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miR-198 regulated the tumorigenesis of gastric cancer by targeting Toll-like receptor 4 (TLR4)

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Abstract. – OBJECTIVE: To investigate the role of miR-198 and its target gene Toll-like receptor 4 (TLR4) of tumorigenesis of gastric cancer (GC).

MATERIALS AND METHODS: The expression of miR-198 in GC cells was detected by quantitative polymerase chain reaction (qPCR). The proliferation, apoptosis, migration, and invasion of GC cells were detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), flow cytometry, transwell chamber, and wound scratch assay. Bioinformatics analysis for the results of protein chip was performed to identify the target genes of miR-198. TLR4 was further confirmed to be the target gene of miR-198 by TLR4 luciferase reporter assay.

RESULTS: miR-198 expression level in GC SGC-7901 cells significantly decreased compared with the normal cells. When the miR-198 was overexpressed, the proliferation, migration, and invasion of GC cells were significantly decreased, while the apoptosis was increased. The expression of TLR4 in SGC-7901 cells was significantly higher, while the expression of TLR4 in SGC-7901 cells transfected with miR-198 significantly lowered, which was consistent with the Western blot for TLR4. The luciferase reporter assay confirmed that TLR4 was the target genes of miR-198 in GC SGC7901 cells.

CONCLUSIONS: miR-198 could induce apoptosis and inhibit the proliferation, migration, and invasion of GC cells through downregulating TLR4 expression.

Key Words:
Gastric cancer, microRNA, miR-198, Toll-like receptor 4 (TLR4).

Introduction

As a common disease threatening human health, gastric cancer (GC) is a malignant tumor derived from the gastric mucosal epithelium, which is the leading cause of gastrointestinal malignancies[1]. MicroRNA (miRNA) is a class of non-coding single-stranded RNA molecules. It consists of about 22 nucleotides encoded by endogenous genes. They are conserved in plants, animals, and some viruses. miRNAs regulated the post-transcriptional gene expression through RNA interference[2,3]. miRNAs in animals are able to recognize their target mRNAs at the 5' end of the miRNA with the 6 to 8 nucleotides[4,5]. Human miR-198 gene located at chromosome 13q13.3. miR-198 has been shown to play an important role in the carcinogenesis and progression of many types of tumors. The miR-198 gene expression was downregulated in hepatocellular carcinoma[6,7], pancreatic cancer[8], colorectal cancer[9], lung cancer[10,11], renal cell carcinoma[12], prostate cancer[13], and breast cancer[14], while upregulation in retinal malignancy, esophageal squamous cell was found. However, the relationship between miR-198 and tumor, and its mechanism of action is still unclear. In the tumor tissue of gastric cancer patients, the expression of miR-198 is also significantly down-regulated. The low expression of miR-198 was associated with early GC metastasis and short survival. Therefore, in this study, miR-198 target gene was identified by protein chip and bioinformatics information analysis, and the target site of Toll-like receptor 4 (TLR4) gene by miR-198 was mutated and verified by luciferase reporter assay. The aim of this investigation was to elucidate the relationship between has-mir-198 and target genes in GC and to provide new biomarkers and drug targets for the diagnosis and treatment of GC, as well as provide effective biomarkers for the accurate diagnosis and treatment of GC.

Materials and Methods

Expression Levels of miR-198 in Gastric Cancer (GC) Cells

5-10 × 10^6 normal gastric epithelial GES-1 cells, GC cell lines (SGC-7901, MGC-803) were

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collected. The total Ribose Nucleic Acid (RNA) was extracted by TRIzol methods (Invitrogen, Carlsbad, CA, USA), and the cDNA was obtained by RNA reverse transcription kits (TaKaRa Bio Inc., Tokyo, Japan). The mRNA expression level of miR-198 in GC cells was detected by reverse transcriptase-polymerase chain reaction (qRT-PCR). The qRT-PCR primers for miR-198 were as follows, has-miR-198 primer L: TCAAGTGCCTCAACCCATCT, has-miR-198 primer R: ATCTCCTCCTCTGTCTGGGT.

Establishment of miR-198 Stable Expression GC Cell Models

The miR-198 overexpression vector was transfected with 293T cells to produce virus, which was used to further infect the SGC-7901 GC cells for establishing the miR-198 stable expression GC cell models. The miR-198 overexpression vector was cotransfected with packaged vectors into 293T cells by FUGENE transfection reagents. 48h after transfection, the virus was collected and the SGC-7901 GC cells were infected with 4 mg/mL polybrene. 48h after infection, the miR-198 stable expression GC cell models were identified by qRT-PCR, Western blot, and EGFP fluorescence intensity observation.

Effect of miR-198 Overexpression on the Biological Characteristics of GC SGC-7901 Cells

To observe the biological characteristics of miR-198 overexpression SGC-7901 cells, taken the empty vector infection as a control, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was performed on 24h, 48h, 72h, 96h under the 450 nm wavelength to detect the proliferation ability of the miR-198 overexpression SGC-7901 cells, as well as the apoptosis, and they were analyzed using flow cytometry for enhanced green fluorescent protein (EGFP). Also, the transwell chamber was used to detect the migration activity of the miR-198 overexpression GC cells, and the wound scratch assay was performed to observe the invasion of the GC cells.

MTT: SGC-7901 cells cultured in logarithmic growth phase were digested with 0.25% trypsin, and then, single cell suspension was prepared with Dulbecco's modified Eagle medium (DMEM) medium. The cell concentration was adjusted to be 1×10^5 cells/mL. The cells were inoculated into a 96-well plate and a blank control group was set with only the cell culture medium. They were placed in 37°C, 5% CO_2 cell incubator cell adherent culture. The medium was changed after 12h. Five wells were set to repeat the experiment. 10 μL MTT solution (5 mg/mL) was added into each well of the culture plate and cultured in the cell incubator for 4 hours. The medium was aspirated and then the culture was terminated after incubation with dimethyl sulfoxide (DMSO 150 μL/well) in a shaker at 37°C for 10 min in the dark. A microplate reader was used to detect the optical density (OD) value under 492 nm wavelength. The results were recorded.

Flow cytometry assay: 1×10^6 GC SGC-7901 cells were collected and centrifuged at 4°C by 1000 rpm for 5 min, the supernatant was discarded, the cells were washed twice with pre-cooled phosphate-buffered saline (PBS). Then, the cells were resuspended in ice-cold 1 × Binding Buffer (100 μL) and placed on ice for 5 minutes. Also, the cells were stained with Annexin V-fluorescein isothiocyanate (FITC, 5 μl) and propidium iodide (PI) 2.5 μL to 100 μL of the cell suspension and they were mixed. The untreated cells were stained separately with Annexin V-FITC tubes and single PI tubes; the tubes were incubated for 15 min in the dark on ice. 400 μL of 1 × Binding Buffer were added to each tube and mixed gently. The results were analyzed within 30 min. The PI was detected by flow cytometry (Partec AG, Arlesheim, Switzerland). The wavelength of the excitation light was 488 nm. The wavelength of 530 nm was used to detect FITC fluorescence and the other wavelength was > 600 nm. The data obtained were analyzed by CellQuest 3.0 software (Franklin Lakes, NJ, USA) and the percentage of cells transferred was calculated.

Transwell chamber: Matrigel 1:8 (BD, Franklin Lakes, NJ, USA) was diluted on the basis of the amount of mmp produced by the cells, coated on the upper chamber surface of the Transwell chamber bottom membrane, and incubated at 37°C for 30 min to polymerize the Matrigel into a gel. The basement membrane was hydrated prior to use. The cell suspension was prepared by the withdrawal of blood cells to be hunger for 12-24h. The GC cells were digested. After centrifugation to discard the culture medium, they were washed for 1-2 times by PBS and re-suspended with se-

rum-free bovine serum albumin (BSA). The cell density was adjusted to 5×10^5/mL. 100 μL GC cell suspension was added into the Transwell chamber. The bottom chamber of the 24-well plate was filled with 600 μL medium containing 20% fetal bovine serum (FBS), paid particularl attention to bubbles. The GC cells were cultured for 12-48h. The Transwell chamber was fetched, the medium in the wells was discarded. The GC cells were washed with calcium-free PBS for 2 times, fixed by methanol for 30 minutes, air-dried appropriately. Then, they were stained with 0.1% crystal violet for 20 min. The non-migratory cells on the upper chamber were gently wiped with a cotton swab and they were washed 3 times with PBS. Five visions were observed under 400× microscope immediately. The cell number was counted.

Wound scratch assay: All equipment should be sterilized, the ruler and marker pen were sterilized by UV irradiation for 30 min before the operation. We drew lines with a marker pen in behind of the 6-well plate, one line every 0.5-1 cm evenly. At least five lines went through each well. 5×10 cells were added to the wells. On the next day, we washed the cells with PBS for 3 times; we cultured them by adding serum-free medium at 37°C and 5% CO_2 incubator. We took pictures on 0, 6, 12, and 24 hours after sampling.

Protein Chip and Bioinformatics Analysis of GC Tissues and the Adjacent Normal Tissues

Tumor tissue wax blocks at different stages with follow-up data were selected. The sections of paraffin specimens were labeled according to HE slices. The typical tumors and corresponding normal tissues were collected to construct tumors tissue microarray. The wax block of tumor tissues was drilled by tissue microarray production machine with a fine needle. The holes were made into 1-1.5 mm. The distance between the holes should be 0.2 mm. We loaded 1-2 points in order to prevent the leakage in the process of dot, slice, staining or immunohistochemical operation. We fixed them tightly to prevent displacement. The wax block of tumor tissues was incubated into 55°C temperature for about 10 minutes. It was drawn out the box before the wax was being completely dissolved and cooled at room temperature. We removed the wax block and stored it at 4°C refrigerator. The 30-50 sections were prepared continuously. RayBio® Biotin Label-based Human 1 (AAH-BLM-1) was used as antibody chip. Then, the tissue chips were analyzed by bioinformatics.

Expression of TLR4 in miR-198 Overexpression GC Cells

5-10 × 10^6 miR-198 overexpression SGC-7901 cells were collected. The total RNA was extracted by TRIzol methods, and the cDNA was obtained by RNA reverse transcription kits (TaKaRa Bio Inc., Otsu, Shiga, Japan). The mRNA expression level of TLR4 in miR-198 overexpression SGC-7901 cells was detected by qRT-PCR. The qRT-PCR primers for TLR4 were as follows, TLR4 primer L: ACCTCCCCTTCTCAACCAAG, TLR4 primer R: GGCTCTGATATGCCCCATCT. Meanwhile, the TLR4 protein was detected by Western blot.

Confirmation of TLR4 to be miR-198 Targeted Genes by Luciferase Reporter Assay

The promoter fragment of TLR4 was cloned from the genomic DNA by PCR and inserted into a luciferase reporter plasmid to construct the pGL3-TLR4; the predicted 3'UTR of TLR4 targeted by miR-198 was mutated as a control. The pGL3-TLR4 wild-type and the mutant ones were transfected with 293T to produce virus; then, the SGC-7901 GC cells were infected. The proteins were extracted and used for luciferase assays. The substrate was added and the luciferase activity was measured. Relative fluorescence intensity was calculated and compared with no-load control.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. All quantitative data were expressed as mean ± standard deviation. The comparison between groups was made using One-way ANOVA test followed by least significant difference (LSD). p-values < 0.05 were considered statistically significant.

Results

Establishment of miR-198 Stable Expression of Gastric Cancer Cell Models

Similar to the expression of miR-198 in gastric cancer (GC), miR-198 expression level in GC SGC-7901 cells significantly decreased compared with that in the normal gastric epithelium GES-1

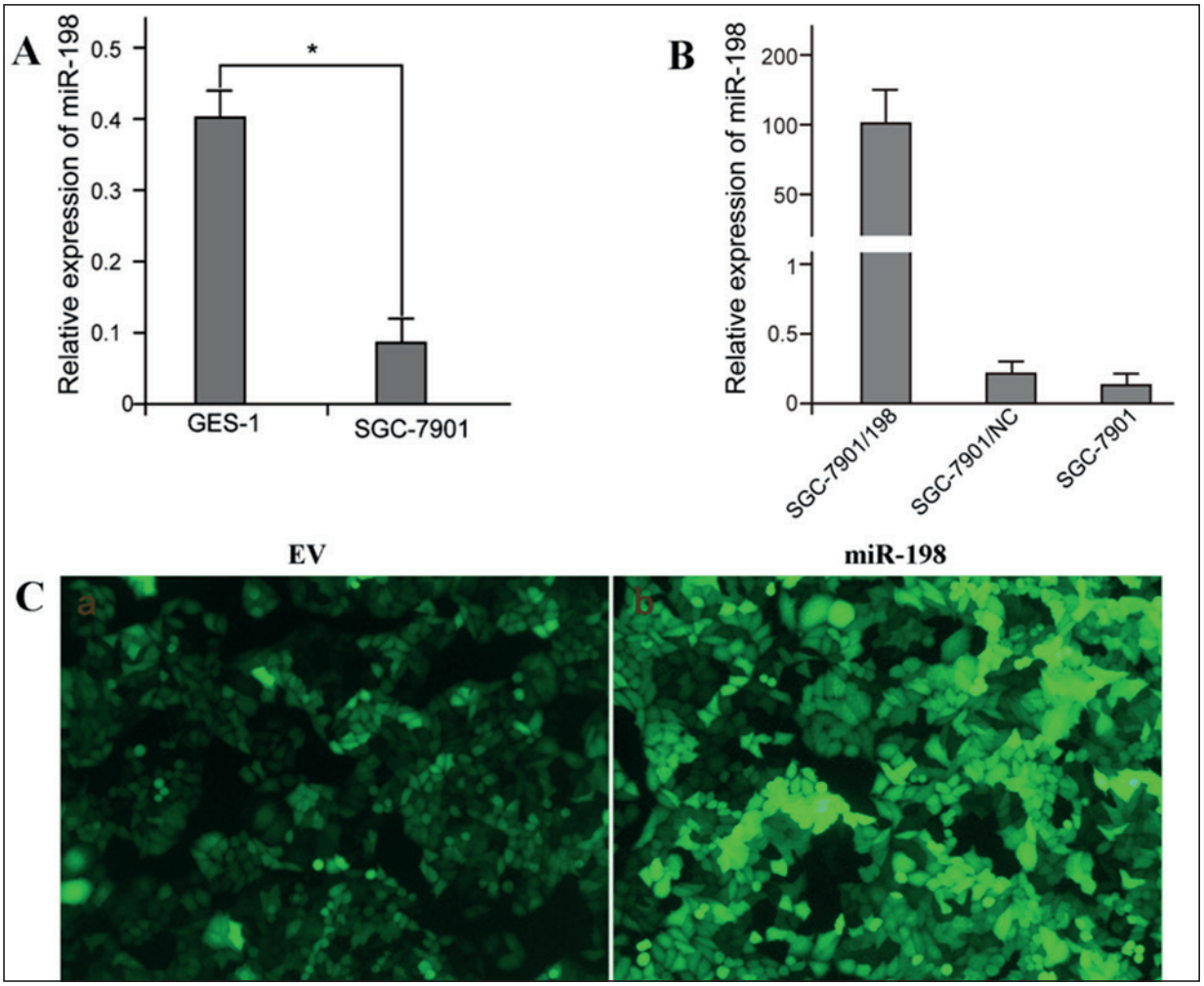

Figure 1. miR-198 expression in GC SGC-7901 cells. ***A,*** The miR-198 expression level in SGC-7901 cells.***B,*** The miR-198 expression level in miR-198 vector and empty vector transfected SGC-7901 cells. ***C,*** The EGFP fluorescent intensity of miR-198 vector in miR-198 stable expression GC cell models.

cells (Figure 1A). So, the miR-198 stable expression GC cell models were established to study the role of miR-198 in the regulation of tumorigenesis of GC. Compared with the untransfected SGC-7901 cells and the empty vector transfected SGC-7901 cells (Figure 1B), the miR-198 in miR-198 overexpression vector transfected SGC-7901 cells had high level of miR-198 expression, which was consistent with the EGFP fluorescent expression of miR-198 stable expression GC cell models detected by fluorescence microscopy (Figure 1C).

Effect of miR-198 Overexpression on the Biological Characteristics of GC SGC-7901 Cells

To investigate the role of miR-198 in the regulation of the formation of GC, the migration, invasion, proliferation, and apoptosis of GC cells were observed. The MTT results on 24h, 48h, 72h, 96h, under the 450 nm wavelength showed that the miR-198 overexpression GC cells had lower growth speed compared with that of the empty vector transfected GC cells (Figure 2A). Similarly, the apoptosis activity of the GC cells detected by flow cytometry showed that the miR-198 overexpression GC cells had apoptosis while the empty vector transfected GC cells had not (Figure 2B). Subsequently, the transwell chamber were used to detect the migration activity of the miR-198 overexpression GC cells; the migration ability of miR-198 overexpression GC cells were significantly reduced compared with that of the empty vector transfected GC cells (Figure 2C). Finally, the wound scratch assay was performed

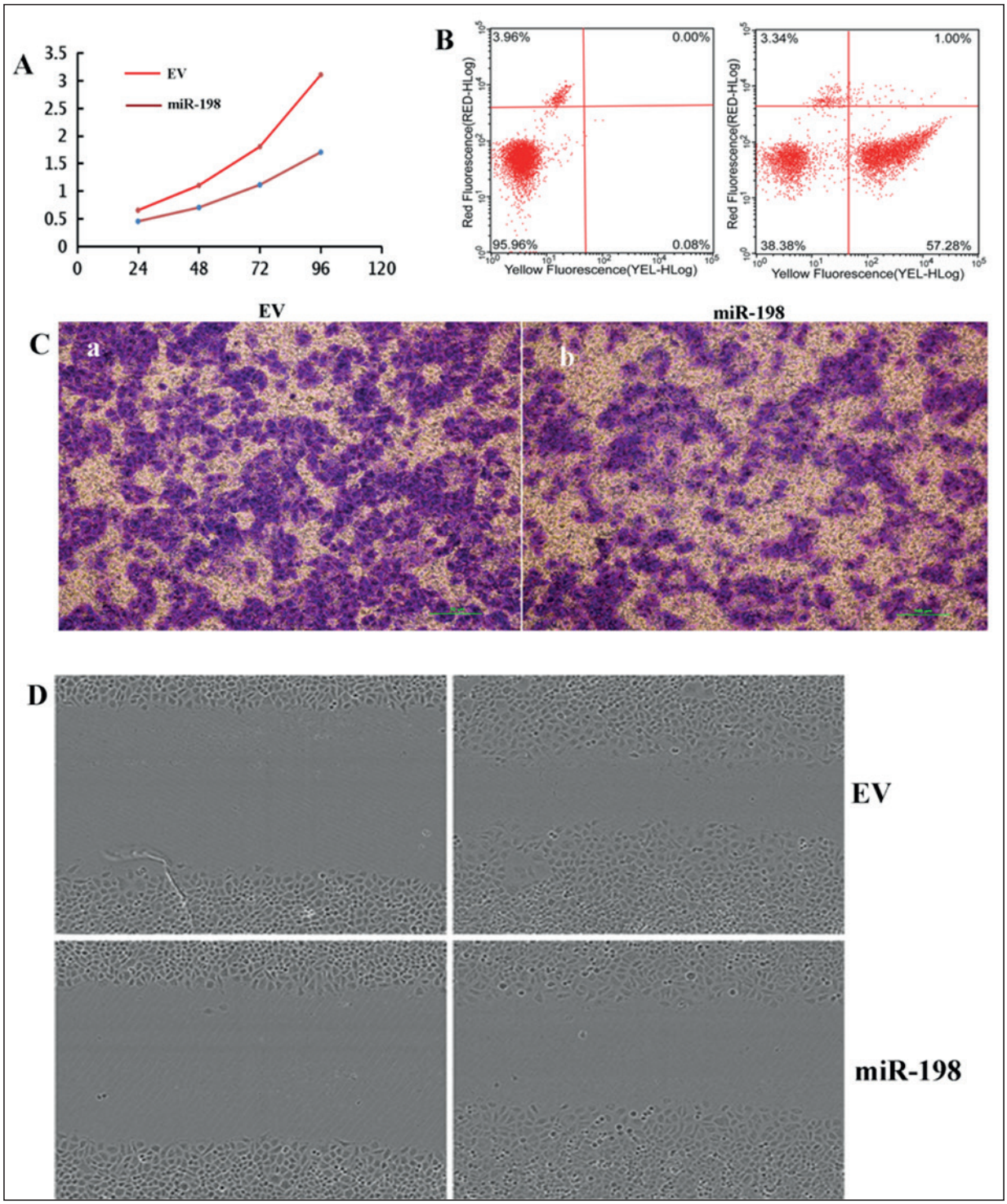

Figure 2. Effect of miR-198 overexpression on the biological characteristics of gastric cancer SGC-7901 cells. ***A,*** The proliferation of GC cells transfected by miR-198 vector detected at 24h, 48h, 72h, and 96h by MTT assay. ***B,*** The apoptosis of the GC cells transfected by miR-198 vector and empty vector measured by flow cytometry. ***C,*** The migration activity of GC cells transfected by miR-198 vector and empty vector detected by transwell chamber experiment. ***D,*** The invasion of GC cells transfected by miR-198 vector and empty vector measured by wound scratch assay.

to observe the invasion of the GC cells; the results showed that the miR-198 overexpression GC cells had significant lower invasion ability compared with that of the empty vector transfected GC cells (Figure 2D).

miR-198 Targeted Genes Identified by High-throughput Protein Chip Assay

To identify the miR-198 targeted genes, high-throughput protein chip assay was performed (Figure 3). The bioinformatics analysis for the high-throughput protein chip assays identified 105 differentially expressed proteins in GC tissues compared with the adjacent non-cancerous tissues, among which, the six up-regulated cytokines were miR-198 candidate targeted genes, including Toll-like receptor 4 (TLR4), growth differentiation factor 1, 5, 11, GDF1, 5, 11), human Fas-associated cell death domain protein (FADD), epidermal growth factor (EGF), and others (Table I).

The further analysis for high-throughput protein chip of GC and adjacent tissues showed that TLR4 and other genes were significantly increased in cancer tissues. Through searching by the three databases [Target Scan (http://www.targetscan.org/), Pictar (http://pictar.mdc-berlin.de/), Diana (http://diana.cslab.ece.ntua.gr/microT/)], it was found that TLR4 may be a potential target for miR-198.

Confirmation of miR-198 Targeted Gene TLR4

Firstly, the expression of TLR4 in GES-1 and SGC-7901 cells was detected by qPCR. The results showed that the expression of TLR4 in SGC-7901 cells was significantly higher than that in SGC-7901 cells transfected by qPCR, while the expression of TLR4 in SGC-7901 cells transfected with miR-198 was significantly lower than that in SGC-7901 cells, which was consistent with the previous results. While the expression of TLR4 was significantly decreased in SGC-7901 cells transfected with miR-198 expression vectors (Figure 4A). The expression of TLR4 was further detected by Western blot at the protein level. Consistent with relative mRNA expression levels, the overexpression of miR-198 apparently inhibited TLR4 expression (Figure 4B).

Subsequently, luciferase reporter assay was further performed to confirm that TLR4 was the target gene of miR-198. The 3'UTR of the TLR4 (position at 3835-3842) targeted by miR-198 was mutated to compared with the luciferase

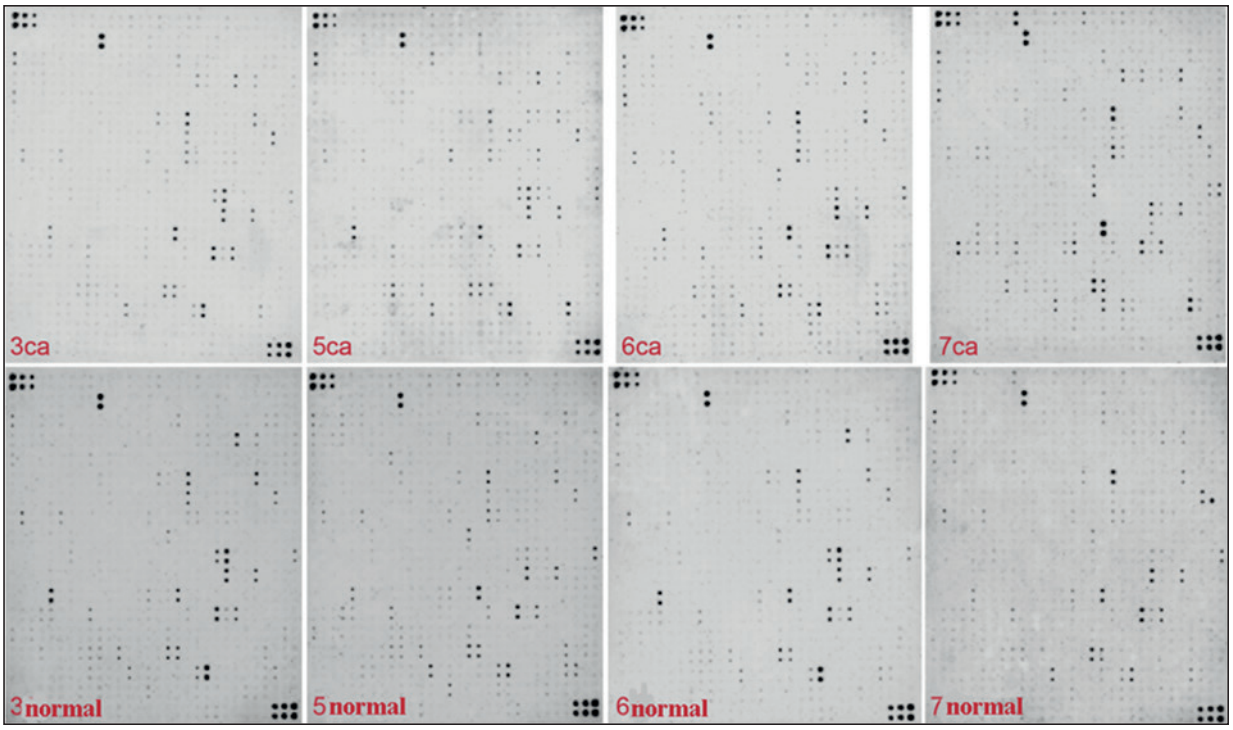

Figure 3. High-throughput protein chip assay for the GC and adjacent tissues. 3ca, 5ca, 6ca, 7ca and 3normal, 5 normal, 6 normal, 7normal indicated 3#, 5#, 6#, 7# cancer samples and the adjacent normal samples.

intensity detected in wild-type TLR4 GC cells (Figure 4C). The results showed that miR-198 overexpression in cancer SGC7901 cells could inhibit the luciferase intensity of TLR4 wild-type, while no effect for the luciferase intensity of TLR4 mutant GC cells were observed. The results demonstrated that TLR4 was a target gene for miR-198 (Figure 4D).

Discussion

miRNAs were small single-stranded RNA molecules composed of 18-23 nucleotides which can inhibit the expression of oncogenes or tumor genes and play an important role in the tumor formation, infiltration, and metastasis. Gastric cancer (GC) had the highest morbidity and mortality in all malignancies, but its treatment was lack of early specific diagnostic markers and effective curative effect. In GC, aberrant expression of miRNAs has been detected in many researches and their involvement, progression, invasion, and metastasis in tumorigenesis were confirmed. miRNA may be a valuable diagnostic marker and treatment targets in GC. The study of the role of microRNA in GC and its relationship with diagnosis and prognostic parameters may help to improve the sensitivity of the diagnosis and treatment of GC[15]. Treece et al[16] detected paraffin sections of 131 patients with malignant GC and benign gastric mucosa by qRT-PCR panel. The results showed that multiple miRNAs were highly expressed in GC tissues, while miR-744 was downregulated. However, the mechanism of regulation for tumorigenesis of GC by miR-198 was still unclear. In this study, the miR-198 expression in GC cells was similar to that in GC. The expression of miR-198 was significantly down-regulated in GC tissue. The low expression of miR-198 may be associated with early GC metastasis and short survival. Our results indicated that miR-198 may play a role in the tumor suppression, in the development, and progression of GC. Our results were consistent with that results of Zhang et al[17] and Yang et al[18] on the inhibition of miRNA on the development of GC.

Invasion and metastasis of tumor were the main features of malignant tumors that caused 90% cancer-related death. Ruoming et al[19] demonstrated that miR-31 could inhibit the proliferation of GC cells, promote apoptosis, and weaken cell migration by regulating SGPP2, Smad4, STAT3, and other target genes by RT-

Table I. Significantly differentially expressed protein factors (105 species, para-cancerous tissue/cancer tissue) associated with GC.

No. of the protein point	Protein factors	Fold
507	XEDAR	2.65
178	GFR alpha-3	0.45
46	BMPR-II	0.21
359	MMP-12	0.24
298	IL-26	0.40
372	NCAM-1/CD56	4.83
99	Dkk-4	0.27
351	MMP-1	0.25
55	CCR5	0.32
237	IL-1 R6/IL-1 Rrp2	0.36
457	TPX	0.50
42	BMP-8	0.32
188	Glypican 5	0.48
44	BMPR-IA/ALK-3	0.43
118	Epiregulin	0.25
358	MMP-11/Stromelysin-3	0.40
245	IL-2 R beta/CD122	0.10
273	IL-17	0.07
497	VE-Cadherin	0.31
260	IL-10 R alpha	0.32
128	Fas Ligand	0.35
75	Coagulation Factor III	0.48
357	MMP-10	0.42
259	IL-10	0.09
20	APJ	0.35
89	CXCR4 (fusin)	0.46
8	Activin RIIA	0.45
125	FADD	0.38
339	M-CSF R	0.39
264	IL-12 p70	0.48
10	AgRP	0.46
168	GDF1	0.10
243	IL-2	0.04
28	BD-1	0.49
199	HCR/CRAM-A/B	0.35
313	Kremen-2	0.33
1	6Ckine	0.40
263	IL-12 p40	0.13
244	IL-2 R alpha	0.23
31	BAX	0.37
123	E-Selectin	0.03
3	Activin B	0.41
309	IP-10	0.50
127	Fas/TNFRSF6	0.09
257	IL-8	0.05
7	Activin RII A/B	0.25
481	TRAIL R1/DR4	0.14
90	CXCR5/BLR-1	0.42
105	EDG-1	0.32
124	Endothelin	0.47
4	Activin C	0.19
271	IL-15 R alpha	0.09
187	Glypican 3	0.46
135	FGF-5	0.11
167	G-CSF R/CD 114	0.10
255	IL-7	0.09

Table Continued

Table I *(Continued)*. Significantly differentially expressed protein factors (105 species, para-cancerous tissue/cancer tissue) associated with GC.

No. of the protein point	Protein factors	Fold
208	ICAM-1	0.26
88	CXCR3	0.08
473	TMEFF1	2.07
38	BMP-4	0.50
327	LRP-6	0.08
348	MIP 2	0.12
166	GCSF	0.04
355	MMP-8	0.21
66	CD 163	0.17
194	GRO-a	0.14
276	IL-17C	0.24
284	IL-18 R alpha /IL-1 R5	0.35
449	TGF-beta 2	0.44
195	Growth Hormone (GH)	0.17
160	Frizzled-6	0.28
283	IL-18 BPa	0.17
226	IGF-II R	0.38
420	SAA	0.09
344	MIG	0.10
409	Progranulin	0.34
227	IL-1 alpha	0.24
356	MMP-9	0.14
218	IGFBP-2	0.28
171	GDF8	0.09
247	IL-3	0.18
338	M-CSF	0.42
173	GDF11	0.29
36	BMP-3	0.19
193	GRO	0.09
288	IL-20 R alpha	0.17
232	IL-1 F8/FIL1 eta	0.36
41	BMP-7	0.50
106	EGF	0.29
54	CCR4	0.43
222	IGFBP-rp1/IGFBP-7	0.25
458	Thrombospondin-1	0.17
418	S100 A8/A9	0.47
329	Lipocalin-2	0.27
39	BMP-5	0.31
472	TLR4	0.21
464	TIMP-1	0.34
431	SLPI	0.46
103	EDA-A2	0.12
114	EN-RAGE	0.29
52	CCR2	0.37
170	GDF5	0.31
5	Activin RIA/ALK-2	0.25
294	IL-22 R	0.33
109	EMAP-II	0.44

PCR, as well as cell apoptosis, invasion and migration analysis, and animal models, inhibit proliferation, thus regulating the tumor development. By RT-PCR, Han et al[20] analyzed the GC and the adjacent non-tumor tissues and demonstrated that let-7b inhibited the invasion and metastasis of GC by regulating the expression of growth-family member 1 (ING1). Li et al[21] studied the expression of miR-25 in plasma and primary tumor tissues of lymph node metastasis and GC patients with lymph node metastasis; they found that miR-25 inhibited GC by down-regulating TOB1 expression. In this study, we used lentivirus vectors transfection to establish stable overexpression of miR-198 in GC cell lines. MTT assay, Transwell chamber, and wound scratch assay were also performed. The results showed that overexpression of miR-198 inhibited the proliferation, invasion, and migration of gastric cancer cells, while promoted apoptosis of GC cells.

With the development of genomics and proteomics, the development of protein microarrays together with the bioinformatics analysis made it easy to select miRNA potential target genes from a pool of genes. By marker-free mass spectrometry and bioinformatics, Korrodi-Gregório et al[22] studied the proteomic profile of two representative lung cancer cell lines and identified a variety of differentially expressed proteins, which was related with cancer transformation, tumor resistance, proliferation, and migration. Liebel et al[23] made a proteomic analysis of pre-treated human liver cancer cells (HepG2) and identified that G protein-coupled receptor (GPCR), heterologous nuclear ribonucleoprotein (hnRNP), and reactive oxygen species (ROS) may be related to cell proliferation and resistance. In the present study, high-throughput protein chip combined with bioinformatics analysis were also used to identify a total of 105 differentially expressed protein-related factors in GC tissues. Six of the up-regulated expression factors were miR-198 candidate targets; they included TLR4, growth differentiation factor 1 (5, 11, GDF1, 5, 11), human Fas-associated cell death domain protein (FADD), and epidermal growth factor (EGF). Then, the three databases [Target Scan (http://www.targetscan.org/), Pictar (http://pictar.mdc-berlin.de/) and Diana (http://diana.cslab.ece.ntua.gr/microT/)] were used to further analyze the high-throughput protein chip results. TLR4 were identified to be a potential target for miR-198 in GC tissue. The expression of TLR4 in GEC-1 and SGC-7901 cells was detected by qPCR and Western Blot. The results showed that the expression of TLR4 in SGC-7901 cells was significantly higher than that in SGC-7901 cells transfected with

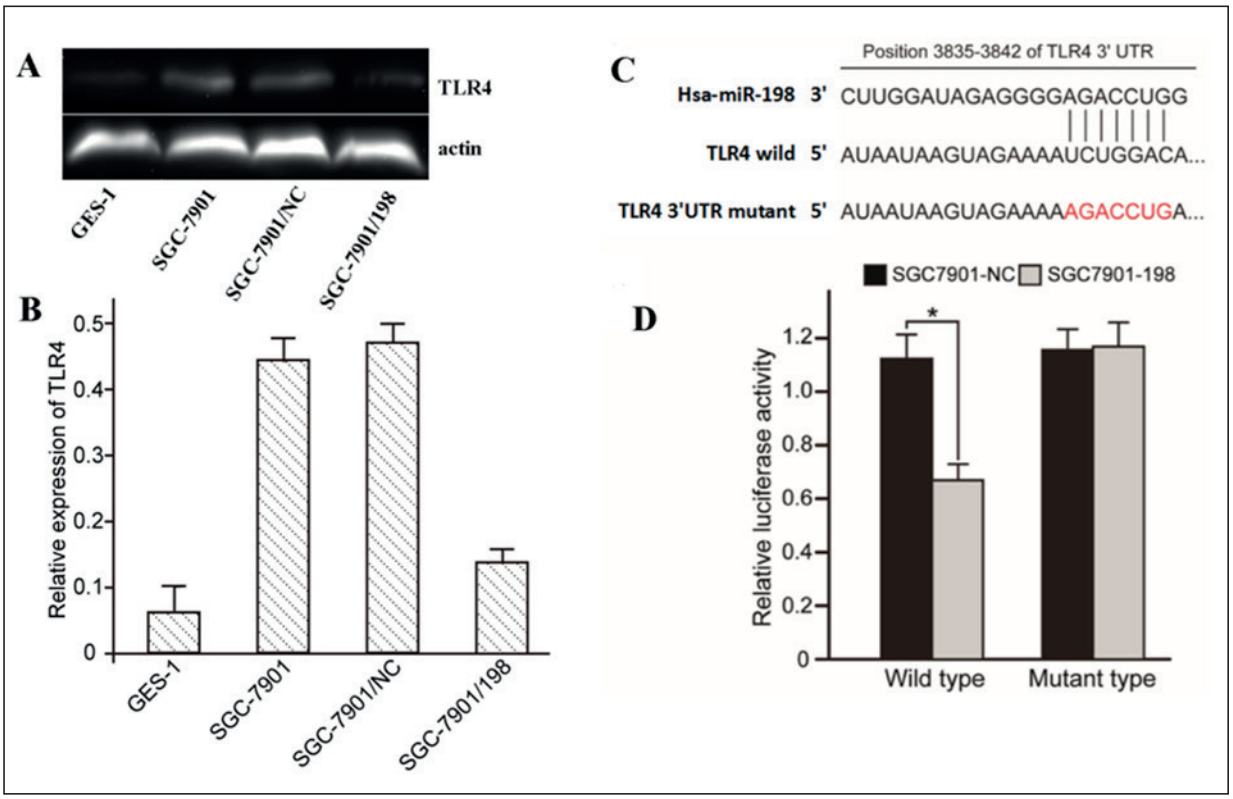

Figure 4. Confirmation of miR-198 targeted gene TLR4. ***A,*** Western blot for TLR4 detected in GC cells. ***B,*** The relative mRNA expression levels of TLR4. ***C,*** The 3'UTR of TLR4 targeted by miR-198 and the mutant types. ***D,*** The luciferase intensity of TLR4 wild-type and the mutant TLR4 type detected in GC cells.

miR-198, indicating that miR-198 overexpression could inhibit the expression of TLR4. By luciferase assays and Western blotting, Gao et al[24] have demonstrated that miR-145 regulated the migration of GC cells by inhibiting the expression of the N-cadherin (CDH2) gene. Zhang et al[17] reported that the mechanism of miR-27 promoted migration, while decreased metastasis of GC through luciferase reporter assay and Western blot. Yang et al[18] identified miR-214 to be the regulator of the proliferation, differentiation, and infiltration of GC cells by targeting the expression of PTEN through qRT-PCRT, luciferase reporter assay, and Western blot. In our investigation, the luciferase reporter assay was also used to confirm that TLR4 was the target of miR-198. Based on the results of bioinformatic analysis, the 3'UTL of TLR4 (the targeted site of miR-198 on TLR4) was mutated, and the fluorescence intensity of GES-1 and SGC-7901 GC cells transfected with wild-type TLR4 and mutant TLR4 vectors were detected, which further confirmed that TLR4 was the target gene of miR-98. Our findings showed that the overexpression of miR-198 significantly reduced the proliferation, migration, and invasion of GC cells, while promoted the apoptosis of GC cells. TLR4 was identified to be the target of miR-198 in GC. miR-198 overexpression significantly reduced the expression of TLR4 in GC cells. Therefore, miR-198 had the effect of inhibiting the development of GC, which was achieved by down-regulating the expression of TLR4.

Conclusions

This study aimed to provide an effective biomarker for identifying miR-198 target genes, as well as to elucidate the molecular mechanism of miR-198 in the development and progression of GC. It provided new biomarkers and drug targets for GC diagnosis and treatment, and also an effective and reliable experimental data for gene therapy of GC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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