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Original Article

MicroRNA-630 acts as a prognostic marker in gastric cancer and its role in cell migration and invasion

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Abstract: Background: MicroRNA-630 (MiR-630) is downregulated in various cancer types, and involved in tumor progression. However, its prognostic value in patients with gastric cancer remains unclear. Methods: Serum samples were collected from healthy individuals and gastric cancer patients. Reverse transcription polymerase chain reaction (RT-PCR) was used to assess miR-630 expression. The diagnostic and prognostic values of miR-630 were analysed by statistical methods. *In vitro*, miR-630 mimics was transfected into SGC-7901 cells, and the biological function of miR-630 was evaluated by MTT assay, transwell assays, and Western blot. A luciferase assay was used to explore whether SOX4-3'-UTR was a target gene of miR-630. Results: Compared with healthy individuals, gastric cancer patients showed reduced serum miR-630 levels. In addition, miR-630 was expressed at significantly lower levels in patients with aggressive tumors. Interestingly, low miR-630 expression was significantly correlated with poor prognostic and short 5-year survival. Multivariate logistic regression analysis indicated that miR-630 was an independent prognostic factor in gastric cancer. *In vitro*, miR-630 upregulation resulted in reduced SGC-7901 cell proliferation and invasion. In addition, an inverse correlation between miR-630 levels and SOX4 mRNA amounts was observed in gastric cancer tissues. More importantly, miR-630 upregulation resulted in reduced SOX4 expression, and the luciferase activity of the SOX4-3'-UTR plasmid was significantly suppressed by miR-630. Conclusion: MiR-630 is an independent prognostic factor for predicting survival of gastric cancer patients, and may inhibit gastric cancer cell proliferation and invasion, by downregulating SOX4.

Keywords: Gastric cancer, miRNAs, prognostic, SOX4

Introduction

Gastric cancer (GC) is the fourth most common cancer, and the second leading cause of cancer related deaths worldwide [1]. The lack of high-efficient diagnosis, treatment and prognosis assessment tools is the biggest challenge in clinic [2]. Patients with metastatic GChave a dismal median survival ranging from 4 to 10 months [3]. Importantly, migration and invasion abilities of GC cells are essential for departing from the original site [4, 5]. Therefore,early diagnosis andaccurate prognostic evaluation of gastric canceras well as deeper understanding of mechanisms surrounding metastasis of GC are very important in improving therapy and long term survival.

MicroRNAs are considered a novel class of biomarkers, and potential therapeutic targets for various diseases. In the past decade, an increasing number of miRNAs have been shown to play important roles in carcinogenesis, with some validated ashaving a significant impact on gastric cancer [6]. For instance, serum miR-29c expression is significantly decreased in GC, and may serve as a diagnostic and therapeutic biomarker for GC [7]. In addition, miR-365 is involved in PTEN/Akt signaling, and its reduction associated with poorly differentiated histology, deep invasion, and advanced stage [8]. Besides, miR-182 overexpression suppresses proliferation and colony formation in gastric cancer cells [9]. MiR-630, identified from the miRNA cluster at chromosome 15q24.1, acts as a tumor suppressor in human cancers, regulating tumor suppressor genes or proteinsinvolved in tumor growth, metastasis, and invasion [10-12]. However, the exact role of MiR-630 in gastric cancer remains unclear. This

Table 1. Clinical characteristics of 118 patients and the expression of miR-630 in GC tissues

Parameters	Case	MiR-630 Median (range)	*P*
Age (years)			0.557
<65	57	3.79 (2.25-5.77)	
≥65	61	3.19 (2.16-5.08)	
Sex			0.470
Male	69	3.34 (2.64-5.81)	
Female	49	3.61 (1.97-6.64)	
Tumor size			0.034
<5 cm	72	3.89 (2.15-5.93)	
≥5 cm	46	3.12 (2.16-5.68)	
Lymph node metastasis			0.021
Negative	34	4.66 (1.37-6.76)	
Positive	84	3.92 (1.23-5.83)	
Depth of invasion			<0.001
T1	24	4.92 (1.15-6.75)	
T2-T4	94	3.33 (0.78-4.98)	
Stage			<0.001
I, II	62	4.53 (1.27-6.15)	
III, IV	56	2.84 (0.32-3.78)	

study, therefore, aimed to assess the role of miR630 in GC, evaluatingclinical data, exploring its biological functionsin gastric cancer cells.

Materials and methods

Patients and sample collection

Preoperative venous blood samples, postoperative GC tissue specimens, and matched non-tumor tissue samples were collected from 131 patients, from 2012 to 2013 at Department of General Surgery of China-Japan Friendship Hospital. Blood samples from 116 subjects receiving physical examination were also obtained. None of thepatients had received previousradio-chemotherapy. Blood samples were centrifugedimmediately for serum preparation; tissue specimens were confirmed pathologically, snap frozen in liquid nitrogen, and stored at -80°C. Written informed consent was obtained from each patient. The study wasapproved by the ethics committee of hospital. The relationship between serum miR-630 level and prognosis was assessed in 97 patients who were observed in follow-up. Each stage was evaluated according to criteria proposed by UICC/AJCC in 2010 [13].

Reagents

Thehuman GC SGC-7901 cell line was purchased from Abcorel Inc. (Shanghai, China). DMEM and trypsin were from Sigma-Aldrich (USA). Fetal bovine serum (FBS), restriction endonucleases BamHI and XhoI, T4 DNA ligase, and RNA extraction reagent were manufactured by In vitrogen Inc. (Carlsbad, CA, USA). Reverse transcription kit was from TaKaRa (China); SYBR Green fluorescent dye kit was a product of Roche (Switzerland). Rabbit or mouse anti-human P21, P27, MMP-2, MMP-9, SOX4, and β-actin polyclonal antibodies were purchased from Abcam (USA). MTTassay kit was manufactured by Gefanbio (China). Horseradish peroxidase-labeled goat anti-rabbit or anti-mouse secondary antibodies and Western blot detection reagents were from Beyotime (China).

Vector construction and cell transfection

For the construction of over-expression plasmid, a miR-630 mimics was designed and synthesized by GenePharma company, Shanghai, China. The sequences were as follows: 5'-AGUAUUCUGUACCAGGGAAGGU-3' and 3'-ACCUUCCCUGGUACAGAAUACU-5'.

We then searched the Sox4 gene sequence via-PubMed, and designed Sox4 mRNA primers according to primer sequences in GenBank. SOX4 3'-UTR sequences were: 5'-CTTGACATGATTAGCTGGCATGATT-3' (forward) and 5'-CCTGTGCAATATGCCGTGTAGA-3' (reverse).

PCR products were cloned into the pcDNA3.0 vector (Addgene, MA, USA). All constructs were verified by sequencing.

For transfection, SGC-7901 cells were seeded into 6 well-plates at a density of 5×10^5 per well. At 50-70% confluency, SGC-7901 cells were transiently transfected with miR-630 mimics or negative control, using Lipofectamine™ 2000 (Invitrogen, CA) according to the manufacturer's instructions.

Quantitative real-time PCR

Total RNA from clinical specimens and GC cells was reverse transcribed into cDNA using

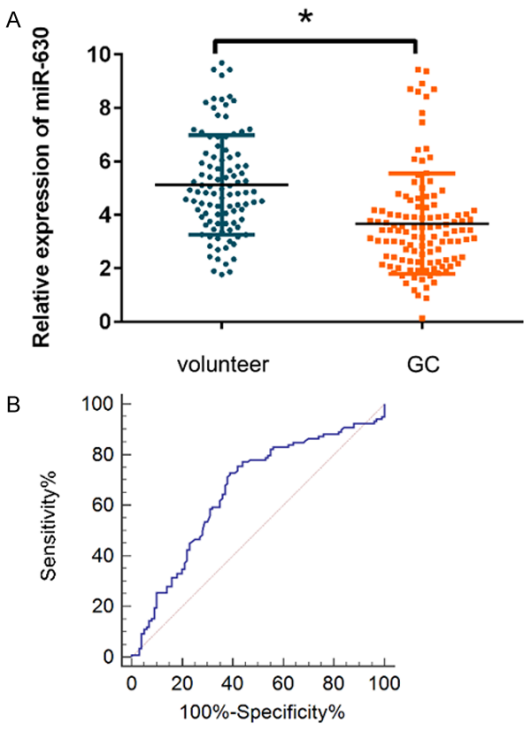

Figure 1. Expression of miR-630 and its ROC analysis on the diagnosis of GC. A: Relative expression of miR-630 in groups of healthy volunteers and gastric adenocarcinoma; miR-630 was significantly in lower expression in patients with GC compared with volunteers. B: ROC analysis of miR-630 on the diagnosis of GC; AUC 0.66 (95% CI, 0.59-0.72), P<0.001; Cutoff value is 4.03. *P<0.01 *vs*. healthy volunteers.

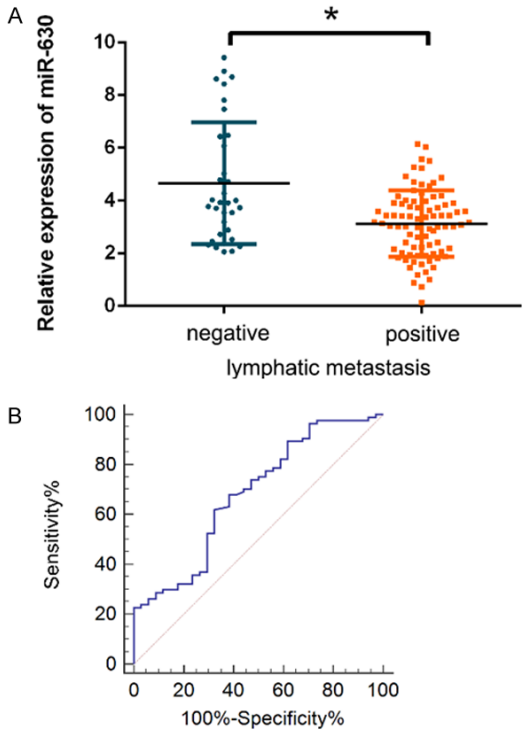

Figure 2. Expression of miR-630 and its ROC analysis in predicting lymph node metastasis. A: Relative expression of miR-630 in GC tissues with or without lymph node metastasis: miR-630 was significantly in lower expression in patients with lymph node metastasis (n=84) compared with non-metastasis (n=34), P<0.01. B: ROC analysis on predicting lymph node metastasis: AUC, 0.68 (95% CI, 0.59-0.76), P<0.001; Cutoff value is 3.64.

Reverse transcription kit, in a reaction system of 20 μL, at 16°C (30 min), 45°C (30 min), and 85°C (5 min). SYBR Green was used to detect miR-630 amplification; RT-PCR was carried out for 40 cycles at 94°C (15 min), 94°C (30 s), 60°C (30 s), and 72°C (30 s), with a final extension at 72°C for 8 min. Data were normalized to the internal control β-Actin. Relative quantification of miR-630 and SOX4 expression levels was performed by the 2-ΔΔCT method. All experiments were repeated three times.

Western blot

Total protein was extracted from cells or tissue specimens. BCA Protein Assay Kit was used for protein quantitation. Equal amounts of proteinwere separated by SDS-PAGE and blotted onto PVDF membranes.After blocking for 1 h, the membranes were incubated with specific primary antibodies against P21, P27, MMP-2, MMP-9, SOX4, and β-actin overnight at 4°C, followed by addition of horseradish peroxidase-labeled goat anti-rabbit or anti-mouse secondary antibodies for 1 h at room temperature. Specific protein bands were visualized by enhanced chemiluminescence (ECL).

Cell proliferation assay

Cells were seeded into 96-well plates at a density of 5×10^3 cells per well. Each group included 3 replicate wells; cells were cultured for 12, 24, 48, and 72 h. After incubation, 20 μL of 5 mg/mL MTT reagent was added to each well, and further incubated for 4-6 h. Finally, 150 μL DMSO was added to each well after culture medium removal, and absorbance was measured at 490 nm on a microtiter plate reader. Growth curves were generated for SGC-7901 cells. All experiments were performed three times.

Cell invasion assay

Boyden chamber assays were usedto assess cell invasion. The medium was supplemented with 1% heat-inactivated FBS, and cells were

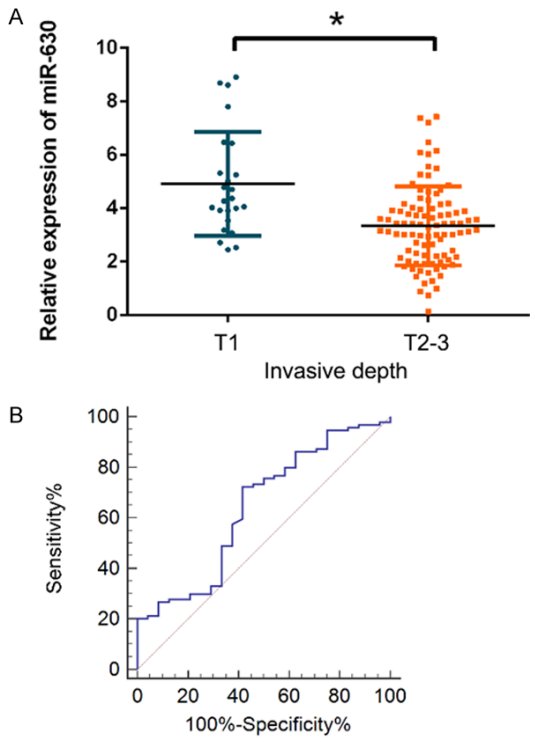

Figure 3. Expression of miR-630 and its ROC analysis on the prognosis of depth of invasion. A: Relative expression of miR-630 in GC tissues with different depth of invasion, which was determined according to endoscopes [38]: miR-630 was significantly in lower expression in patients with deeper invasion T2-4 (n=94), P<0.01. B: ROC analysis onpredicting depth of invasion: AUC 0.64 (95% CI, 0.55-0.73), P=0.021; Cutoff value is 3.90.

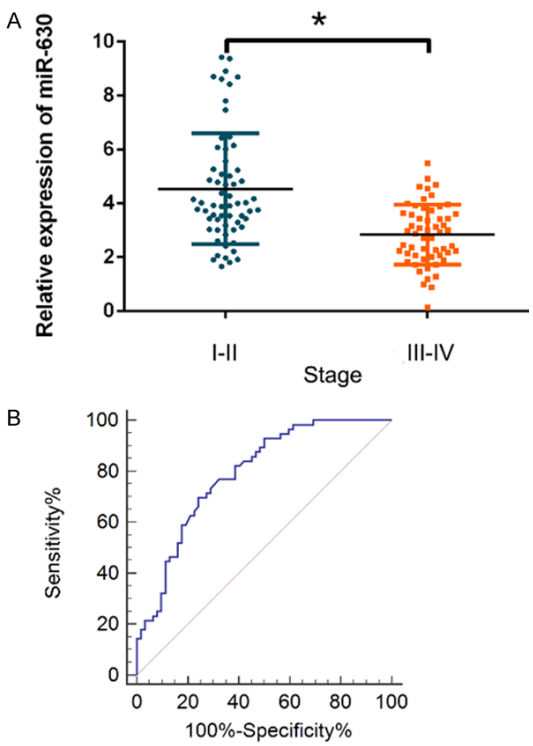

Figure 4. Expression of miR-630 and its ROC analysis on the prognosis of tumor stage. A: Relative expression of miR-630 in GC tissues with different tumor stage: miR-630 was significantly in lower expression in patients with higher tumor stage T3-4 (n=62), P<0.01. B: ROC analysis on prognosis of tumor stage: AUC 0.79 (95% CI, 0.71-0.86), P<0.001; Cutoff value is 3.18.

seededat a density of $5*10^4$/ml in the upper chamber; the lower chamber was filled with 500 μl complete medium with 20% FBS. After incubation for 24 h at 37°C, non-invading SGC-7901 cells were gently removed from the upper chamber. Cells migrating to the lower compartment were fixed and stainedwith 0.1% crystal violet. Cells were washed 3 times with PBS, and five random high power fields in each sample were analyzed by light microscopy.

Luciferase reporter assay

Wild-type (WT) 3'-UTR of SOX4 and the mutated sequence were inserted into the pGL3 control vector (Promega, Madison, USA) to generateWT *SOX4*-3'-UTR and mutant *SOX4*-3'-UTR vectors, respectively. In the luciferase reporter assay, SGC-7901 cells were seeded into 48-well plates and allowed to adhere for 24 h before co-transfection with a mixture of 100 ng pGL3-WT SOX4-3'-UTR or pGL3-MUT SOX4-3'-UTR and 60 pmol miR-630 mimics or controlsequence, using Lipofectamine 2000 reagent. Three independent experiments were performed. At 48 h post-transfection, firefly and Renilla luciferase activity was determined using the Dual-luciferase Reporter Assay System (Promega).

Statistical analysis

Data are mean ± standard deviation (SD). Based on day of death or discharge, Kaplan-Meier survival analysis was performed. Log rank test was used to compare survival between different serum concentrations of miR-630. Receiver operating characteristic (ROC) curves were used to assess the clinical value of miR-630. Categorical variables were presented as count or percentage. Student's t-test was used to assess continuous variables,

Table 2. Logistic regression analysis for patients with GC

Parameters	OR	95% CI	*P*-value
Age	1.22	1.03-1.29	0.047*
Male	1.17	1.01-1.21	0.312
Tumor size	1.86	1.14-4.10	0.015*
Lymph node metastasis	1.35	0.75-2.06	0.073
Depth of invasion	2.29	1.03-4.77	0.029*
Stage	3.43	1.78-8.27	0.017*
Expression of miR-630	0.37	0.11-0.51	0.005*

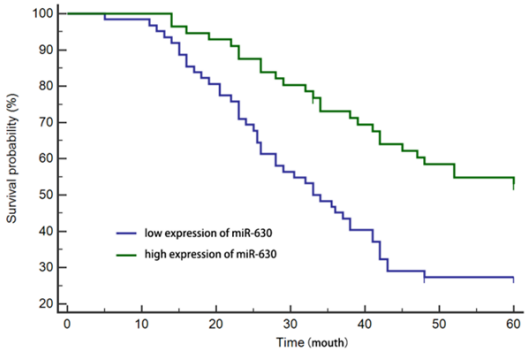

Figure 5. Kaplan-Meier survival curve on the prognostic role of serum miR-630 in GC patients. The expression level of miR-630 was determined by qRT–PCR assay in 97 GC patients divided into two groups according to median ratio of relative expression of miR-630 (3.31). After a follow-up of 60 months, the survival data were compared using log-rank test. GC patients with high miR-630 expression showed shorter survival compared with patients exhibiting low miR-630 expression (P<0.001).

with χ^2-test employed for categorical variables. P<0.05 was considered statistically significant. Statistical analyses were performed using the SPSS20.0 software, Medcalc and GraphPad Prism 6.0.

Results

Patient characteristics

The relationship between patient characteristics and serum miR-630 expression is shown in **Table 1**. There were no statistically significant differences among various age and gender groups (P>0.05). However, miR-630 levels were higher in patients with tumor size >5 cm compared with values obtained for individuals with small size tumors (P=0.016). Compared with patients without lymph node metastasis, those with lymph node metastasis showed lower miR-630 levels (P<0.001). Similarly, miR-630 amounts were higher in patients with tumor-invasion at T1 than in those at T2-4; miR-630 expression was lowerin advanced pathological stages (P<0.001).

Serum miR-630 for diagnosis and severity evaluation in gastric adenocarcinoma

To assess the value of serum miR-630 in gastric adenocarcinoma diagnosis, 100 healthy volunteers were enrolled. Interestingly, miR-630 expression was significantly lower in patients with GC compared with the volunteers (P<0.01; **Figure 1A**). ROC curve analysis indicated an AUC value for miR-630 in diagnosing GC of 0.66 (95% CI, 0.59-0.72) (P<0.01); with a cut-off value of 4.03, sensitivity and specificity were 72.9% and 61.0%, respectively (**Figure 1B**), suggesting that miR-630 could be used as a biomarker for GC diagnosis.

According to other clinical parameters, GC patients were divided into two groups. ROC curve analysis was performed to determine the associations of miR-630 with clinical indexes. The results indicated an AUC value for miR-630 of 0.68 (95% CI, 0.59-0.76) (P<0.001; **Figure 2**), in predicting lymph node metastasis. In addition, when predicting depth of invasion and tumor stage, AUC values were 0.64 (95% CI, 0.55-0.73; P=0.021) (**Figure 3**) and 0.79 (95% CI, 0.71-0.86; P<0.001) (**Figure 4**), respectively. These data showed good prospective application of miR-630 in evaluating the severity of gastric adenocarcinoma.

Risk factors in 5-year mortality

As shown in **Table 2**, multivariate logistic regression analysis indicated that age, tumor size, depth of invasion and tumor stage were risk factors for 5-year mortality. Meanwhile, serum miR-630 level was a protective factor with OR=0.27 (95% CI, 0.07-0.31; P=0.008). Based on the above data, Kaplan-Meier survival analysis was carried out to further correlate miR-630 expression with GC prognosis. The 118 patients were divided into high- and low-miR-630 groups, according to the median ratio of relative miR-630 expression (3.31). A total of 97 patients were followed up for 60 mouths. Interestingly, patients with high miR-630 expression (n=45) lived longer than those with low miR-451 amounts (n=52) (P<0.001 in log-

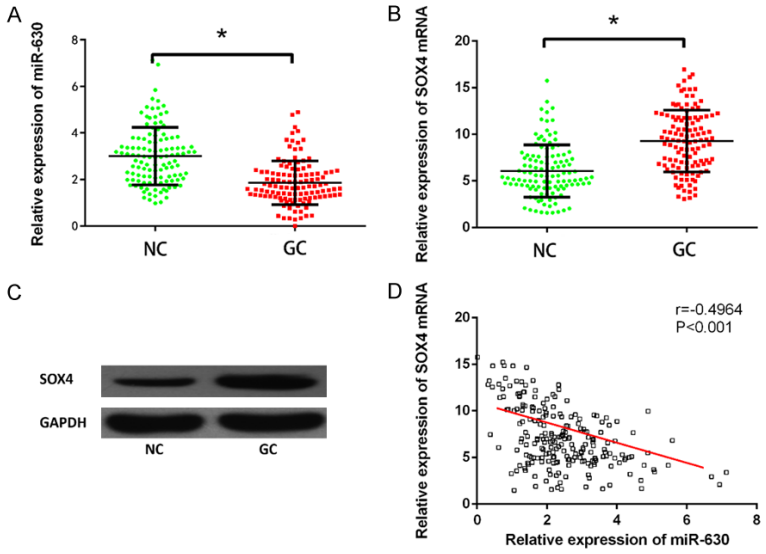

Figure 6. Expression of miR-630 and SOX4 mRNA in the two groups. A, B: Analysis of the expression of miR-630 and SOX4 mRNA in normal gastric tissue and GC tissues by RT-PCR. The expression of miR-630 was decreased in GC tissues whereas the expression of SOX4 mRNA was significantly increased (P<0.01), compared with adjacent normal tissues. B: SOX4 expression was also overexpressed in GC tissues confirmed by Western blot (P<0.01), using GAPDH as an internal control. C: Pearson correlation was used to analyze the relationship between miR-630 and SOX4 mRNA. D: The result showed that miR-630 was negatively correlated with SOX4 (P<0.01).

rank test; **Figure 5**). These findings suggested that miR-1630 could serve as a prognostic biomarker in patients with gastric adenocarcinoma.

Gene expression levels of miR-630 and SOX4 in GC tissues and matched normal tissues

Since an important role for miR-630 in clinic was found, the underlying molecular mechanisms were further investigated. SOX4, a potential target of miR-630 found via software programs such as TargetScanS, miRanda and DIANA-microT, was detected in GC and corresponding normal tissues, together with miR-630 by RT-PCR. Lower expression of miR-630 was found in GC tissues compared with normal ones (P<0.05); in contrast, SOX4 mRNA expression was higher, and negatively correlated with miR-630 level, as shown in **Figure 6** (P<0.05).

MiR-630 suppresses SGC-7901 cell proliferation and invasion in vitro

In vitro experiments were carried out to further explore the biological function of miR-630 in GC cells. RT-PCR was used to detect miR-630 expression levels in SGC-7901 cells after transfection with miR-630 mimics or the corresponding negative control. Compared with miR-NC amounts, miR-630 expression in SGC-7901 cells transfected with miR-630 mimics was significantly increased (P<0.01, **Figure 7A**). Subsequently, the effects of miR-630 on SGC-7901 cell proliferation and invasion were evaluated. As shown in **Figure 7B**, the cell proliferativeability was distinctly inhibited in the miR-630 mimics group compared with the other two groups (P<0.05), with the level of proliferation related protein p21/27 increased (P<0.01, **Figure 7C**). In addition, cell invasion ability was inhibited by miR-630 as assessed byTranswell assay (P<0.01, **Figure 7D**), and the expression of invasion-related proteins was suppressed by miR-630 mimics (P<0.05), as shown by Western blot (**Figure 7E**). Taken together, these data indicated that miR-630 inhibited proliferation and invasion in SGC-7901 cells.

SOX4is a potential target of miR-630 in SGC-7901 cells

To test the hypothesis that miR-630 modulated SOX4 expression, WB was used to assess protein expression of SOX4 in miR-630 mimics, miR-NC and control groups; interestingly, SOX4levels were significantly reduced in the miR-630 mimics group compared with the other two groups (**Figure 8A**, P<0.05). A putative miR-630-binding sequence in the 3'-UTR of *SOX4* mRNA was suggested in **Figure 8B**. To further validate *SOX4* as a target gene, luciferase reporter vectors with the putative SOX4 3'-UTR target site for miR-630 (pGL3-wt-SOX4, set as wild-type) and mutant version in the seed region (pGL3-mut-SOX4, set as mutant type) were generated. Indeed, luciferase activity was suppressed by miR-630 in SGC-7901 cells harboring the WT plasmid (P<0.05), but not the MUT plasmid (**Figure 8C**). These findings suggested that miR-630 targeted the 3'UTR of SOX4 in HGC-27 cells.

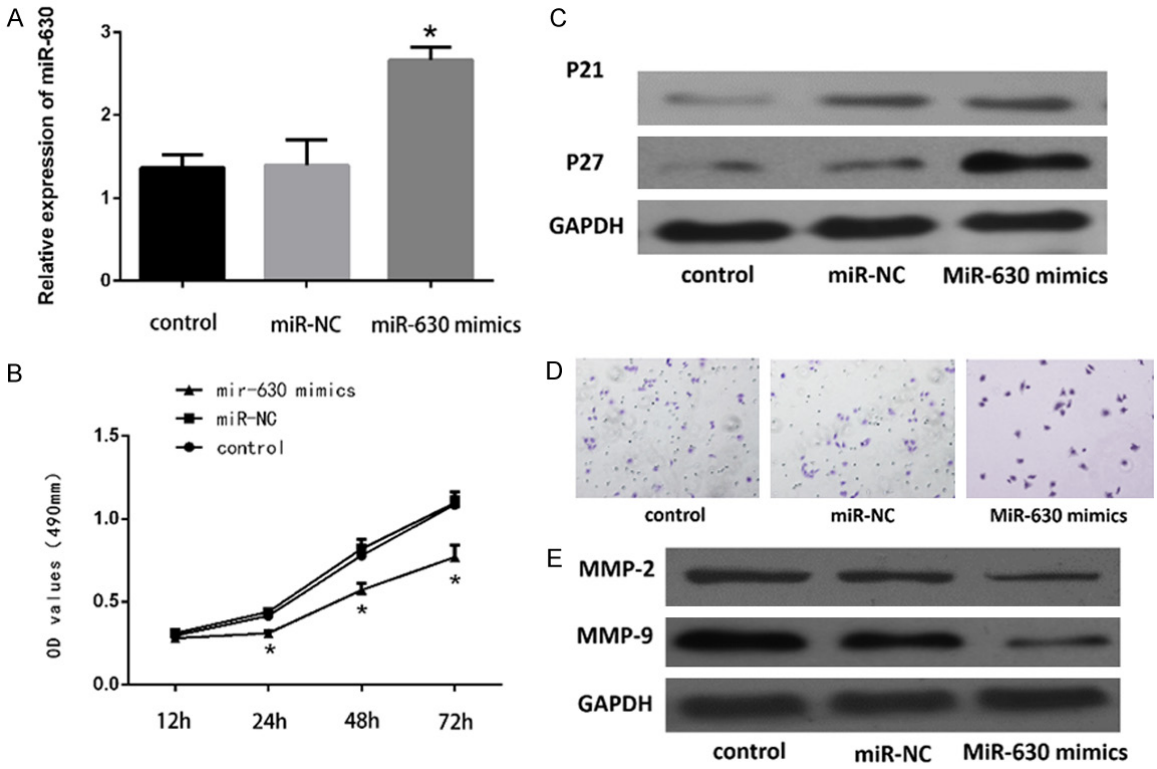

Figure 7. MiR-630 inhibited SGC-7901 proliferation and invasion in vitro. A: MiR-630 was significantly upregulated in SGC-7901 cells when successfully transfected with miR-630 mimics, (P<0.01). B: MTT assay showed that overexpression of miR630 markedly inhibited SGC-7901 cell proliferation compared with miR-NC and control groups (P<0.01). C: Western blot showed that the proliferation-related protein P21/27 were increased in group of miR-630 mimics, P<0.01. D: Transwell migration assay revealed that miR-630 remarkably inhibited cell invasion (P<0.01). E: Invasion-related proteins MMP-2/9 were distinctly suppressed in the miR-630 group compared with the other two groups, P<0.01.

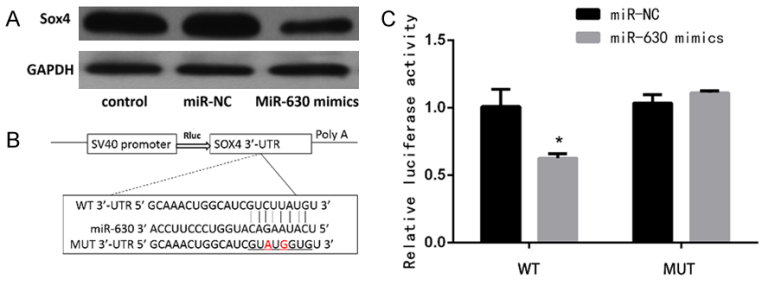

Figure 8. MiR-630 directly targeted SOX4 in SGC-7901 cells. A: Western blot of SOX4 expression in each group. SOX4 was significantly suppressed in miR-143 mimics group (P<0.01). B: A human SOX4 3'-UTR fragment containing wild type or mutant miR-630-binding sequence was cloned downstream of the luciferase reporter gene, sequences were shown. C: Luciferase reporter assay showed that miR-630 inhibited luciferase activity in SOX4 with WT-binding sites but not with the MUT-binding sites in 3'UTR of SOX4 mRNA (P<0.01).

Discussion

It is well known that GC is a complex, heterogeneous and multifactorial disease, and over one million new cases are diagnosed per year with >70% occurring in developing countries. Although incidence and mortality rates are slowly declining, GC remains a significant public health problem [14]. Various risk factors contribute to tumorigenesis, especially *Helicobacter pylori* and its molecular virulence characteristics [15]. On the other hand, powerful tools, such as next-generation sequencing, may definitely help understandthis complex disease; molecular targeted agents were shown to extend survival of patients with advanced gastric cancer [16]. However, validated predictive biomarkers, as well as personalized orprecision medicine, still need to be improved.

MicroRNAs (miRNAs) are short (20-24nt) non-coding RNAs involved in post-transcriptional regulation. By entirely or partially base-pairing to the 3'-untranslated region of target mRNAs, miRNAs play crucial roles during various biological progresses [17-19]. Recent studies have demonstrated that miRNAs contribute to gastric cancer by regulating the expression of oncogenes and tumor suppressors to affect cell proliferation, migration, invasion and apoptosis [20]. In addition,many have been associated with tumor type or stage, and patient survival, and even developed as diagnostic or prognostic markers [6, 21]. Previous studies indicated that miR-630 could up-regulate the cell cycle inhibitor p27 to arrest cells in G0-G1 phase and reduce proliferation rates in non-small cell lung cancer cells [22]. Corcoran et al. found that miR-630 is decreased in breast cancer and plays an important role in modulating the response to HER-targeting drugs as well asin overall aggressive phenotypic characteristics of HER2-overexpressing breast cancer cells [10]. Cao et al.demonstrated that miR-630 promotes apoptosis by downregulating cell-cycle kinase 7 (CDC7), and inhibits CDC7-mediated proliferationin the human lung cancer A549 cells [23]. Similarly, Song et al. assessed 22 patients with NSCLC and found that miR-630 expression was significantly decreased, demonstrating that miR-630 suppresses proliferation, migration, and invasion in NSCLC cells by down-regulating LMO3 [24]. Sakurai demonstrated that gefitinib induces miR-630, leading toefficient growth arrest and apoptosis of human prostate cancer cells [25]. Farhana et al. considered IGF-1R a potential target gene of miR-630, and pre-miR-630 overexpression was shown to significantly enhance inhibition and apoptosis in pancreatic cancer cells [26]. In addition, Zhou et al. revealed that MTDH mediates the functions of miR-630 in suppressing migration, invasion, and colony formation in breast cancer cells [12]. We, therefore, assessed the function of miR-630 in gastric cancer; as expected; lower expression was found in GC patients compared with healthy volunteers. ROC curve analysis indicated an AUC value for miR-630 in diagnosing GC of 0.66 (95% CI, 0.59-0.72), suggesting that miR-630 could contribute to diagnosing GC. Next, the associations of miR-630 with clinical parameters in patients with gastric cancer were statistically analyzed. Interestingly, miR-630 showed a good prospective application in predicting-lymph node metastasis, depth of invasion, and tumor stage in gastric cancer, indicating that miR-630 is a biomarker in evaluating severity of gastric adenocarcinoma. Furthermore, we found that patients with high miR-630 expression showed significantly longer survival than those with low amounts, after follow-up for about 60 months. These findingssuggested that miR-630 might play a critical role in GC progression and development.What's more, RT-PCR was carried out to assess miR-630 expression in GC and corresponding normal tissues, andlower miR-630 levels were obtained in GC tissues. Taken together, these results suggested that miR-630 downregulation might be a common event in GC. In subsequent *in vitro* experiments, we found that miR-630 overexpression markedly inhibits proliferation and invasion in SGC-7901 cells.

Furthermore, SOX4 was identified in this study as a direct target of miR-630 in gastric cancer cells. Interestingly, SOX4 mRNA levels were negatively correlated with miR-630 expression. Meanwhile, lower SOX4 expression levels were found in SGC-7901 cells transfected with miR-630 mimics, which were further validated by a luciferase assay. The discovery of the sex-determining region Y (SRY) generevealed the existence of a family of evolutionary conserved SRY-related high-mobility-group (HMG)-box (SOX) transcription factors [27]. A total of 20 members of this family have been identified. Several studies have shown that increased expression of SOX4, which belongs to the highly conserved group C SOX (SOXC) transcription factors, is correlated with prolonged patient survival and slower disease progression in gastric, colon, prostate, breast, lung, and endometrial cancers [28-32]. Wang et al. reported that SOX4 knockdown by RNAi suppresses proliferation, metastasis, and invasion in colorectal cancer cells and reversesepithelial mesenchymal transition (EMT) via vimentin upregulation and E-cadherin downregulation [33]. It was also demonstrated that SOX4 inhibition is associated with decreased invasion and metastasis in breast cancer cells [34]. Furthermore, patients with high SOX4 expression levels were shown to have shorter OS and DSS than those with low SOX4 amounts in oral squamous cell carcinoma, indicating that SOX4 might contribute to invasive and oncogenic phenotypes of head

and neck squamous cell carcinoma by promoting cellsurvival and causing chemoradio-resistance [35]. Besides, Li et al. found that SOX4 is highly expressed in esophageal cancer cells, revealing it as a target of miR-133a in inhibiting cell migration and invasion [36]. Similarly, Sun et al. found that SOX4 is upregulated in cervical cancer cells, with its regulation affecting tumor proliferation and drug sensitivity [37]. In the current study, SOX4 was revealed as the executor of pathological functions of miR-630; these findings suggested an important functional role for SOX4-miR-630 interaction, making the latter a potential therapeutic target for gastric cancer treatment.

In summary, this studydemonstrated that miR-630 has good application in diagnosing GC and evaluating disease severity, also actingas a prognostic biomarker. MiR-630 targets SOX4, thereby inhibiting tumor proliferation and invasion. These findings suggest a novel therapeutic strategy targeting miR-630/SOX4 interaction in gastric cancer.

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Disclosure of conflict of interest

None.

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