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Research paper

MiR-455-5p acts as a novel tumor suppressor in gastric cancer by down-regulating RAB18

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ABSTRACT

Aim: To detect the potential regulation pathway of microRNA-455-5p (miR-455-5p) and RAB18, member RAS oncogene family (RAB18) in gastric cancer (GC) cells and tissues and discuss the clinical significance of miR-455-5p in GC genesis and progression.
Methods: Real-time PCR was used to measure mRNA level of miR-455-5p in GC, TargetScan and dual luciferase assay were used to predict and demonstrate the candidate target gene of miR-455-5p, Western blot were utilized to detect the protein level of RAB18. Cell function assays were also performed to determine the function of miR-455-5p in GC.
Results: miR-455-5p was reduced significantly in gastric cancer cells and tissues compared with the corresponding normal control, the lower expression of miR-455-5p was related to advanced clinical stage in gastric cancer, re-expression of miR-455-5p could inhibit human GC cell proliferation and invasion, overexpression of miR-455-5p could also promote GC cell apoptosis. Furthermore, we found that RAB18 was a candidate target of miR-455-5p. Re-expression of miR-455-5p could inhibit the protein level of RAB18.
Conclusion: MiR-455-5p might serve as a novel biomarker in gastric cancer by targeting RAB18.

1. Introduction

Gastric cancer (GC) is one of the most common digestive system tumors all over the world (Cidon et al., 2013). Because of the lack of early diagnostic indicators, most of the patients were diagnosed with advanced gastric carcinoma. Although surgery and chemotherapy strategies have improved, the overall prognosis for gastric cancer remains grim. Thus, it is important to find a new early diagnostic biomarkers of gastric cancer.

MicroRNAs (miRNAs) are a kind of small endogenous noncoding RNA molecules and they do not have the function of encoding protein (Engels and Hutvagne, 2006). They can regulate the expression of target genes through completely or incompletely combined with the target mRNAs in post-transcriptional level (Zhang et al., 2013). Recent researches indicated that miRNAs could act as an oncogene or tumor suppressor genes in many cancer. Accumulating evidences have proved that miRNAs have associated with a series of cell functional assays including cell proliferation, apoptosis, invasiveness, and migration (Cai et al., 2013; Challagundla et al., 2014). For example, miR-148a is obviously decreased in gastric cancer and overexpression of miR-148a can suppress gastric cancer cells proliferation (Yan et al., 2014); Li et al. proved that miR-10b was obviously reduced in gastric cancer and could accelerate the apoptosis of SGC-7901 and MGC-803 cells (Li et al., 2015); Lei indicated that miR-219-1-3p was identified as a tumor suppressor in gastric cancer (Lei et al., 2013). In general, previous researches suggest that miRNAs may contribute to tumorigenesis and were believed to act as a class of new molecular biomarkers for gastric cancer (Jiang et al., 2015; Tong et al., 2014; Zhao et al., 2013). MiR-455-5p is located on chromosome 6. Accumulating evidence predicts that miR-455-5p may play key roles in human cancers. Chai has reported that miR-455-5p was decreased in colorectal carcinoma and re-expression of miR-455-5p could inhibit the invasion of colon cancer cells(SW480) by target RAF-1 oncogene (Chai et al., 2015). Shoshan E. showed that miR-455-5p negatively regulated the expression of the CPEB1 gene which was regarded as a tumor suppressor and promoted melanoma growth and metastasis (Shoshan et al., 2015). Hudson J. had reported miR-455-5p was down-regulated in medullary thyroid carcinoma and played a significantly function on tumor development (Hudson et al., 2013). Moreover, the down-regulation of miR-455-5p was also reported in large-cell lymphomas, large B cell lymphoma, and esophageal cancer (Liu et al., 2013; Song et al., 2014; Hummel et al., 2011). However, the potential mechanism of miR-455-5p in gastric cancer remains unknown.

In the study, we analyzed the expression of miR-455-5p in GC, the results suggested that miR-455-5p was obviously decreased in gastric

Abbreviations: miRNA, microRNA; miR-455-5p, microRNA-455-5p; RAB18, RAB18, member RAS oncogene family; GC, gastric cancer; TCGA, The Cancer Genome Atlas.

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cancer. Furthermore, the restored expression of miR-455-5p inhibited CC cells proliferation and promoted apoptosis. Moreover, we proved that miR-455-5p played a significant role in inhibiting cell proliferation and invasion of GC cells by targeting RAB18. The result indicated that miR-455-5p could play a role of tumor suppressor in gastric cancer by regulating RAB18.

2. Materials and methods

2.1. Clinical samples

82 paired gastric cancer samples and the corresponding normal specimens were acquired from the Cancer Research Institute of China Medical University (Shenyang, China) between 2013 and 2014. All patients received surgical resection and were diagnosed with GC by histopathological confirmation. All patients did not receive chemotherapy before surgery. The samples were immediately stored in liquid nitrogen after surgical removal until RNA extraction. Informed written consents were approved by all patients and the study was approved by China Medical University ethics committee. (See Table 1.)

Table 1
Clinicopathological characteristics of patients with gastric cancer (GC).

Parameter	Total samples	Percentage(%)	95% CI of mean of log2 fold change ± SEM	P
Age (years)	82			0.894
≥60		62	0.396 (−1.324 to 0.259)	
<60		38	0.656 (−2.22 to 0.549)	
Gender	82			0.714
Male		78	0.373 (−1.313 to 0.186)	
Female		22	0.664 (−2.013 to o.698)	
Location	80			0.665
Proximal		24	0.666 (−2.046 to 0.753)	
Body		60	0.432 (−1.166 to 0.573)	
Distal		16	0.913 (−3.465 to 0.452)	
Classification	75			0.520
Ulcer		53	0.404 (−1.047 to 0.578)	
Protrude		25	0.723 (−2.265 to 0.771)	
Erosion		22	1.105 (−3.996 to 0.428)	
Grade	78			0.375
Well and moderately differentiated		36	0.624 (−1.458 to 1.103)	
Poorly differentiated		64	0.402 (−1.624 to 0.108)	
Tumor size	78			0.121
T1–T2		30	0.649 (−2.789 to 0.957)	
T3–T4		70	0.393 (−1.274 to 0.762)	
Stage	76			0.015
I–II		41	0.506 (−0.584 to 1.482)	
III–IV		59	0.430 (−2.100 to 1.718)	
Lymphatic invasion	72			0.248
N0–N1		65	0.418 (−1.738 to 0.59)	
N2–N3		35	0.576 (−1.257 to 1.098)	

2.2. Cell culture

The gastric cancer cell lines MKN-45, MGC-803, SGC-7901 and a normal human gastric cell line GES-1 used in this study were purchased from Typical China Academy Culture Collection Commission Cell Library (Shanghai, China). The cells were propagated in RPMI 1640 medium (Invitrogen, Calsbad, CA, USA) added with 10% Fetal Bovine Serum (Gibco, BRL, UK) and were maintained at 37 °C in 5% CO2.

2.3. Cell transfection

The miR-455-5p mimics, and the scramble mimics were obtained from GenePharma (Shanghai, China). The miR-455-5p mimics and the scramble were transfected into MGC-803 and SGC-7901 cells using Lipofectamine TM 3000 Transfection Reagent (Invitrogen) according to the manufacturer's instructions. Each experiment was carried out three times.

2.4. Real-time PCR analysis

Total RNA was isolated from GC cells and tissues with Trizol reagent (Tiangen, Beijing, China). The RNA concentration and purity were determined spectroscopically and was reverse transcribed for 30 min at 25 °C, 30 min at 42 °C and 5 min at 85 °C using miRNAs RT-PCR Quantitation Kit (GenePharma). The U6 RNA served as an internal control for normalization. Stem-loop primers used for reverse transcription of miR-455-5p and U6 were 5′-GTCGTATCGAGT GGAGCGTC GGAGCTATACGCACTCGATACGACACAAA-3′ and 5′-GTCC TATCCAGTGCAGGGT CCGAGGTGCACTGGATACGACAAAATATGGAAC-3′. The resulting cDNA was used for real-time PCR. MiRNAs RT-PCR Quantitation Kit was used to perform the quantitative real-time PCR with the following steps: 3 min at 95 °C, followed by 40 cycles of 12 s at 95 °C, 40 s at 62 °C. The primer pairs used for amplification of miR-455-5p and U6 were: 5′-CGAGCTTCCTTCTGCAGGT-3′ (F) and5′-CACCACTGCCATCCCACA-3′ (R), and 5-TGCGGGTGCTCGCT TCGCAGC -3 (F) and 5- CCAGTGCAGGGTCCGAGGT -3 (R).

2.5. Cell proliferation and apoptosis assays

The miRNA-455-5p mimics-transfected cells (SGC-7901 and MGC-803) were grown on 96-well plastic dishes in normal culture medium. Then, CCK8 working solution (Keygen, Jiangsu, China) was added into the medium for 4 h. Then, a microplate reader was used to detect the absorbance of the wells at 0, 12, 24, 48, and 72 h. Each experiment was performed at least three times. For cell apoptosis assay, 1×10^6 GC cells transfected with miRNA-455-5p mimics or scramble were seeded into 6-well plates for 72 h and the cells were harvested. The Annexin V-PE/7AAD Apoptosis Detection Kit (Keygen) was utilized to conduct the experiments in accordance with the manufacturer's instructions. All experiments were performed three times.

2.6. Cell migration and invasion assays

Gastric cancer cells (SGC-7901 and MGC-803) were grown in 6-well plates and transfected with miRNA-455-5p mimics or scramble for 24 h, Then, a 200ul pipette tip was used to create a linear scratch on confluent monolayer cells. An inverted microscope was utilized to visualize the wound healing at 0, 24, 48 and 72 h. For the invasion assay, a Matrigel-coated membrane matrix (Univ-bio, Shanghai, China) was used. 24 h after transfection,1×10^5 GC cells were cultured into the top chamber with medium out of serum. Then, the lower chamber was filled with medium containing 10% FBS. A cotton tip was used to remove the noninvasive cells. Crystal violet (Tiangen) was used to dye the invasive cells and an inverted microscope was used to count the numbers of the invasive cells. Each sample was performed three times.