated and invaded cells in the bottom chambers were stained with 0.1% crystal violet and quantified using a microscope (200× magnification).

Dual-luciferase reporter assay

The binding sites between miR-301-3p and *Cx43* mRNA were predicted using the TargetScanHuman v7.2 database (http://www.targetscan.org/vert_72/). The wild-type *Cx43* 3′ UTR containing the miR-301-3p binding sites was cloned into the pGL3 luciferase reporter vector (Promega, Madison, WI, USA). A mutant version of this vector was generated by mutating the miR-301-3p binding sites. The luciferase reporter plasmid, miR-301-3p mimic, or miR-301-3p inhibitor (100 nM, Guangzhou RiboBio) were co-transfected into gastric cancer cells for 48 hours. The relative luciferase activity was evaluated with the Dual-Luciferase Reporter Assay Kit (Promega) according to the manufacturer's instructions.

Statistical analysis

All data are presented as mean ± standard deviation (SD) obtained from at least three experiments. Statistical analyses were performed with the help of SPSS 23.0 software (IBM Corp., Armonk, NY, USA) and GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego, CA, USA). Differences among three or more groups were analyzed by a one-way analysis of variance, while the differences between two groups were evaluated by a Student's t-test. The differences were considered to be statistically significant when $P < 0.05$.

Results

Correlation of miR-301-3p and Cx43 expression in gastric cancer

miR-301-3p was significantly upregulated in well-differentiated and poorly differentiated gastric cancer tissues compared with normal tissues ($P < 0.001$), while there was no significant difference between the various differentiated tissues ($P > 0.05$, Figure 1a). miR-301-3p expression levels were also dramatically increased in well-differentiated and poorly differentiated gastric cancer cell lines compared with the normal gastric epithelial cell line GES-1 ($P < 0.001$, Figure 1b). On the contrary, *Cx43* mRNA expression was dramatically downregulated in well-differentiated and poorly differentiated tissues ($P < 0.001$, Figure 1c). Moreover, relatively lower *Cx43* expression was also found in gastric cancer cell lines compared with GES-1 cells ($P < 0.001$, Figure 1d). The expression levels of miR-301-3p were significantly negatively correlated with *Cx43* mRNA levels, with a correlation coefficient value of -0.602 ($P < 0.001$, Figure 1e).
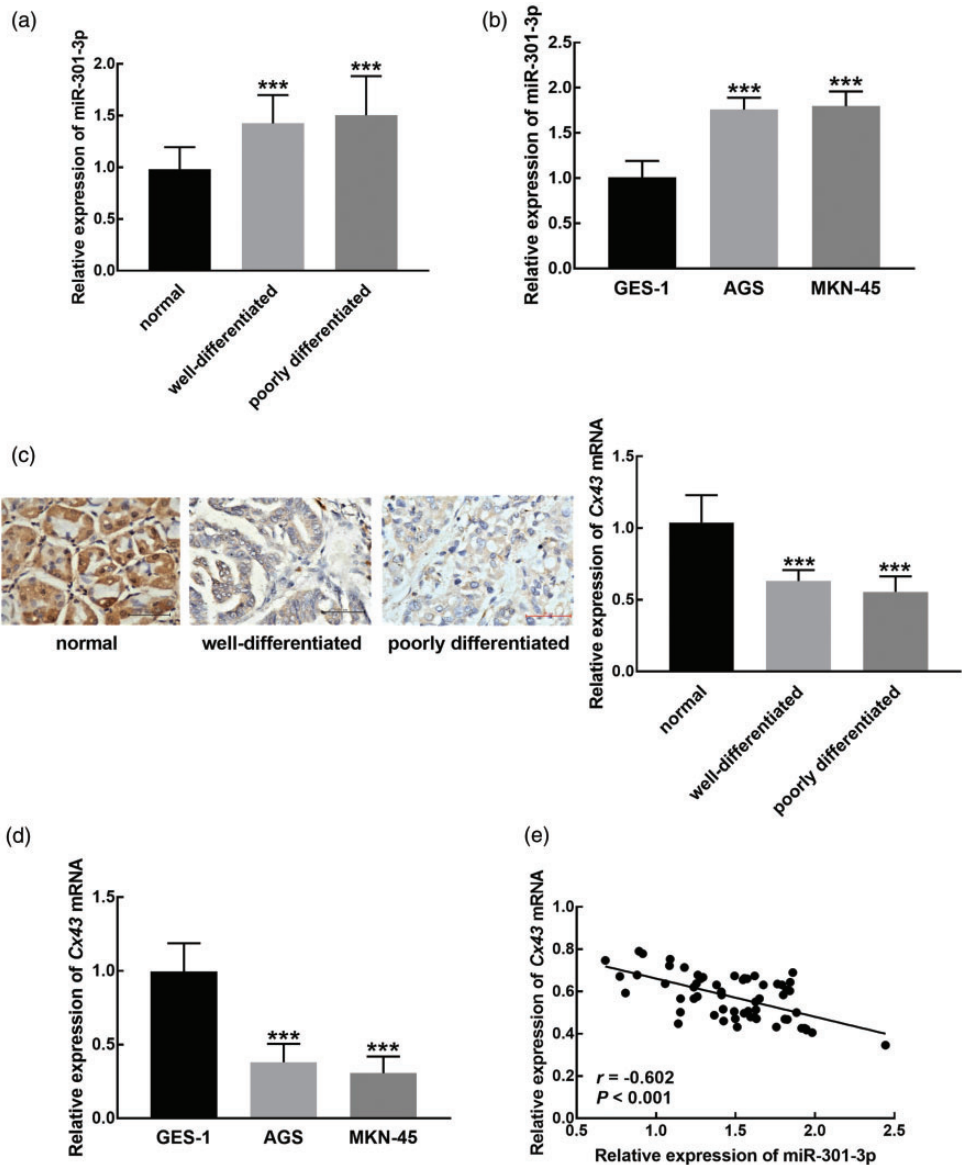
Figure 1. The expression levels and correlation of miR-301-3p and *Cx43* mRNA in gastric cancer patient samples. (a) miR-301-3p was significantly upregulated in well-differentiated and poorly differentiated gastric cancer patient tissue samples compared with normal gastric tissues. (b) MiR-301-3p was significantly upregulated in AGS (well-differentiated) and MKN-45 (poorly differentiated) cells compared with the normal gastric cell line, GES-1. (c) *Cx43* mRNA was significantly downregulated in gastric cancer patient tissue samples with various differentiation degrees compared with normal gastric tissues. (d) *Cx43* mRNA was downregulated in AGS (well-differentiated) and MKN-45 (poorly differentiated) cells compared with GES-1 cells. (e) The expression levels of miR-301-3p were negatively correlated with *Cx43* mRNA levels. $r = -0.602$, $P < 0.001$. ***$P < 0.001$ relative to normal tissues and cell lines.

Clinical significance of Cx43 and miR-301-3p in gastric cancer

According to the median *Cx43* expression value in gastric cancer tissues, the 60 patients were divided into low expression and high expression groups. Patients that had relatively low *Cx43* expression showed poor differentiation (n = 29, P = 0.001) and advanced TNM stage (n = 25, P = 0.004), and possessed vascular invasion (n = 26, P = 0.007) and lymph node metastasis (n = 23, P = 0.020). Similarly, miR-301-3p upregulation was also associated with poor tumor differentiation (P = 0.002), advanced TNM stage (P = 0.006), positive vascular invasion (P = 0.002), and positive lymph node metastasis status (P = 0.001) of gastric cancer patients (Table 1).

Cx43 is a direct target of miR-301-3p

miR-301-3p could bind to the 3′ UTR of *Cx43* mRNA, and these binding sites are shown in Figure 2a. The relative luciferase activity of the *Cx43* reporter vector was inhibited following transfection of the miR-301-3p mimic, but was enhanced when the miR-301-3p inhibitor was transfected. However, the relative luciferase activity of the mutant vector was not affected by any of these transfections. These results indicate that a direct interaction occurs between miR-301-3p and *Cx43* (P < 0.001, Figure 2b).

The biological function of Cx43 in the progression of gastric cancer

MKN-45 and AGS cells were co-transfected with pcDNA 3.1-*Cx43* and miR-301-3p mimic to co-regulate the expression of *Cx43* and miR-301-3p. As depicted in Figure 3a, the transfection of miR-301-3p mimic notably inhibited the expression of *Cx43* (P < 0.001). *Cx43* expression was significantly enhanced by the transfection of pcDNA 3.1-*Cx43*, which was reversed by the co-transfection of miR-301-3p mimic (P < 0.01, P < 0.001). The proliferation rates of MKN-45 and AGS cells were promoted by miR-301-3p mimic transfection and inhibited by *Cx43* overexpression (P < 0.01, Figure 3b). miR-301-3p mimic transfection could also reduce the inhibitory effect of *Cx43* upregulation on the proliferation of MKN-45 and AGS cells (P < 0.05, Figure 3b).

Additionally, the migration and invasion rates of MKN-45 and AGS cells were inhibited by *Cx43* overexpression, but promoted by miR-301-3p upregulation (P < 0.001, P < 0.01, Figure 3d and e). However, the co-transfection of miR-301-3p mimic and pcDNA 3.1-*Cx43* could reverse the inhibited migration and invasion of gastric cancer cells (P < 0.01, Figure 3d and e).

Discussion

The differentiation degree of cancer tissues is associated with disease development and clinical outcome of patients, together with other factors.[16,17] Exploring molecular biomarkers associated with the development of gastric cancer could improve the diagnostic efficiency and risk prediction accuracy. Previous studies have identified a variety of biomarkers that affect the proliferation, migration, and invasion of gastric cancer cells and are involved in the diagnosis and prognosis of this disease.[18,19] For example, prospero homeobox protein-1 (PROX1) could influence the proliferation, migration, and invasion of MKN-45 cells and was positively correlated with the cancer stage of patients, indicating that PROX1 is a possible indicator and molecular therapeutic target of gastric cancer.[20]

Cx43 is a vital component of gap junction intercellular communication, which has previously been associated with the recurrence and metastasis of cancers, as well as

Table 1. Associations between the expression levels of Cx43 or miR-301-3p and the clinical characteristics of gastric cancer patients.

	Total (n = 60)	Cx43 expression			miR-301-3p expression		
		Low (n = 31)	High (n = 29)	P-value	Low (n = 27)	High (n = 33)	P-value
Age (n, %)				0.201			0.212
<60 years	28 (47%)	12 (43%)	16 (57%)		15 (54%)	13 (46%)	
≥60 years	32 (53%)	19 (59%)	13 (41%)		12 (38%)	20 (62%)	
Sex (n, %)				0.620			0.492
Male	53 (88%)	28 (53%)	25 (47%)		23 (43%)	30 (57%)	
Female	7 (12%)	3 (43%)	4 (57%)		4 (57%)	3 (43%)	
Differentiation (n, %)				0.001			0.002
Well + Moderate	15 (25%)	2 (13%)	13 (87%)		12 (80%)	3 (20%)	
Poor	45 (75%)	29 (64%)	16 (36%)		15 (33%)	30 (67%)	
TNM stage (n, %)				0.004			0.006
I–II	22 (37%)	6 (27%)	16 (73%)		15 (68%)	7 (32%)	
III	38 (63%)	25 (66%)	13 (34%)		12 (32%)	26 (68%)	
Vascular infiltration (n, %)				0.007			0.002
Negative	19 (32%)	5 (26%)	14 (74%)		14 (74%)	5 (26%)	
Positive	41 (68%)	26 (63%)	15 (37%)		13 (32%)	28 (68%)	
Lymph node metastasis status (n, %)				0.020			0.001
Negative	24 (40%)	8 (33%)	16 (67%)		17 (71%)	7 (29%)	
Positive	36 (60%)	23 (64%)	13 (36%)		10 (28%)	26 (72%)	
Helicobacter pylori status (n, %)				0.261			0.157
Negative	23 (38%)	14 (61%)	9 (39%)		13 (57%)	10 (43%)	
Positive	37 (62%)	17 (46%)	20 (54%)		14 (38%)	23 (62%)	
Smoking (n, %)				0.586			0.549
Yes	33 (55%)	16 (48%)	17 (52%)		16 (48%)	17 (52%)	
No	27 (45%)	15 (56%)	12 (44%)		11 (41%)	16 (59%)	
Drinking (n, %)				0.599			0.311
Yes	31 (52%)	15 (48%)	16 (52%)		12 (39%)	19 (61%)	
No	29 (48%)	16 (55%)	13 (45%)		15 (52%)	14 (48%)	
Tumor sites (n, %)				0.334			0.405
Antrum	56 (93%)	28 (50%)	28 (50%)		26 (46%)	30 (54%)	
Other	4 (7%)	3 (75%)	1 (25%)		1 (25%)	3 (75%)	

with poor survival of patients. *Cx43* inhibited the invasion and migration of cisplatin-resistant cells in testicular cancer.[21] *Cx43* was downregulated in gastric cancer and was associated with the positive peritoneal metastasis of gastric tumors.[11] Several miRNAs were reported to regulate *Cx43* expression in various diseases. For example, miR-382-3p could suppress the inflammatory response of chondrocytes induced by IL-1β via mediating *Cx43* levels.[22] The miR-218-5p-*Cx43* axis contributes to gemcitabine chemoresistance of breast tumors and can regulate the growth, migration, and invasion of breast cancer cells.[23] However, the clinical significance and
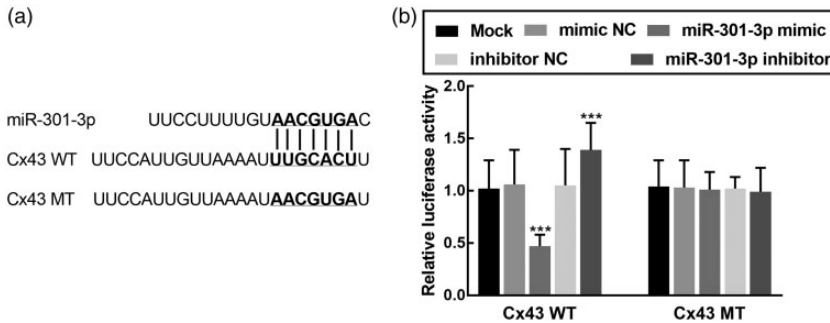
Figure 2. *Cx43* mRNA is a direct target of miR-301-3p. (a) The binding sites between miR-301-3p and *Cx43* wild-type (*Cx43* WT) or *Cx43* mutant-type (*Cx43* MT). (b) Overexpression of miR-301-3p through mimic transfection significantly suppressed the luciferase activity of *Cx43* WT, while knockdown of miR-301-3p with a specific inhibitor enhanced the luciferase activity of *Cx43*. The luciferase activity of *Cx43* MT was not affected by miR-301-3p expression modulation. ***$P < 0.001$ relative to the mock group.

biological function of *Cx43*, as well as its interaction with miR-301-3p, were still unclear for gastric cancer.

Consistent downregulation of *Cx43* mRNA was observed in both gastric cancer tissues and cell lines with various differentiation degrees. Downregulated *Cx43* was closely associated with the differentiation, TNM stage, vascular invasion, and lymph node metastasis status of gastric cancer patients, suggesting that these important clinical features could possibly indicate the disease development of these patients. Furthermore, these findings indicate that *Cx43* is involved in the progression of this cancer.[24] miR-301-3p expression levels were also related to the critical characteristics of patients, as well as negatively correlated with *Cx43* mRNA levels. This implies a potential interaction between miR-301-3p and *Cx43* mRNA in the development of gastric cancer. miR-301-3p was reported to bind to the 3′ UTR of *Cx43* mRNA and be involved in bacterial meningitis pathogenesis.[12] Interestingly, although both *Cx43* and miR-301-3p expression were significantly associated with the differentiation of patients and showed a decreased trend in poorly-differentiated gastric cancer tissues,

the differences were not statistically significant. The limited sample size may have contributed to these results. Therefore, a relatively large sample size is needed in further studies. Additionally, bias was potentially a factor during patient enrollment and may have limited the obtained clinical results.

For the biological function of *Cx43*, we found that overexpression of *Cx43* could suppress the proliferation, migration, and invasion of gastric cancer cells, indicating that *Cx43* has a tumor suppressive role in these cells. miR-301-3p reportedly serves as a tumor promoter in gastric cancer by accelerating cell proliferation and suppressing apoptosis, which is consistent with the results of our present research.[14] Moreover, transfection of a miR-301-3p mimic significantly inhibited the expression of *Cx43* and reversed the inhibitory effect of *Cx43* overexpression on proliferation, migration, and invasion of gastric cancer cells. Overall, these results suggest that the miR-301-3p-*Cx43* axis could indicate the development of gastric cancer and mediate the proliferation, migration, and invasion of gastric cancer cells.

A further mechanism underlying the function of the miR-301-3p-*Cx43* axis was
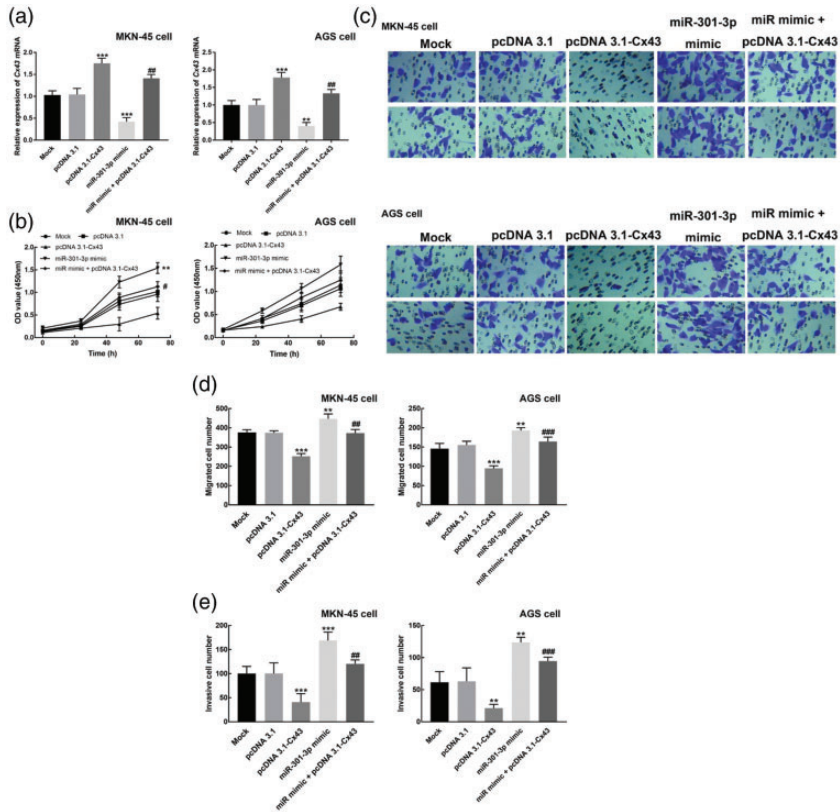
Figure 3. *Cx43* affects various cell processes of gastric cancer cells. (a) *Cx43* expression was inhibited by miR-301-3p mimic transfection, but promoted by pcDNA3.1-*Cx43* transfection. The co-transfection of miR-301-3p mimic and pcDNA 3.1-*Cx43* could reverse the upregulation of *Cx43* by pcDNA3.1-*Cx43*. (b) The overexpression of *Cx43* significantly suppressed the proliferation of MKN-45 cells, while miR-301-3p mimic transfection exerted the opposite effect. The co-transfection of miR-301-3p mimic and pcDNA 3.1-*Cx43* could reverse the inhibitory effect of *Cx43* overexpression on the proliferation of gastric cancer cells. (c) Representative images of the Transwell assays of the migration (top) and invasion (bottom) of MKN-45 and AGS cells. (d) The overexpression of *Cx43* significantly suppressed the migration of MKN-45 cells, while miR-301-3p mimic transfection promoted the migration of MKN-45 cells dramatically. The co-transfection of miR-301-3p mimic and pcDNA 3.1-*Cx43* could reverse the inhibitory effect of *Cx43* overexpression on the migration of gastric cancer cells. (e) The overexpression of *Cx43* significantly suppressed the invasion of MKN-45 cell, while miR-301-3p mimic transfection promoted the invasion of MKN-45 cells dramatically. The co-transfection of miR-301-3p mimic and pcDNA 3.1-*Cx43* could reverse the inhibitory effect of *Cx43* overexpression on the invasion of gastric cancer cells. $**P < 0.01$, $***P < 0.001$ relative to the mock group. $^{#}P < 0.05$, $^{##}P < 0.01$ relative to the pcDNA3.1-*Cx43* group.

not uncovered in this study. The mTOR/ p70s6k signaling pathway was considered to be critical for the growth and apoptosis of various cancer cells, such as esophageal squamous cell carcinoma, ovarian cancer, and prostate cancer.[25–27] Whether the mTOR/p70s6k signaling pathway is involved in the functional processes of the miR-301-3p-*Cx43* axis requires validation in further studies. Additionally, *Cx43* is an important member of the connexin protein family, which could improve the

intercellular communication between cells and facilitate the transfer of signaling molecules.[4] The effects of the miR-301-3-*Cx43* interaction on gap junctions were not evaluated, so future studies are needed.

In conclusion, we show that the miR-301-3p-*Cx43* axis is significantly associated with the disease development of gastric cancer patients. miR-301-3p can regulate *Cx43* expression to mediate proliferation, migration, and invasion of gastric cancer cells.

Declaration of conflicting interest

Funding

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References

1. Siegel RL, Miller KD and Jemal A. Cancer statistics, 2016. *CA Cancer J Clin* 2016; 66: 7–30.
2. Mihmanli M, Ilhan E, Idiz UO, et al. Recent developments and innovations in gastric cancer. *World J Gastroenterol* 2016; 22: 4307–4320.
3. Strand MS, Lockhart AC and Fields RC. Genetics of Gastric Cancer. *Surg Clin North Am* 2017; 97: 345–370.
4. Grek CL, Rhett JM, Bruce JS, et al. Connexin 43, breast cancer tumor suppressor: Missed connections? *Cancer Lett* 2016; 374: 117–126.
5. Langlois S, Cowan KN, Shao Q, et al. The tumor-suppressive function of Connexin43 in keratinocytes is mediated in part via interaction with caveolin-1. *Cancer Res* 2010; 70: 4222–4232.
6. Liu W, Hua S, Dai Y, et al. Roles of Cx43 and AKAP95 in ovarian cancer tissues in G1/S phase. *Int J Clin Exp Pathol* 2015; 8: 14315–14324.
7. Ming J, Zhou Y, Du J, et al. miR-381 suppresses C/EBPα-dependent Cx43 expression in breast cancer cells. *Bioscience Reports* 2015; 35: e00266.
8. Zhao L, Jiang L, He L, et al. Identification of a novel cell cycle-related gene signature predicting survival in patients with gastric cancer. *J Cell Physiol* 2019; 234: 6350–6360.
9. Li CH, Hao ML, Sun Y, et al. Ultrastructure of gap junction and Cx43 expression in gastric cancer tissues of the patients. *Arch Med Sci* 2020; 16: 352–358.
10. Liu D, Zhou H, Wu J, et al. Infection by Cx43 adenovirus increased chemotherapy sensitivity in human gastric cancer BGC-823 cells: not involving in induction of cell apoptosis. *Gene* 2015; 574: 217–224.
11. Tang B, Peng ZH, Yu PW, et al. Aberrant expression of Cx43 is associated with the peritoneal metastasis of gastric cancer and Cx43-mediated gap junction enhances gastric cancer cell diapedesis from peritoneal mesothelium. *PLoS One* 2013; 8: e74527.
12. Dong Y, Wang J, Du K, et al. MiR-301a-3p in the pathogenesis of bacterial meningitis by targeting Cx43. *Neuroreport* 2019; 30: 174–181.
13. Guo Y, Yao B, Zhu Q, et al. MicroRNA-301b-3p contributes to tumour growth of human hepatocellular carcinoma by repressing vestigial like family member 4. *J Cell Mol Med* 2019; 23: 5037–5047.
14. Fan H, Jin X, Liao C, et al. MicroRNA-301b-3p accelerates the growth of gastric cancer cells by targeting zinc finger and BTB domain containing 4. *Pathol Res Pract* 2019; 215: 152667.
15. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *PLoS Med* 2007; 4: e296.
16. Jögi A, Vaapil M, Johansson M, et al. Cancer cell differentiation heterogeneity and aggressive behavior in solid tumors. *Ups J Med Sci* 2012; 117: 217–224.
17. Scott RE. Differentiation, differentiation/gene therapy and cancer. *Pharmacol Ther* 1997; 73: 51–65.

18. Li G, Hu J and Hu G. Biomarker Studies in Early Detection and Prognosis of Breast Cancer. *Adv Exp Med Biol* 2017; 1026: 27–39.

19. Zou J and Wang E. Cancer Biomarker Discovery for Precision Medicine: New Progress. *Curr Med Chem* 2019; 26: 7655–7671.

20. Ueta K, Otowa Y, Kakeji Y, et al. PROX1 Is Associated with Cancer Progression and Prognosis in Gastric Cancer. *Anticancer Res* 2018; 38: 6139–6145.

21. Wu D, Li B, Liu H, et al. *In vitro* inhibited effect of gap junction composed of Cx43 in the invasion and metastasis of testicular cancer resistance to cisplatin. *Biomed Pharmacother* 2018; 98: 826–833.

22. Lei J, Fu Y, Zhuang Y, et al. miR-382-3p suppressed IL-1beta induced inflammatory response of chondrocytes via the TLR4/MyD88/NF-kappaB signaling pathway by directly targeting CX43. *J Cell Physiol* 2019; 234: 23160–23168.

23. Xia C, Jiang H, Ye F, et al. The multifunction of miR-218-5p-Cx43 axis in breast cancer. *Onco Targets Ther* 2019; 12: 8319–8328.

24. Lyons K, Le LC, Pham YT, et al. Gastric cancer: epidemiology, biology, and prevention: a mini review. *Eur J Cancer Prev* 2019; 28: 397–412.

25. Liu Y, Wang X, Jia Y, et al. Effects of bufalin on the mTOR/p70S6K pathway and apoptosis in esophageal squamous cell carcinoma in nude mice. *Int J Mol Med* 2017; 40: 357–366.

26. Lian X, Gu J, Gao B, et al. Fenofibrate inhibits mTOR-p70S6K signaling and simultaneously induces cell death in human prostate cancer cells. *Biochem Biophys Res Commun* 2018; 496: 70–75.

27. Zhou H, Zhao H, Liu H, et al. Influence of carboplatin on the proliferation and apoptosis of ovarian cancer cells through mTOR/p70s6k signaling pathway. *J BUON* 2018; 23: 1732–1738.