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Aberrant expression of microRNAs in gastric cancer and biological significance of miR-574-3p

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ABSTRACT

The discovery of microRNAs (miRNAs) provides a new and powerful tool for studying the mechanisms, diagnosis and treatments of cancer. In this study, we employed AFFX miRNA expression chips to search for miRNAs that may be aberrantly expressed in gastric cancer tissues and to investigate the potential roles that miRNAs may play in the development and progression of gastric cancer. 14 miRNAs were found to be down-regulated and 2 miRNAs up-regulated in gastric cancer tissues compared to the normal gastric tissues. Among the aberrantly expressed miRNAs, miR-574-3p was selected to further study its expression features and functional roles. Interestingly, the reduced expression of miR-574-3p occurred mainly in the early stages of gastric cancer or in cancers with high level of differentiation, suggesting that it can be used as a marker for a mild case of gastric cancer. Functional study revealed that cell proliferation, migration and invasion were significantly inhibited in miR-574-3p-transfected gastric cancer SGC7901 cells. Computational prediction and experimental validation suggest that Cullin2 may be one of the targets of miR-574-3p. Overall our study suggests that the aberrantly expressed miRNAs may play regulatory and functional roles in the development and progression of gastric cancer.

1. Introduction

Cancer is a polygenic disease processing in many steps, and many genes have been found to be involved in the formation and progression of cancer. Gene alterations play important roles in uncontrollable proliferation, apoptosis evasion, angiogenesis, metastasis and invasion of cancer [1–4]. However, the mechanisms of controlling the expression of the genes are still not fully understood. The discovery of microRNA provides a powerful tool for studying the mechanisms, diagnosis and treatments of cancers [5,6].

MicroRNAs (miRNAs), approximately 19–24 nucleotides in length, are evolutionarily conserved small single-stranded non-coding RNA molecules. It was reported that more than 60% of human protein-coding genes have been under selective pressure to maintain their pairing with miRNAs [7]. They work mainly at post-transcriptional level by binding to the sequences in the 3′ untranslated regions (3′UTR) of their targeted mRNAs resulting in translational repression or gene silencing [7–9]. So far, the regions encoding some miRNAs have been found to be located in cancer-associated genomic regions or at fragile genomic sites [10]. MiRNAs are involved in regulation of wide array of biological processes, such as cell proliferation, differentiation and apoptosis [11,12]. These processes are deregulated during the development and progress of a cancer, which may be associated with abnormal changes of miRNA. Indeed, aberrant expressions of miRNAs have been studied in several types of human cancers, including lung [13], colon [14], breast [15], prostate [16], hepatocellular [17] and ovarian [18] cancers. They may play roles in the development and progression of cancers similar to those played by oncogenes or tumor suppressor genes [19,20].

Although aberrant expression of miRNAs in gastric cancer has been reported recently, the results are various between groups [21–24]. In addition, the function roles of these aberrant expressed miRNAs in gastric cancer remain largely unknown and their mRNA targets are barely studied. In order to gain more information on this issue, we employed AFFX miRNA expression chips to study the aberrant expressions of miRNAs and quantitative real-time PCR to identify and confirm miRNAs with aberrant expressions in gastric cancer. Among the aberrantly expressed miRNAs, we chose miR-574-3p to further study their functional roles concerning proliferation, migration and invasion of gastric cancer cells. We also computationally predicted and experimentally validated the target of miR-574-3p.

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Table 1
Clinicopathological characteristics of the patients.

Clinical parameters	Number of cases
Gender	
Male	12
Female	8
Age	
<40	13
≥40	7
Level of differentiation	
Poorly differentiated	7
Moderately differentiated	8
Highly differentiated	5
Clinical stage	
I–II	11
III–IV	9
Lymph node metastasis	
Positive	8
Negative	12

2. Materials and methods

2.1. Materials

Trizol was purchased from Invitrogen, and MirVana™ miRNA Isolation Kit was purchased from Ambion. AFFX miRNA expression chips and RNA Labeling Kit were purchased from Affymetrix (CA, USA). miRNA cDNA Synthesis Kit, miRNA PrimeScript®RT Enzyme Mix, and SYBR®Premix Ex Taq™ were all purchased from Takara, China. Cell counting kit-8 (CCK-8) was purchased from Dojindo Laboratories (Dojindo, Japan). PVDF membrane was purchased from Bio-Rad (CA, USA). Rabbit anti human CUL2 and mouse anti human β-actin monoclonal antibodies, goat anti-rabbit and goat anti-mouse secondary antibody were purchased from Proteintech Group, Inc (IL, USA).

2.2. Patients and specimens

Gastric cancer tissues and their adjacent normal tissues were collected from 20 patients with primary gastric cancer during their initial cancer-removal surgery. All samples were snap-frozen within 10 min after excision and stored at −196 °C in liquid nitrogen. The written informed consent was obtained from each patient at the First Hospital and the Second Hospital of Jilin University, Changchun, China where the specimens were obtained. The research was approved by the Chinese IRB overseeing human subjects at the First Hospital and the Second Hospital of Jilin University and by the Institutional Review Board (IRB) at the University of Georgia, USA.

The histological subtype and pathologic stage of each tumor were determined according to the World Health Organization (WHO) and the tumor, lymph node and metastasis (TNM) classification system. The clinicpathological characteristics of the tumor tissues from these patients are summarized in Table 1.

2.3. MicroRNA preparation

Total RNA from tissue samples was extracted using the Trizol RNA, and its content was analyzed using the UV2800 ultraviolet spectrophotometer (UNIC, NY, USA) having the A260/A280 ratio between 1.8 and 2.0, representing that the RNA samples are highly purified and not degraded. The miRNA was isolated from the total RNA and purified using the mirVana™ miRNA Isolation Kit.

2.4. MicroRNA microarrays

AFFX miRNA expression chips were used to perform the miRNA expression assay. Total RNA from 16 cancer tissues and their adjacent normal tissues was isolated and miRNAs were labeled using the RNA Labeling Kit. The labeled miRNA hybridization solution was added to the chip for hybridization for 17 h at 45 °C, 60 rpm. After being washed and stained using the GeneChip® Fluidics Station 450 (Affymetrix), the chip was inserted into the Affymetrix autoloader carousel using an appropriate fluidics script and scanned with the GeneChip® Scanner 3000 with GeneChip® Operating Software.

2.5. Quantitative real-time PCR

SYBR Green quantitative real-time PCR was used to measure the content of miRNA and mRNA in the tissue and cell. We first obtained cDNA using One Step PrimeScript® miRNA cDNA Synthesis Kit. 1 μg total RNA, 2 μl miRNA PrimeScript®RT Enzyme Mix, 10 μl miRNA Reaction Buffer Mix and 2 μl 0.1% BSA were mixed, incubated in 37 °C, water-bath for 60 min. The reaction was inactivated by dipping the reaction tube into 85 °C water-bath for 5 s. The real-time PCR was prepared with the SYBR®Premix Ex Taq™ quantitative augmentation reaction system. The total reaction volume was 20 μl including 10 μl SYBR®Premix Ex Taq™II (2×), 2 μl reverse transcription reaction products, 0.4 μl ROX Reference Dye (50×), 0.8 μl Uni-miR qPCR Primer (10 μM) and 0.8 μl purposely primer (10 μM). The PCR was performed in the ABI PRISM 7300 Fast Real-Time PCR System (Ambion) at 95 °C for 30 s, then at 95 °C for 5 s and 60 °C for 31 s for 40 cycles. The primers used for the real-time PCR are shown in Table 2. All the primers were synthesized by Shanghai ShengGong Biotechnology Company. The results of real-time PCR are expressed as $2^{-\Delta\Delta Ct}$, ΔCt (for miRNA: measured Ct subtracting the internal control U6 Ct; for mRNA: measured Ct subtracting the GAPDH Ct), or ΔΔCt (cancer ΔCt subtracting the normal

Table 2
Sequences of primers and miRNA mimics.

Name	Primer or miRNA mimic sequences
U6-F	CGCTTCGGCAGCACATATACTA
U6-R	CGCTTCACGAATTTGCGTGTCA
miR-31	AGGCAAGATGCTGGCATAGCT
miR-574-3p	CACGCTCATGCACACACCCCACA
GAPDH-F	AGAAGGCTGGGGCTCATTTG
GAPDH-R	AGGGGCCATCCACAGTCTTC
CUL2-F	GAGTGCGTTGGATAAGGCCCT
CUL2-R	CTCTGTCATCCCTTTCGCTGACT
RXRA-F	CGCCATCTTTGACAGGGTGCT
RXRA-R	GGTTCGAGAGCCCCTTGGAGT
miR-574-3p mimic sense	CACGUCAUGCACACACCCACA
miR-574-3p mimic antisense	UGUGGGUGUGUGCUGGCGUG
Negative control sense	UUCUCCGAACGUGUCACGUTT
Negative control antisense	ACGUGACACGUUCGGAGAATT

Table 3
Expression changes of miRNAs in gastric cancer.

Down-regulated	Up-regulated
hsa-let-7g	hsa-miR-455-3p
hsa-miR-200b	hsa-miR-34a
hsa-miR-768-3p	
hsa-let-7d	
hsa-miR-104-3p	
hsa-miR-27b	
hsa-miR-1207-5p	
hsa-miR-663	
hsa-miR-486-5p	
hsa-miR-222	
hsa-miR-574-3p	
hsa-miR-768-5p	
hsa-miR-378	
hsa-miR-31	