Gastric Cancer

MicroRNA Expression Signature in Gastric Cancer

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ABSTRACT

Objective: To identify the miRNA specific signature as novel diagnostic and prognostic tools for gastric cancer.

Methods: miRNAs expression profiling of 3 normal gastric tissues, 24 malignant tissues, gastric cancer cell SGC7901 and normal gastric cell GES-1 were detected using microarray technology. The hierarchical clustering algorithm of the Cluster software was used to analyse the miRNAs expression of all samples. The expression levels of miR-433 and miR-9 which were significantly down-regulated in gastric cancer tissues and SGC7901 cells by microarray technology were validated by quantitative Real-time PCR (qRT-PCR).

Results: Differential expressions of 26 individual miRNAs between normal samples (including 3 normal gastric tissues and GES-1 cells) and carcinomas (including 24 malignant tissues and SGC7901 cells) were discovered,19 of them showing down-regulation and 7 up-regulation in carcinoma samples. Hierarchical clustering of the cancer samples by their miRNA expression accurately separated the carcinomas from normal samples and further their histotypes of carcinomas. The expression levels of miR-433 and miR-9 were significantly down-regulated in gastric cancer tissues and SGC7901cells

Conclusion: The differential expression of miR-433 and miR-9 may be used as a novel diagnostic tool for gastric cancer.

Key words: miRNA; Real-time PCR; Gastric cancer; Microarray

INTRODUCTION

Gastric cancer is the fourth most common malignancy and the second leading cause of death due to cancer worldwide[1]. Many study results show that gastric cancer is a polygenic disease, and its development is a multistep process, a result of many kinds of related genes developing abnormally[2]. But, in the process, the regulation mechanism of gene activity involved in canceration is still not understood.

A new class of small noncoding RNAs, named microRNAs (miRNAs) that are endogenously expressed in animal and plant cells, have been discovered recently[3–5]. They regulate the expression of protein-coding genes, either by triggering degradation or preventing translation of the target mRNAs[6]. Primary transcripts of miRNAs (pri-miRNAs) are generated by RNA polymerase II[7]. Then, they are sequentially processed by RNase III, Drosha and Dicer, to first produce –70 nt intermediate hairpin structures (pre-miRNAs) and finally the 17–24 nt-long, mature miRNAs[8]. Mature miRNAs which are single-stranded, recognize their targets mainly through limited base-pairing interactions between the 5'-end of the miRNA (nucleotides 2–8, the seed

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region) and complementary sequences in the 3'untranslated regions (3'-UTRs) of the target mRNAs[9−11]. Bioinformatic methods predict that approximately one-third of all human genes may be targets of miRNAs[12, 13]. Several studies have shown that miRNAs play important roles in essential processes, such as cell growth, differentiation and cell death[14, 15]. Moreover, it has been shown that miRNAs are aberrantly expressed or mutated in cancers, suggesting that they may play a role as a novel class of oncogenes or tumor suppressor genes[16−20].

Recently, miRNA expression profiling studies have been done to identify cancer-specific miRNA signatures[18, 21−29]. In this study, we presented expression profiling of 328 miRNAs in 2 cell lines, 24 gastric cancer samples and 3 normal gastric tissue samples, to reveal the miRNA signature of gastric cancer. Furthermore, hierarchical clustering of all the samples by their miRNA expression was done, suggesting that it can serve as a new tool in defining biologic differences of gastric cancer.

MATERIALS AND METHODS

Gastric Tissues and Cell Lines

All human gastric tissue samples were obtained from surgical specimens at The First and Second Affiliated Hospital of Chongqing University of Medical Science, China, including 3 normal gastric tissues and 24 malignant tissues (2 early gastric cancers and 22 advanced gastric cancers). The details of these samples are shown in Table 1. All tissues were preserved in liquid nitrogen after removing from the body. The gastric cancer cell SGC7901 was derived from The Institute for Viral Hepatitis, Chongqing University of Medical Science, China. And normal gastric cell GES-1 was derived from Cancer Institute and Hospital, Chinese Academy of Medical Sciences. Both of the cells were cultured in RPMI1640, supplemented with 10% fetal bovine serum and 100 U/ml, penicillin/streptomycin.

Total RNA Preparation

Total RNAs were extracted from the gastric cancer tissues, normal gastric mucous tissues, SGC7901 and GES-1 cells by using Trizol (Invitrogen) according to the manufacturer's instructions. The total RNAs were quantified by ultraviolet spectrophotometer at a wavelength of 260 nm.

Table 1. Details of the samples of this study

Sample	Number	Stage
Papillary carcinoma	1	Early
	2	Early
	3	Advanced
	4	Advanced
	5	Advanced
	6	Advanced
	7	Advanced
	8	Advanced
	9	Advanced
	10	Advanced
Mucinous carcinoma (MC)	11	Advanced
	12	Advanced
	13	Advanced
	14	Advanced
	15	Advanced
	16	Advanced
	17	Advanced
	18	Advanced
	19	Advanced
	20	Advanced
Medullary carcinoma	21	Advanced
	22	Advanced
	23	Advanced
Squamous carcinoma (SC)	24	Advanced
Normal Tissue (NT)	25	Advanced
	26	Advanced
	27	Advanced
GES-1		
SGC7901		

miRNA Labeling and miRNA Microarray Hybridization

Total RNAs 5 μg from each sample were used for miRNA labeling (miRCURY^{TM} Array Labeling Kit, Cat #208032, exiqon). Then, miRNA array hybridization was carried out on miRNA microarray (miRCURY^{TM} Array microarray Kit, Cat #208002V8.1, Exiqon, Hybridization chamber II, Cat #40080, corning, Bioarray LifterSlip coverslip, Ambion). Every microarray chip was repeated 4 times for the same sample. Hybridization signals were detected with streptavidin-Alexa Fluor 647 conjugate using a GenePix 4000B scanner (Axon Instruments). The 635 nm laser was used. Images were quantified by the GenePix Pro 6.0 (Axon Instruments). The samples were clustered by their miRNA expression profiles using the hierarchical clustering algorithm