Pathology - Research and Practice 215 (2019) 152511

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

miR-199a-3p targets ETNK1 to promote invasion and migration in gastric cancer cells and is associated with poor prognosis

Li Li[a,c], Yi-Ping Mou[b,c], Yuan-Yu Wang[b,c,*], Hui-Ju Wang[a,c], Xiao-Zhou Mou[a,c]

[a] Clinical Research Institute, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, 310014, Zhejiang, China
[b] Departments of Gastrointestinal and Pancreatic Surgery, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, 310014, PR China
[c] Key Laboratory of Gastroenterology of Zhejiang Province, Hangzhou, 310014, PR China

ARTICLE INFO

Keywords:
Gastric cancer
miR-199a-3p
ETNK1
Invasion
Migration
Prognosis

ABSTRACT

Purpose: To investigate the prognostic significance of miR-199a-3p and its role in invasion and metastasis in gastric cancer.

Methods: miR-199a-3p expression in 436 formalin-fixed and 39 frozen gastric cancer tissues was investigated by in situ hybridization and RT-PCR, respectively. The role of miR-199a-3p in the migration and invasion of gastric cancer cells was determined in overexpression and inhibitor studies using transwell assays and the SGC-7901, BGC-823 and MGC-803 gastric cancer cells lines. The effect of miR-199a-3p expression on ethanolamine kinase 1 (ETNK1) levels was determined by western botting.

Results: miR-199a-3p was significantly up-regulated in AGS, SGC-7901, BGC-823 and MGC-803 gastric cancer cells, when compared with GES-1 non-malignant gastric epithelial cells. In situ hybridization studies revealed that human non-tumor gastric mucosa samples were negative for miR-199a-3p expression, while 162 of 436 (37.16%) cases of gastric cancer demonstrated positive expression. miR-199a-3p overexpression was associated with tumor size, Lauren classification, depth of invasion, lymph node and distant metastasis, TNM stage and prognosis. In patients with I, II and III stage tumors, high miR-199a-3p expression was associated with a significantly lower 5-year survival rate. miR-199a-3p overexpression was associated with increased cell migration and invasion. ETNK1 expression was inhibited following miR-199a-3p overexpression in BGC-823 and SGC-7901 cells, and elevated following miR-199a-3p suppression in MGC-803 cells.

Conclusion: miR-199a-3p is highly expressed in gastric cancer, and correlates with invasion, metastasis and prognosis. miR-199a-3p regulates the invasion and migration of gastric cancer cells by targeting ETNK1. Consequently, miR-199a-3p may serve as a prognostic indicator in gastric cancer.

1. Introduction

The mainstay of treatment for gastric cancer is surgery. However, in stage II (excluding T1 disease) and III (moderately advanced) disease, an appreciable proportion of patients have recurrence, even after curative resection [1]. Thus, there is an urgent need to identify new molecular markers for early diagnosis, and prediction of progression and metastasis in patients with gastric cancer. Several genetic alterations have been identified as carcinogenic changes involved in the development of cancer. These changes include the activation of proto-oncogenes, the inactivation of tumor suppressor genes, reduced expression or loss of the cell adhesion molecule E-cadherin, telomerase reactivation, and microsatellite instability [2]. Nonetheless, the precise molecular mechanisms driving tumorigenesis and cancer progression remain largely unknown.

MicroRNAs (miRNAs) are a family of 21–25 nucleotide, noncoding small RNAs that influence gene expression by regulating mRNA translation and degradation [3]. miRNAs play a role in the promotion and prevention of cancer by modulating the expression of their downstream mRNA targets [4]. miR-199a-3p expression has been reported to be elevated in head and neck cancers, and is higher in head and neck adenoid cystic carcinoma when compared with head and neck squamous cell carcinoma [5]. Microarray data have shown that miR-199a-3p is highly expressed in gastric cancer [6]. Plasma levels of miR-199a-3p have been shown to be significantly elevated in osteosarcoma patients [7]. miR-199a-3p has also be shown to be upregulated in gastric cancer cell lines and patient tissues [8,9].

ETNK1 encodes an ethanolamine kinase, which functions in the first

* Corresponding author at: Key Laboratory of Gastroenterology of Zhejiang Province, Hangzhou. No. 158 Shangtang Road, Hangzhou, Zhejiang, 310014, PR China.
E-mail address: lywyy1979@126.com (Y.-Y. Wang).

https://doi.org/10.1016/j.prp.2019.152511
Received 9 April 2019; Received in revised form 28 May 2019; Accepted 20 June 2019
0344-0338/

committed step of the phosphatidylethanolamine synthesis pathway. This cytosolic enzyme is specific for ethanolamine and exhibits negligible kinase activity on choline. microRNAs contribute to the response of larynx and tongue-derived squamous cell carcinoma (SCC) cells to platinum chemotherapy by regulating the levels of ETNK1 [10]. ETNK1 also related with chemosensitivity of gastric cancer cells [11].

While changes in miR-199a-3p expression have been observed in a number of cancer types, its role in gastric cancer progression and prognosis has not been explored. We have previous shown that miR-199a-3p is highly expressed in gastric cancer [9]. In this study we aimed to elucidate the role of miR-199a-3p in gastric cancer invasion and metastasis, and to determine its potential utility in the prognosis of this disease.

2. Materials and methods

2.1. Frozen gastric cancer tissues

Gastric cancer tissues were obtained from the Zhejiang Provincial People's Hospital between February 2013 and October 2013. After surgical removal, tissues were immediately frozen in liquid nitrogen and stored at −80 °C until use. Of the 39 samples collected, two were categorized as stage I, seven as stage II, 26 as stage III and four as stage IV tumors, according to the 2010 AJCC histological classification of gastric carcinoma. The clinicopathological characteristics of the gastric cancer patients are summarized in Table 1. All patients provided informed consent for the use of their tissues prior to surgery. The study was approved and monitored by the ethics committee of Zhejiang Provincial People's Hospital(KT2017030).

2.2. Archived gastric cancer samples

Gastric cancer tissues were obtained from gastrectomy specimens from 436 patients who underwent surgery at the Department of Surgery, Zhejiang Provincial People's Hospital, between January 1998 and January 2004. Tissues were formalin-fixed, paraffin-embedded, and diagnosed clinically and histopathologically at the Departments of Gastrointestinal Surgery and Pathology. The follow-up deadline was December 2008. The survival time was calculated from the date of surgery to the follow-up deadline or date of death as a consequence of carcinoma recurrence or metastasis. Ninety-two non-cancerous human gastric tissues (distance from tumor site > 5 cm) were obtained from gastrectomies. Routine chemotherapy was given to all patients with advanced-stage disease after surgery, but no radiation treatment was administered to any patients included in our study. The study was approved and monitored by the ethics committee of Zhejiang Provincial People's Hospital.

Table 1
Summary of clinicopathologic characteristics of gastric cancer patients.

Clinical parameters	N (%)
Gender	
Men	25 (64.10%)
women	14 (35.90%)
Location	
Proximal	10 (25.64%)
Middle	12 (30.77%)
Distal	17 (43.59%)
Size of tumor	
<5cm	21 (53.85%)
≥5cm	18 (46.15%)
Histologic differentiation	
Moderately	13 (33.33%)
Poorly	19 (48.72%)
undifferentiation	7 (17.95%)
Lymphatic metastasis	
No	15 (38.46%)
Yes	24 (61.45%)
TNM Stages	
I	2 (5.13%)
II	7 (17.93%)
III	26 (66.7%)
IV	4 (10.26%)
Distant metastasis	
No	36 (92.31%)
Yes	3 (7.69%)

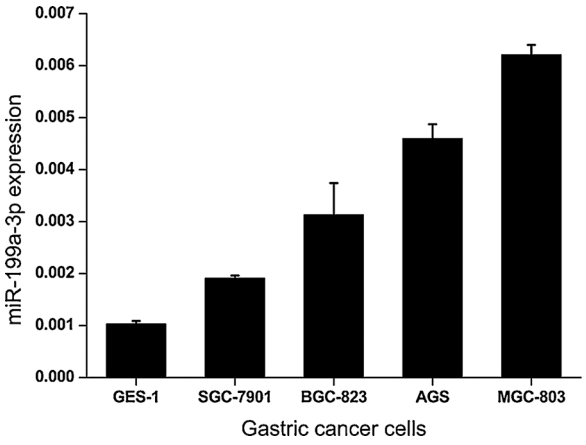

Fig. 1. miR-199a-3p was significantly upregulated in AGS, BGC-823, MGC-803, and SGC-7901 cells compared with the non-malignant gastric epithelial cell line GES-1 ($P < 0.05$).

2.3. Cell culture

The human gastric cancer cell lines AGS, BGC-823, SGC-7901 and MGC-803, and the non-malignant gastric epithelial cell line GES-1 were obtained from the Key Laboratory of Gastroenterology of Zhejiang Province (Hangzhou, China), and were cultured in RPMI1640 medium containing 10% fetal bovine serum (FBS), 50 U/ml penicillin and 50 μg/ml streptomycin. All cells were maintained at 37 °C in a humidified incubator under an atmosphere of 5% CO_2.

2.4. RT-PCR

RT-PCR was used to determine miR-199a-3p expression in cultured cells and frozen gastric cancer tissues. Total RNA was extracted from the AGS, BGC-823, MGC-803 and SGC-7901 human gastric cancer cell lines, the GES-1 non-malignant gastric epithelial cell line, and from gastric cancer tissue using Trizol (Invitrogen) according to the manufacturer's instructions. cDNA synthesis was conducted using the miScript Reverse Transcription Kit using the manufacturer's reverse primers for miR-199a-3p and U6 (Qiagen). The resulting cDNA was amplified using QuantiTect SYBR Green PCR Master Mix (Qiagen) and reactions were performed on an ABI 7500 FAST Real-time PCR system (Applied Biosystems). Forward and reverse primer sequences for miR-199a-3p amplification were CTTTCTAGAAACTGGAGGCCCAG and TGGTGTCTAGACATGGCTACACTTTATAC, respectively. Forward and reverse primer sequences for U6 amplification were CTCGCTTCGGCA GCACA and AACGCTTCACGAATTTGCGT, respectively. PCR parameters were as follows: 95 °C for 15 min, followed by 40 cycles of 94 °C for 15 s, 55 °C for 30 s, and 72 °C for 34 s. Melting curve analysis was performed subsequent to thermocycling. The expression of miR-199a-3p in cancer tissues was compared with that of matched normal samples using the $2^{-\triangle\triangle CT}$ method, while the expression of miR-199a-3p in gastric cancer cells was compared with that of GES-1 cells.

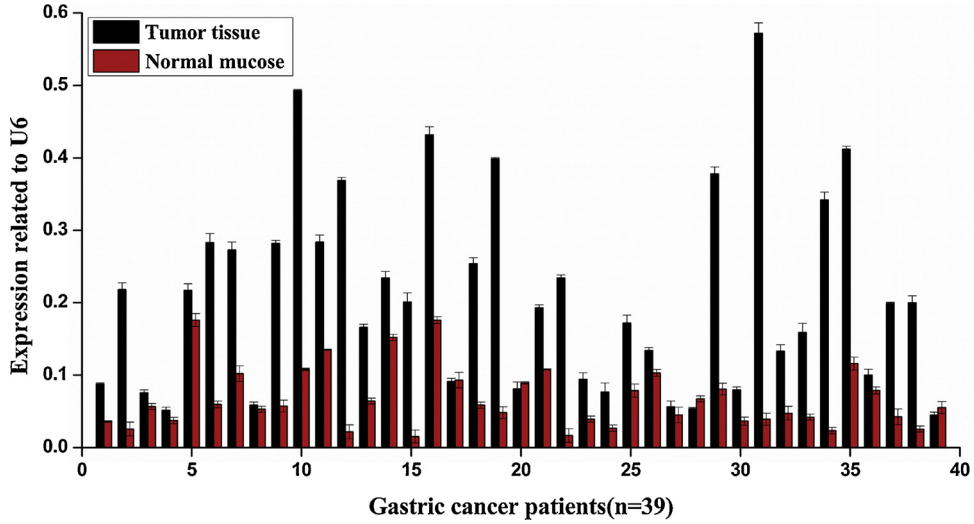

Fig. 2. miR-199a-3p were up-regulated in gastric cancer tissues,compared with non-tumor mucosa ($P < 0.05$).

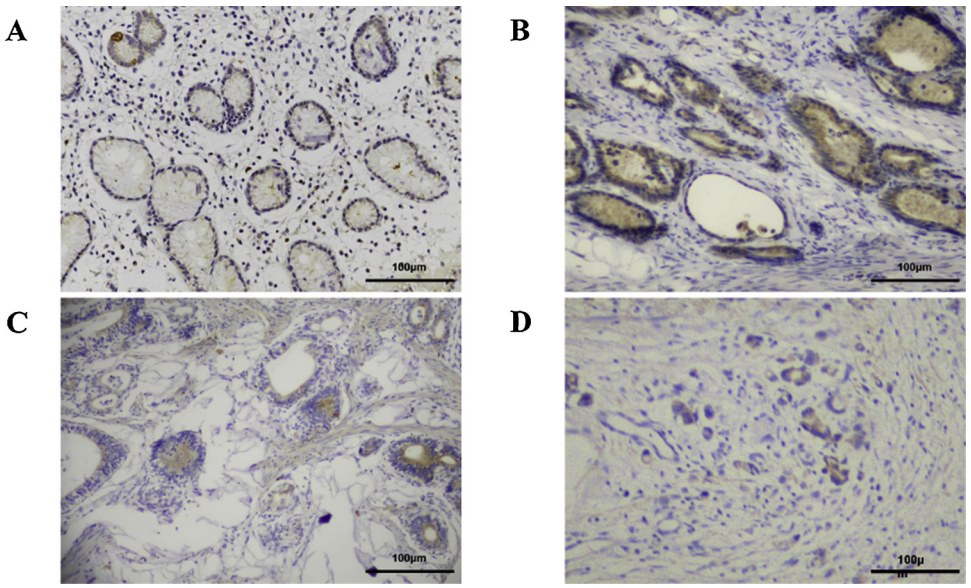

Fig. 3. ISH analysis of miR-199a-3p expression in gastric cancer tissue and non-cancerous human gastric mucosa, magnificationx200. **A:** miR-199a-3p was negative in noncancerous gastric mucosa ; **B, C:** miR-199a-3p was highly expressed in moderately differentiated adenocarcinoma ; **D:** miR-199a-3p washighly expressed in poorly differentiated adenocarcinoma.

2.5. In situ detection of miR-199a-3p

In situ hybridization was performed using a sensitivity-enhanced in situ hybridization kit according to manufacturer's instructions (MK1030; Boster Biological Technology, China). A 3′-digoxin-labelled miR-199a-3p probe was used to detect cytoplasmic miR-199a-3p in formalin-fixed, paraffin-embedded tissue specimens (sequence: CACA AATTCGGTTCTACAGGGTA; miRCURY LNA detection probe, Exiqon, Denmark). Specimens were reviewed by two independent observers who scored samples based on the proportion of positively stained tumor cells and the intensity of cell staining [12,13]. The cutoff value for high and low expression was chosen based on a measure of heterogeneity using the log-rank test with respect to overall survival. An optimal cutoff value was identified where a staining index score of ≥ 4 was used to define tumors with high miR-199a-3p expression, and a score of ≤ 3 to define tumors with low miR-199a-3p expression.

2.6. miR-199a-3p transfection

Control (mirna-nc) and hsa-mir-199a-3p expression plasmids, as well plasmids encoding control and hsa-mir-199a-3p-specific sponge inhibitors (mir-sponge-nc and hsa-mir-199a-3p-sponge, respectively) were purchased from Invitrogen (Carlsbad, CA, USA). SGC-7901, BGC-823 and MGC-803 cells were seeded in six-well dishes at a density of 2.0×10^5 cells per well 24 h prior to transfection. Transfection was performed with Lipofectamine 2000 (Invitrogen). Twenty-four hours after transfection, cells were assayed for migration, and invasion.

2.7. In vitro cell migration and invasion assays

Transwell chambers with 8-μm diameter membrane pores were used to evaluate cell migration and invasion. Twenty-four hours after transfection, cells were harvested by trypsinization and seeded in triplicate into the top wells of transwell chamber inserts (Millicell Hanging Cell Culture Inserts, PIEP12R48, Millipore Corporation). Cells were seeded at a density of 1.0×10^5 cells per chamber in a final volume of 200 μl serum-free RPMI1640 media. The chamber inserts were then placed into the 24-well transwell plate containing RPMI1640 media supplemented with 30% FBS as a chemo-attractant. After a 24 h incubation, cells remaining on the upper side of the chamber were removed with a cotton swab, and cells on the lower surface of the membrane were fixed in 100% methanol for 15 min, followed by staining with Giemsa solution. Cells in five random fields (100× magnification) per chamber were counted and the average cell number

Table 2
Relationship of miR-199a-3p expression with pathological parameters of tumor (number/%).

Clinical parameters	miR-199a-3p			
	Low	High	t/χ^2	*P*
Age(yrs)	57.35 ± 11.44	61.93 ± 12.72	3.877	< 0.01
Gender			0.314	0.576
Male	198(63.7%)	113(36.3%)		
Female	76(60.8%)	49(39.2%)		
Location			6.101	0.047
Proximal	27(49.1%)	28(50.9%)		
Middle	101(62.0%)	62(38.0%)		
Distal	146(67.0%)	72(33.0%)		
Size			14.812	< 0.01
< 5cm	180(70.3%)	76(29.7%)		
≥5cm	94(52.2%)	86(47.8%)		
Lauren classification			131.593	< 0.01
Intestinal	198(88.8%)	25(11.2%)		
Diffuse	76(35.7%)	137(64.3%)		
Histology classification			2.059	0.560
Papillary adenocarcinoma	9(56.2%)	7(43.8%)		
Tubular adenocarcinoma	209(64.1%)	117(35.9%)		
Mucinous adenocarcinoma	15(51.7%)	14(48.3%)		
Signet-ring cell carcinoma	41(63.1%)	24(36.9%)		
Histologic differentiation			3.971	0.265
Well	11(84.6%)	2(15.4%)		
Moderately	80(62.5%)	48(37.5%)		
Poorly	181(61.8%)	112(38.2%)		
Others	2(100.0%)	0(0%)		
Invasion depth			49.704	< 0.01
T1	53(93.0%)	4(7.0%)		
T2	83(76.1%)	26(23.9%)		
T3	129(52.9%)	115(47.1%)		
T4	9(34.6%)	17(65.4%)		
TNM Stages			130.402	< 0.01
I	85(94.4%)	5(5.6%)		
II	89(85.6%)	15(14.4%)		
III	87(50.3%)	86(49.7%)		
IV	13(18.8%)	56(81.2%)		
Lymphatic metastasis			68.938	< 0.01
No	145(87.3%)	21(12.7%)		
Yes	129(47.8%)	141(52.2%)		
Regional lymph nodes			93.712	< 0.01
PN0	145(87.3%)	21(12.7%)		
PN1	83(61.0%)	53(39.0%)		
PN2	39(39.4%)	60(60.6%)		
PN3	7(20.0%)	28(80.0%)		
Distant metastasis			60.991	< 0.01
No	263(70.1%)	112(29.9%)		
Yes	11(18.0%)	50(82.0%)		

Table 3
The expression of miR-199a-3p in gastric cancer and 5-year survival rates.

	Low expression of miR-199a-3p	High expression of miR-199a-3p	χ2	P
stage I	87.1%	60.0%	6.605	0.01
stage II	67.4%	33.3%	22.086	0.001
stage III	31.0%	8.1%	38.596	0.001
stage IV	7.7%	1.8%	4.12	0.042

then determined. For invasion assays, transfected cells (2.0×10^5) were seeded into the top well of the chamber, and the bottom well then filled with conditioned medium. After a 48 h incubation, cells that had migrated onto the lower side of the membrane were counted as described above.

2.8. Western blot analysis

Whole cell protein lysates were extracted and protein concentrations then quantified using a BCA protein assay kit (Pierce, Rockford, IL, USA). Western blotting was performed according to standard procedures. Rabbit polyclonal anti-ethanolamine kinase 1 (ETNK1) antibody was from Abcam (USA), and rabbit monoclonal anti-GAPDH antibody was from Cell Signaling Technology (Danvers, MA, USA). GAPDH served as the loading control.

2.9. Statistical analysis

All statistical analyses were performed using SPSS 19.0 software (Chicago, IL, USA). Data were analyzed using the Student's t test, while categorical data were analyzed using the chi-square or Fisher exact test. Survival curves were estimated using the Kaplan–Meier method, and the log-rank test was used to calculate differences between the curves. Multivariate analysis using the Cox proportional hazards regression model was performed to assess the prognostic value of miR-199a-3p expression. Correlation coefficients between miR-199a-3p expression and clinicopathological findings were estimated using the Pearson correlation method. Statistical significance was set at $P < 0.05$.

3. Results

3.1. miR-199a-3p is upregulated in gastric cancer cell lines and frozen gastric cancer tissues

RT-PCR analysis revealed that miR-199a-3p was significantly up-regulated in AGS, BGC-823, MGC-803, and SGC-7901 cells, when compared with GES-1 non-malignant gastric epithelial cells ($P < 0.05$; Fig. 1). Analysis of 39 frozen primary gastric cancer tissues revealed that miR-199a-3p was up-regulated in 30 (76.9%) cases (Fig. 2).

3.2. miR-199a-3p expression is associated with clinicopathological features in gastric cancer

Expression of miR-199a-3p in 436 formalin-fixed cancer tissues and 92 non-tumor mucosa samples was investigated by in situ hybridization. miR-199a-3p expression was not detected in non-tumor mucosa samples (Fig. 3A). High levels of miR-199a-3p expression were detected in 162 (37.16%) tumors, and low levels of expression were detected in 274 (62.84%) tumors. miR-199a-3p was mainly localized in the cytoplasm of gastric cancer cells (Fig. 3B–D). High miR-199a-3p expression correlated with age, tumor location and size, Lauren classification, depth of invasion, lymph node and distant metastasis, and TNM stage ($P < 0.05$), but was not associated with gender, differentiation, and histological type (Table 2 and Table 3).

3.3. miR-199a-3p expression is associated with poor prognosis in gastric cancer

We also analyzed the relationship between miR-199a-3p expression and prognosis (Fig. 4, Table 4). For stage I, II and III disease, the 5-year survival rates of patients with high miR-199a-3p expression were significantly lower than those of patients with low miR-199a-3p expression. In stage IV disease there was no significant association between miR-199a-3p expression and the 5-year survival rate. Factors with possible prognostic effects in gastric carcinoma were analyzed by Cox regression analysis. Lauren classification ($P = 0.012$), distant metastases ($P = 0.009$), TNM stage ($P = 0.001$), and miR-199a-3p expression ($P = 0.008$) were independent prognostic factors in patients with gastric carcinoma (Table 4). However, age, sex, tumor location and size, histological classification, tumor differentiation, and regional lymph node stage had no prognostic value.

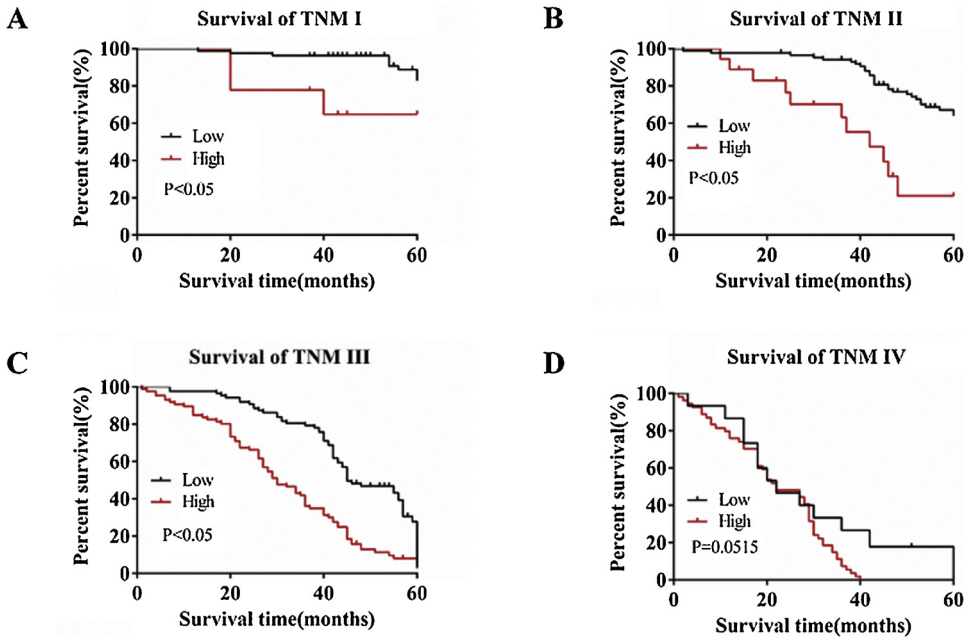

Fig. 4. Kaplan-Meier curves with univariate analyses (log-rank) for patients with low miR-199a-3p expression versus high miR-199a-3p expression.

Table 4
Multivariate analysis as determined by Cox regression analysis.

	95% confidential interval		B	Hazard ratio	p
	Lower	Upper			
Lauren classification	1.675	3.108	0.835	1.604	0.012
Distant metastasis	1.260	2.665	0.780	1.717	0.009
TNM Stages	1.166	2.308	0.458	2.634	0.001
miR-199a-3p expression	1.385	2.543	0.635	1.869	0.008

3.4. miR-199a-3p promotes gastric cancer cell migration and invasion in vitro

To study the biological role of miR-199a-3p in gastric cancer, we analyzed the effects of its altered expression on the migratory and invasive behavior of gastric cancer cell lines using miR-199a-3p overexpression and inhibitor sponge plasmids. In BGC-823 and SGC-7901 cells, overexpression of miR-199a-3p led to significantly increased migration and invasion, when compared with controls ($P < 0.05$; Fig. 5A & B). In MGC-803 cells, downregulation of miR-199a-3p expression following transfection with a miR-199a-3p sponge plasmid resulted in decreased invasion ($P < 0.05$; Fig. 5D). miR-199a-3p inhibition had no significant effect on the migration of MGC-803 cells (Fig. 5C).

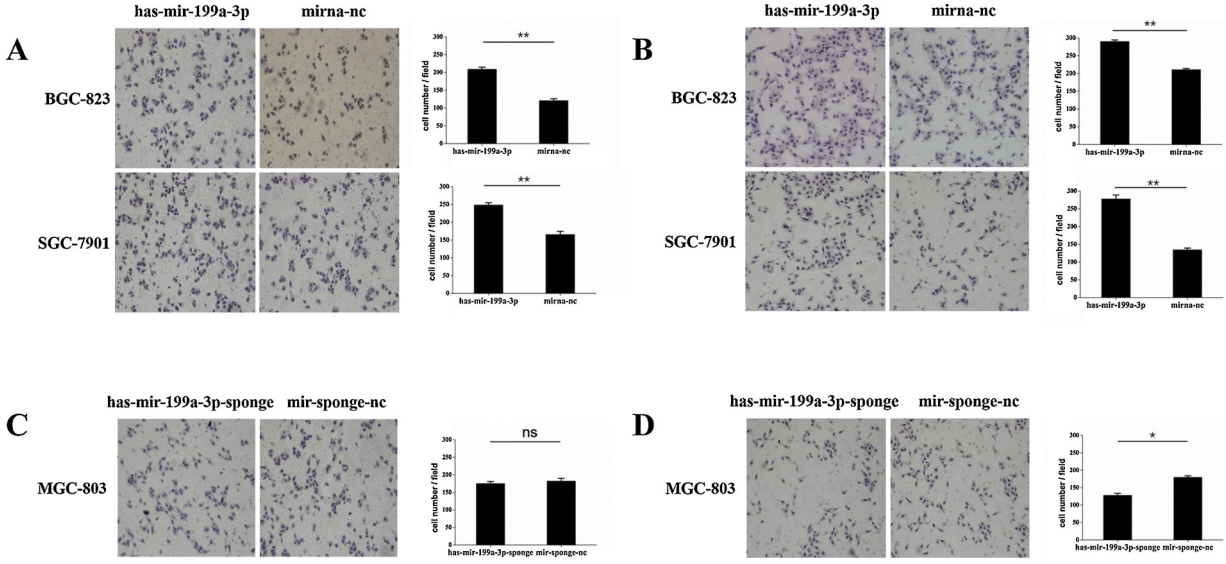

Fig. 5. 199a-3p promotes invasion and migration of gastric cancer cells, magnification, x100. **A**: High ectopic expression of miR-199a-3p led to significantly increased abilities of cell migration compared with the control cells ($P < 0.01$). **B**: High ectopic expression of miR-199a-3p led to significantly increased abilities of cell invasion compared with the control cells ($P < 0.01$). **C**: In MGC-803, there was no significant difference in migration of MGC-803 between the has-mir-199a-3p-sponge group and the mir-sponge-nc group.**D**: In MGC-803, downregulated miR-199a-3p expression decreased the invasion cells in the has-mir-199a-3p-sponge group compared with the mir-sponge-nc group ($P < 0.05$).

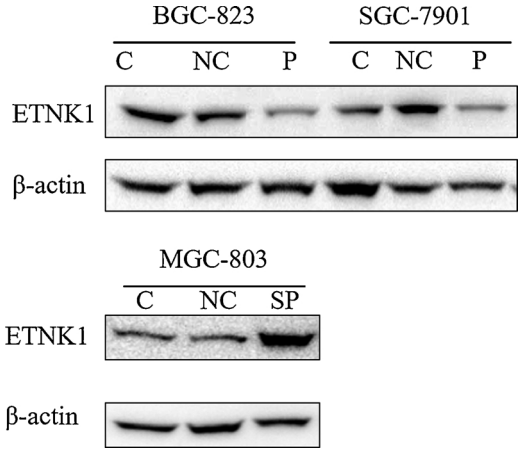

Fig. 6. miR-199a-3p and ETNK1 expression are inversely associated in gastric cancer cells.
A: Overexpression of miR-199a-3p in BGC-823 and SGC-7901 cells inhibited ETNK1 protein expression. **B:** Inhibition of miR-199a-3p enhanced ETNK1 protein expression in MGC-803 cells.

3.5. miR-199a-3p and ETNK1 expression are inversely associated in gastric cancer cells

miR-199a-3p and ETNK1 protein expression were inversely associated in gastric cancer cell lines. Overexpression of miR-199a-3p in BGC-823 and SGC-7901 cells inhibited ETNK1 protein expression, while inhibition of miR-199a-3p enhanced ETNK1 protein expression in MGC-803 cells(Fig. 6).

4. Discussion

Stomach cancer is the third leading cause of cancer death in both sexes worldwide [14]. Though the development of surgery and adjuvant chemotherapies has improved, the prognosis for gastric cancer remains poor. Therefore, developing novel and effective molecular markers for early diagnosis is essential for predicting the prognosis of gastric cancer patients.

miRNAs have been implicated in both the promotion and prevention of cancer by adjusting the expression of downstream target mRNAs. miR199a-3p expression has been reported to be elevated in colorectal cancer, and gastric cancer cell lines and patient tissues [8,15,16]. In this study, we have shown that miR-199a-3p was significantly upregulated in 77% of frozen and 37% of formalin-fixed gastric cancer tissues. miR-199a is highly expressed in the stroma of pancreatic tumors [17] but is significantly decreased in hepatocellular carcinoma, prostate cancer and ovarian cancer [18–20]. The methylation status of the miR-199a promoter may lead to differences in the expression of miR-199a-3p in different tumor tissues [20]. miR-199a-3p expression correlates with invasion, metastasis, and prognosis. It is significantly increased in osteosarcoma patients, and correlates with metastasis status and histological subtype [7]. High expression of miR-199a-3p is significantly associated with deep wall invasion in colorectal cancer [15], and contributes to more advanced lymphatic invasion, lymph node metastasis, liver metastases, late TNM stage and shorter overall survival in this disease [16]. miR-199a-3p expression was shown to be reduced in muscle-invasive bladder cancer samples, when compared with non-malignant tissue samples, and was associated with tumor and lymph node status [21]. The role of miR-199a-3p in gastric cancer progression and prognosis has not previously been explored. Our research has revealed that high levels of miR-199a-3p expression correlate with Lauren classification, depth of invasion, lymph node and distant metastasis, TNM stage and poor prognosis.

While miR-199a-3p expression has been reported to promote cell proliferation and suppress cell apoptosis in gastric cancer and colorectal cancer cells [8,16], it has been shown to suppress the proliferation and invasion of hepatocellular carcinoma and prostate cancer cells [19,22]. In this study, we sought to determine the role of miR-199a-3p in the migratory and invasive behavior of gastric cell lines. High miR-199a-3p expression resulted in a significant increase in the migration and invasion of gastric cancer cells, while inhibition of miR-199a-3p inhibited these processes.

We identified ETNK1 as a putative target of miR-199a-3p regulation following a search of the miRBase database, and subsequently investigated the relationship between miR-199a-3p and ETNK1 expression in gastric cancer cell lines. Our data show that ETNK1 and miR-199a-3p expression were inversely associated; overexpression of miR-199a-3p in BGC-823 and SGC-7901 cells inhibited ETNK1 protein expression, while suppression of miR-199a-3p in MGC-803 cells enhanced ETNK1 expression.

Our study shows that miR-199a-3p is highly expressed in gastric cancer, and is associated with invasion, metastasis and poor prognosis. In addition, our data suggest that miR-199a-3p regulates the invasion and migration of gastric cancer cells by targeting ETNK1. miR-199a-3p may serve as a prognostic indicator in gastric cancer.

Author contributions

YYW conceived and designed the experiments. LL and HJW performed the experiments. FH and YYW analyzed the data. LL provided reagents, materials and analysis tools. XZM and YYW wrote the manuscript.

Funding support

This study was supported by Zhejiang Provincial Medical Science Research Foundation (2013RCA005 and 2016KYA017 to YYW, and 2018KY270 to LL)

Acknowledgments

This study was supported by Zhejiang Provincial Medical Science Research Foundation (2013RCA005 and 2016KYA017 to YYW, and 2018KY270 to LL). We thank James Monypenny, PhD, from Liwen Bianji, Edanz Group China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

References

[1] M. Sasako, S. Sakuramoto, H. Katai, T. Kinoshita, H. Furukawa, T. Yamaguchi, A. Nashimoto, M. Fujii, T. Nakajima, Y. Ohashi, Five-year outcomes of a randomized phase III trial comparing adjuvant chemotherapy with S-1 versus surgery alone in stage II or III gastric cancer, J. Clin. Oncol. 29 (33) (2011) 4387–4393.
[2] I. Mizrahi, H. Mazeh, R. Grinbaum, N. Beglaibter, M. Wilschanski, V. Pavlov, M. Adileh, A. Stojadinovic, I. Avital, et al., Colon Cancer Associated Transcript-1 (CCAT1) expression in adenocarcinoma of the stomach, J. Cancer 6 (2) (2015) 105–110.
[3] G. Hutvágner, P.D. Zamore, A microRNA in a multiple-turnover RNAi enzyme complex, Science 297 (5589) (2002) 2056–2060.
[4] C.L. Bartels, G.J. Tsongalis, MicroRNAs: novel biomarkers for human cancer, Clin. Chem. 55 (4) (2009) 623–631.
[5] J.A. Veit, K. Scheckenbach, P.J. Schuler, S. Laban, S.P. Wiggenhauser, J. Thierauf, J.P. Klussmann, T.K. Hoffmann, MicroRNA expression in differentially metastasizing tumors of the head and neck: adenoid cystic versus squamous cell carcinoma, Anticancer Res. 35 (3) (2015) 1271–1277.
[6] V.Y. Shin, E.K. Ng, V.W. Chan, A. Kwong, K.M. Chu, A three-miRNA signature as promising non-invasive diagnostic marker for gastric cancer, Mol. Cancer 14 (1) (2015) 202.
[7] F. Lian, Y. Cui, C. Zhou, K. Gao, L. Wu, Identification of a plasma four-microRNA panel as potential noninvasive biomarker for osteosarcoma, PLoS One 10 (3) (2015) e0121499.
[8] Z. Wang, X. Ma, Q. Cai, X. Wang, B. Yu, Q. Cai, B. liu, Z. Zhu, C. Li, MiR-199a-3p promotes gastric cancer progression by targeting ZHX1, FEBS Lett. 588 (23) (2014) 4504–4512.
[9] Y.Y. Wang, Z.Y. Ye, Z.S. Zhao, L. Li, Y.X. Wang, H.Q. Tao, H.J. Wang, X.J. He, Clinicopathologic significance of miR-10b expression in gastric carcinoma, Hum.

Pathol. 44 (7) (2013) 1278–1285.
[10] E.A. Ratovitski, Delta Np63 alpha – responsive microRNA modulate the expression of metabolic enzymes, Curr. Pharm. Biotechnol. 16 (9) (2015) 832–850.
[11] H. Chang, S.Y. Rha, H.C. Jeung, J.J. Jung, T.S. Kim, H.J. Kwon, B.S. Kim, H.C. Chung, Identification of genes related to a synergistic effect of taxane and suberoylanilide hydroxamic acid combination treatment in gastric cancer cells, J. Cancer Res. Clin. Oncol. 136 (12) (2010) 1901–1913.
[12] L.B. Song, W.T. Liao, H.Q. Mai, H.Z. Zhang, L. Zhang, M.Z. Li, J.H. Hou, et al., The clinical significance of twist expression in nasopharyngeal carcinoma, Cancer Lett. 242 (2006) 258–265.
[13] Z.S. Zhao, Y.Y. Wang, Y.Q. Chu, Z.Y. Ye, H.Q. Tao, SPARC is associated with gastric cancer progression and poor survival of patients, Clin. Cancer Res. 16 (2010) 260–268.
[14] J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D.M. Parkin, D. Forman, F. Bray, Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012, Int. J. Cancer 136 (5) (2015) E359–86.
[15] R. Nonaka, J. Nishimura, Y. Kagawa, H. Osawa, J. Hasegawa, K. Murata, S. Okamura, H. Ota, M. Uemura, et al., Circulating miR-199a-3p as a novel serum biomarker for colorectal cancer, Oncol. Rep. 32 (6) (2014) 2354–2358.
[16] D. Wan, S. He, B. Xie, G. Xu, W. Gu, C. Shen, Y. Hu, X. Wang, Q. Zhi, L. Wang, Aberrant expression of miR-199a-3p and its clinical significance in colorectal cancers, Med. Oncol. 30 (1) (2013) 378.
[17] P.R. Kuninty, L. Bojmar, V. Tjomsland, M. Larsson, G. Storm, A. Östman, P. Sandström, J. Prakash, MicroRNA-199a and -214 as potential therapeutic targets in pancreatic stellate cells in pancreatic tumor, Oncotarget 7 (13) (2016) 16396–16408.
[18] C. Giovannini, F. Fornari, R. Dallo, M. Gagliardi, E. Nipoti, F. Vasuri, C.A. Coadă, M. Ravaioli, L. Bolondi, L. Gramantieri, MiR-199-3p replacement affects E-cadherin expression through Notch1 targeting in hepatocellular carcinoma, Acta Histochem. 120 (2) (2018) 95–102.
[19] F. Qu, J. Zheng, W. Gan, H. Lian, H. He, W. Li, T. Yuan, Y. Yang, X. Li, C. Ji, X. Yan, L. Xu, H. Guo, MiR-199a-3p suppresses proliferation and invasion of prostate cancer cells by targeting Smad1, Oncotarget. 8 (32) (2017) 52465–52473.
[20] Y. Deng, F. Zhao, L. Hui, X. Li, D. Zhang, W. Lin, Z. Chen, Y. Ning, Suppressing miR-199a-3p by promoter methylation contributes to tumor aggressiveness and cisplatin resistance of ovarian cancer through promoting DDR1 expression, J. Ovarian Res. 10 (1) (2017) 50.
[21] T.H. Ecke, K. Stier, S. Weickmann, Z. Zhao, L. Buckendahl, C. Stephan, E. Kilic, K. Jung, miR-199a-3p and miR-214-3p improve the overall survival prediction of muscle-invasive bladder cancer patients after radical cystectomy, Cancer Med. 6 (10) (2017) 2252–2262.
[22] A. Ghosh, D. Dasgupta, A. Ghosh, S. Roychoudhury, D. Kumar, M. Gorain, R. Butti, S. Datta, S. Agarwal, S. Gupta, G. Krishna Dhali, A. Chowdhury, T.D. Schmittgen, G.C. Kundu, S. Banerjee, MiRNA199a-3p suppresses tumor growth, migration, invasion and angiogenesis in hepatocellular carcinoma by targeting VEGFA, VEGFR1, VEGFR2, HGF and MMP2, Cell Death Dis. 8 (3) (2017) e2706.