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MiR-129-5p functions as a tumor suppressor in gastric cancer progression through targeting ADAM9

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ABSTRACT

MicroRNAs (miRNAs) are identified as key regulators in cancer initiation, progression and metastasis including gastric cancer (GC). The aim of the study is to explore clinical significance and potential mechanism of miR-129-5p in GC development. In the study, our results found that miR-129-5p expression was significantly down-regulated in GC tissues, compared with adjacent normal tissues using qRT-PCR analyses. Furthermore, lower miR-129-5p expression closely associated with tumor size and lymph node invasion and poor prognosis of GC patients. Using CCK8 assay, cell colony formation, transwell invasion assay, we demonstrated that miR-129-5p overexpression reduced cell proliferation, cell colony formation and cell invasion capacity in MKN45 (higher miR-129-5p expression) and SGC-7901 (lower miR-129-5p expression). However, downregulation of miR-129-5p had reverse effects on cell proliferation and invasion. Targeting association analysis, dual luciferase assay, qRT-PCR and western blot analysis results verified that miR-129-5p could target the 3′UTR of ADAM9 mRNA and regulated its protein expression. Furthermore, we confirmed that miR-129-5p suppressed cell proliferation and invasion ability through regulating ADAM9. In vivo, upregulation of miR-129-5p also inhibited tumor growth. Therefore, these results indicated that miR-129-5p functioned as a tumor suppressor in GC and may be a potential target of GC treatment.

1. Introduction

Gastric cancer (GC) imposes a global health burden and ranks the second most common cause of cancer-associated death worldwide. In 2012, approximately 951,600 new GC cases occur and 723,100 cases die from gastric cancer [1,2]. Surgical resection is the main method of gastric cancer treatment. However, recurrence and tumor metastasis cause poor survival time for advanced stage GC patients after standard adjuvant treatment [3,4]. Thus, to explore novel therapeutic methods for GC patients is important.

MicroRNAs (miRNAs) are small non-coding RNA and exert post-transcriptional regulation by suppressing or degrading the translation of its targeted mRNA [5,6]. According to recent reporters, some miRNAs are involved in GC development and progression by affecting their downregulating targets [7]. For instance, miR-520c enhances cell proliferation, migration, and invasion by suppressing IRF2 in gastric cancer [8]. MiR-186 affects the cell proliferation, invasion and migration of human gastric cancer by inhibition of Twist1 [9]. MiR-137 plays tumor suppressor role in gastric cancer cell lines by targeting KLF12 and MYO1C [10]. These studies indicated miRNAs act as key regulators of GC development.

MiR-129-5p has been reported to act as a tumor suppressor in some tumors including GC. For example, down-regulation of miR-129-5p inhibits growth and induces apoptosis in laryngeal squamous cell carcinoma by targeting APC [11]. In GC, miR-129-5p is down-regulated and involved in migration and invasion of gastric cancer cells by targeting interleukin-8 [12]. MiR-129-5p suppresses gastric cancer cell invasion and proliferation by inhibiting COL1 A1 [13]. However, the underlying role of miR-129-5p in GC still needs to be explored.

In the study, we demonstrated that miR-129-5p expression levels were downregulated in GC tissues and cells. Lower miR-129-5p expression associated with poor prognosis of GC patients. Furthermore, miR-129-5p overexpression reduced cell proliferation and invasion capacities in vitro. In addition, we demonstrated that miR-129-5p inhibited cell proliferation and invasion through targeting ADAM9. In vivo, miR-129-5p also inhibited tumor growth. Thus, these findings indicated that miR-129-5p functioned as a tumor suppressor in GC and may be a potential target of GC treatment.

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Table 1
Correlation between miR-129-5p expression and clinicopathologic factors were shown.

Clinicopathologic factors	Number of patients	Lower expression (n = 26)	Higher expression (n = 24)	P-value
Age (years)				0.355
≤55	32	14	16	
> 55	18	12	8	
Gender				0.488
Male	33	16	17	
Female	17	10	7	
Tumor size				0.009*
< 3cm	28	10	18	
> 3cm	22	16	6	
Local invasion				0.355
T1,T2	30	14	16	
T3,T4	20	12	8	
Differentiation				0.860
High and middle	36	19	17	
Poor	14	7	7	
Lymph node metastasis				0.019 *
Negative	29	11	18	
Positive	21	15	6	
TNM stage				0.233
I/II	29	13	16	
III/IV	21	13	8	

* P < 0.05.

2. Materials and methods

2.1. Patient tissue samples

A total of 50 human GC tissue samples and adjacent normal tissue samples were obtained from patients who radical resection at Department of Gastrointestinal Surgery, The First Affiliated Hospital of Zhengzhou University. The clinical data was showed in Table 1.The tissue samples were collected and were rapidly frozen in liquid nitrogen and stored at −80 °C until RNA analysis. The overall survival time was calculated from the day of the first operation to death or the last day of follow-up. Written inform consent and the study was approved by the First Affiliated Hospital of Zhengzhou University.

2.2. Cell line culture

Three human gastric cancer cell lines (MKN-45, BGC-823 and SGC-7901) and the normal gastric mucosal cell line GES-1 cell were purchased from the Chinese Academy of Science Cell Bank (Shanghai, China). All cells was cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO, Carlsbad, CA, USA), and supplemented with 10% fetal bovine serum (GIBCO, Carlsbad, CA, USA), streptomycin (100 μg/ml), and penicillin (100 U/ml) at 37 °C with 5% CO_2 in a humidified incubator.

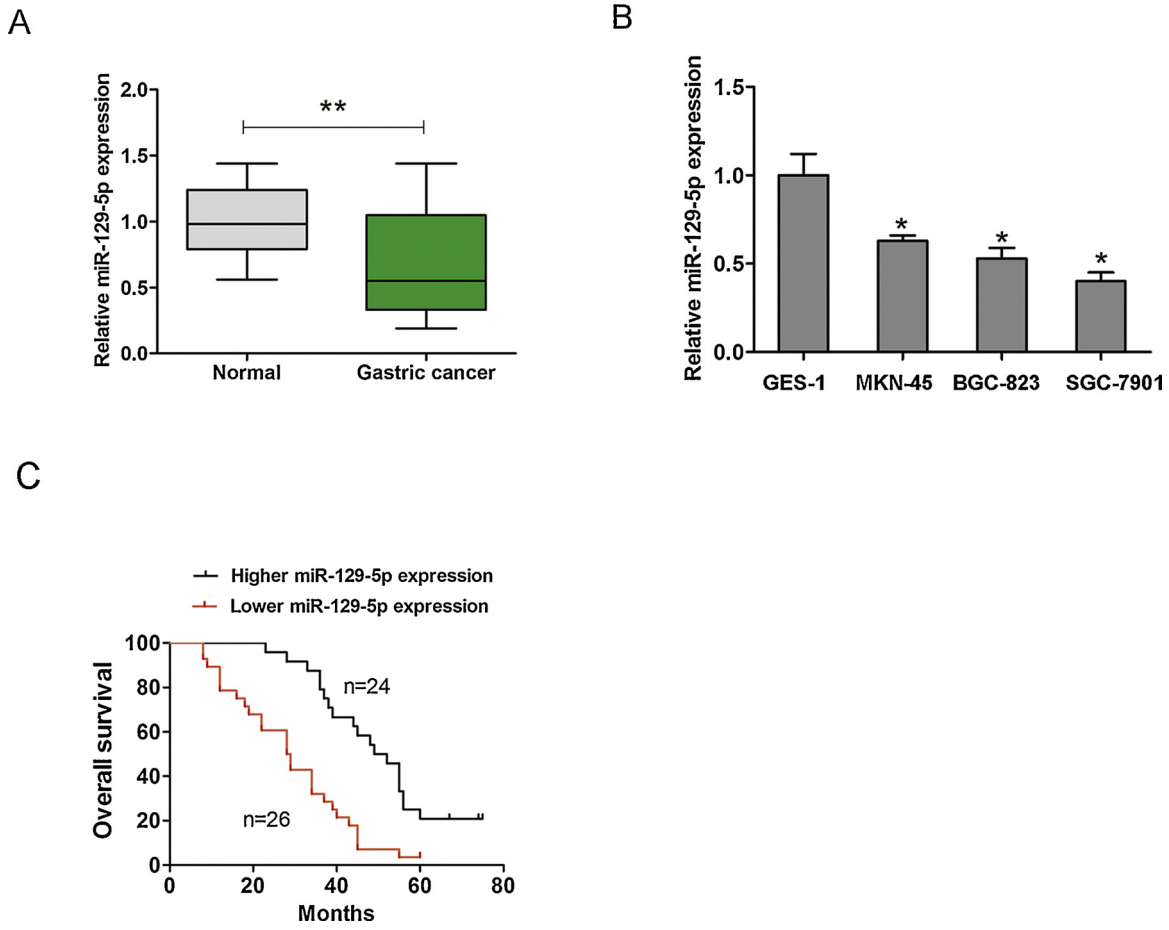

Fig. 1. MiR-129-5p is downregulated in gastric cancer tissues and cell lines. (A) MiR-129-5p expression was examined by qRT-PCR in 50 GC tissues and adjacent normal tissues. U6 was used as an internal control. (B) MiR-129-5p expression was examined by qRT-PCR in GC cell lines (MKN-45, BGC-823 and SGC-7901) and GES-1 cell lines. U6 was used as an internal control. All data are representative of three independent experiments, * represents P < 0.05. (C) K–M survival plot and log rank test were used to examine the association between miR-129-5p expression and overall survival time of GC.