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Original Article

MiR-217 is involved in the carcinogenesis of gastric cancer by down-regulating CDH1 expression

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Abstract GC is one of the most leading malignancies all over the world, and is also the leading cause of cancer-related mortalities. At present, GC remains difficult to diagnose at an early stage. In this study, we first detected the expression of 9 selected miRNAs in the exosomes from 67 GC patients' circular exosomes and found 4 miRNAs level was significantly altered. Meanwhile, one out of 4 candidate miRNAs also had a higher expression in the GC tissue samples, and negative correlated with CDH1 expression. Predicted by bioinformatics tools, confirmed by dual luciferase assay and immunoblotting, we identified that CDH1 is a direct target of miR-217. MiR-217 overexpression enhanced gastric cancer cells proliferation, and reduced exosomal CDH1 level which can be delivered into microenvironment. In conclusion, we constructed the negative correlation between miR-217 and CDH1 level in GC patients and cells; unveiled part of the miR-217 function during the pathogenesis of GC. These findings may give insight into understanding the mechanism of GC pathogenesis and provide new biomarkers for clinical diagnosis.

Introduction

Gastric cancer (GC) is the fourth most common cancer and the second most common cause of cancer related death worldwide [1–4]. Although the incidence and mortality of gastric cancer have gradually decreased, there are about 700,000 confirmed mortalities annually worldwide [5]. Furthermore, many patients are diagnosed with advanced

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gastric cancer and have a poor prognosis. Thus, more sensitive GC markers for improving screening, diagnosis, prognostic evaluation and tumor grading is urgently needed.

MicroRNA (miRNA) is a group of short non-coding RNAs that suppress the expression of protein coding genes by partial complementary binding, especially to the 3' untranslated regions (UTRs) of messenger RNAs (mRNAs). MiRNA expression alterations are involved in the initiation, progression, and metastasis of human cancer and it is believed that miRNAs function both as tumor suppressors and oncogenes in cancer development [6,7]. Deregulated miRNA was also found in the serum samples of GC patients and may be used as biomarker for GC diagnosis and classification.

Cadherin-1 (CDH1) is a calcium-dependent cell-cell adhesion glycoprotein. Loss of CDH1 function or expression has been implicated in cancer progression and metastasis. The intracellular domain of E-cadherin associates with a group of catenins including β-catenin and γ-catenin [8,9]. There is a report indicates that overexpression of β-catenin can induce cytoplasmic accumulation of endogenous β-catenin, cell cycle G1-S phase arrest and cell proliferation reduction [10]. Meanwhile, prolonged expression of ectopic E-cadherin leads to apoptosis.

Exosomes ranging in size from 40 to 100 nm in diameter, secreted by cells are proposed to be mechanism through which secreted cells pass signals to targeted cells. Tumor-derived exosomes are essential in tumor migration and metastases such as angiogenesis is enhanced by exosomes. Meanwhile, altered exosomal miRNAs in serum has been found in the patients with several kinds of cancers [11,12].

In this study, we detected the expression of 9 miRNAs in the plasma exosomes from 67 GC patients and the expression of 4 miRNA in the GC tissue samples. The biological function of miR-217 during the carcinogenesis of GC was partially unveiled.

Materials and methods

Patients and samples

A total of 67 specimens of primary gastric adenocarcinoma, corresponding adjacent non-tumorous gastric tissue and plasma samples were obtained between 2016 and 2017 at Department of Gastroenterology, Qingdao Municipal Hospital. All the 67 matched fresh frozen gastric adenocarcinoma tissues and adjacent non-cancerous tissues were selected for RNA extraction and qRT-PCR. Control plasma samples were from 67 age and sex matched healthy participants.

Tumor staging was determined according to the tumor—node—metastasis classification system of the American Joint Committee on Cancer, 7th edition. Follow-up was performed every 6 months by specially trained staff according to standard epidemiologic procedures. Patients and controls were acquired with informed consent, under the protocol approved by Department of Gastroenterology, Qingdao Municipal Hospital research ethics committee.

Exosome isolation

Exosomes from GC cells were isolated using serial centrifugation method as described [13]. Patient plasma samples were diluted with phosphate buffer saline (PBS) in a 1:1 ratio [13]. Plasma samples were then pre-treated with anti-IgG antibody (1:500 dilution) coupled to A/G sepharose beads (25 μl) to precipitate excessive non-specific immunoglobulins. After an overnight incubation at 4 °C, the pre-treated samples were centrifuged at 5000 × g for 15 min and the pre-cleared serum was used for exosomes isolation using a standard ultracentrifugation method. Briefly, the pre-cleared serum samples went through a series of centrifugation steps (2000 × g for 20 min and 20,000 × g for 45 min) and then transferred onto 30% sucrose solution, followed by ultra-centrifugation at 100,000 g for 2 h. Isolated exosomes were recovered from the sucrose solution and stored at −80 °C until further analyses.

miRNA real-time RT-qPCR

Quantitive RT-PCR analysis was used to determine the relative expression level of candidate miRNAs. Total RNA was extracted from clinical samples and cells, using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The expression level of miRNAs was detected by TaqMan miRNA RT-Real Time PCR. Single-stranded cDNA was synthesized by using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) and then amplified by using TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) together with miRNA-specific TaqMan MGB probes (Applied Biosystems, Foster City, CA, USA). The U6 snRNA was used for normalization.

Cell culture

Human gastric cancer cell line (BGC-823) was purchased from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China) and maintained in humidified incubator at 37 °C in a CO2 incubator in Dulbecco's modified Eagle's media (DMEM) or RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin.

Dual luciferase assay

490bp of CDH1 3′UTR contains the miR-217 target site was cloned into downstream of firefly luciferase coding region in pmirGLO vector (promega, Madison, WI, USA) to generate luciferase reporter vector. For luciferase reporter assays, HEK293T cells were seeded in 48-well plates. MiR-217 mimic and luciferase reporter vectors were co-transfected by using lipofectamine 2000 (Invitrogen, Carlsbad, CA USA). After 48 h, cells were harvested and assayed with the Dual-Luciferase Assay (Promega, Madison, WI USA). Each treatment was performed in triplicate in three independent experiments. The results were expressed as relative luciferase activity (Firefly LUC/Renilla LUC).

Immunoblotting

Protein extracts were boiled in SDS/β-mercaptoethanol sample buffer, and 30 μg samples were loaded into each lane of 10% polyacrylamide gels. The proteins were separated by

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