Clinica Chimica Acta 400 (2009) 97–102

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Detection of miR-106a in gastric carcinoma and its clinical significance

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ARTICLE INFO

Article history:
Received 4 September 2008
Received in revised form 16 October 2008
Accepted 20 October 2008
Available online 30 October 2008

Keywords:
Gastric cancer
MicroRNA
miR-106a
Gene diagnosis

ABSTRACT

Background: MicroRNAs (miRNAs) play important roles in carcinogenesis. miRNA-106a (miR-106a) has oncogenic activity in humans, and often has altered expression. The clinical significance of miR-106a in the diagnosis of gastric carcinoma is poorly understood.
Methods: The level of miR-106a in 55 gastric carcinoma and 17 non-tumor tissues was quantified by real-time reverse transcriptase-polymerase chain reaction, and the relationship between miR-106a level and clinical and pathological factors was explored.
Results: The level of miR-106a in cancer tissues was significantly higher than that in non-tumor tissues, with an average 1.625-fold increase. miR-106a level was significantly associated with tumor stage, size and differentiation; lymphatic and distant metastasis; and invasion ($P<0.01$). The altered expression of miR-106a was confirmed in gastric cancer cell lines.
Conclusion: miR-106a may be a potential biomarker in the diagnosis of gastric carcinoma.

1. Introduction

MicroRNAs (miRNAs) are non-coding RNAs that regulate expression of target mRNAs. They are encoded by genes that are presumably transcribed into single or clustered primary transcripts, which are processed to produce the mature miRNAs. Altered expression of specific miRNAs in several tumor types has been reported [1,2]. They are deemed to play a crucial role in the initiation and progression of human cancer, and those with a role in cancer are designated as oncogenic miRNAs (oncomiRs) [3]. It has been confirmed that they regulate cell differentiation, proliferation and death [4]. The oncomiRs expression profiling of human malignancies has also identified a number of diagnostic and prognostic cancer signatures [3].

Human gastric cancer is still a major cause of cancer mortality worldwide [5]. However, conventional strategies for treatment of gastric cancer are not yet satisfactory. Ideal therapeutic targets should be causally associated with disease and amenable to designing therapeutic interventions, whereas ideal biomarkers should be easy to measure and have strong associations with clinical outcomes [6]. miRNAs are thought to match both criteria [7,8]. Several miRNAs have been thought to be associated with gastric cancer [9–12]. Volinia et al. first found that 26 miRNAs and 17 miRNAs were overexpressed and downexpressed in six kinds solid cancers including stomach, respectively [9]. The miR-106b-25 cluster, upregulated in a subset of human gastric tumors, is involved in the posttranscriptional regulation of transcription factor E2F1 [10]. miR-15b and miR-16 modulate multidrug resistance by targeting B cell lymphoma/leukmia-2 (BCL2) in human gastric cancer cells [11]. miR-21 was found overexpressed in 92% (34/37) of gastric cancer samples and could serve as an efficient diagnostic marker for gastric cancer [12]. Recently, Ji et al. reported that restoration of tumor suppressor miR-34 inhibited cell growth and induced apoptosis in human p53-mutant gastric cancer cells, indicating that miR-34 might restore p53 function [13].

miRNA-106a (miR-106a), located in Xq26.2, has oncogenic activity in humans, and often has altered expression [14]. In fact, 3 miRNA clusters, miR-17-92 (located in 13q31.3), miR-106b-25 (located in 7q22.1), and miR-106a-92, which share high homology in their gene structures, have oncogenic potential [10,15]. miR-17-92 cluster includes miR-17-5p, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92-1. miR-106b-25 cluster includes miR-106b, miR-93, and miR-25. And miR-106a-92 cluster includes miR-106a, miR-18b, miR-20b, miR-19b-2, and miR-92-2. Among them, the relationships between members of miR-17-92 or miR-106b-25 clusters and gastric cancer have been studied by Petrocca et al. [10]. They found that miR-106b-25 clusters might play a key role in the development of transforming growth factor-β (TGF-β) resistance and apoptosis in gastric cancer. They also found that miR-17-5p was highly expressed in gastric cancer cells. However, despite its high homology with miR-17-92 and miR-106b-25 clusters, the oncogenic potential of miR-106a-92 has never

Abbreviations: RT, reverse transcription; PCR, polymerase chain reaction; miRNAs, microRNAs; microRNA-106a, miR-106a; ANOVA, analysis of variance; TNM, tumor node metastasis; FFPE, formaldehyde-fixed, paraffin-embedded; WHO, World Health Organization; SPSS, Statistical Program for Social Sciences; LOH, loss of heterozygosity.

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doi:10.1016/j.cca.2008.10.021

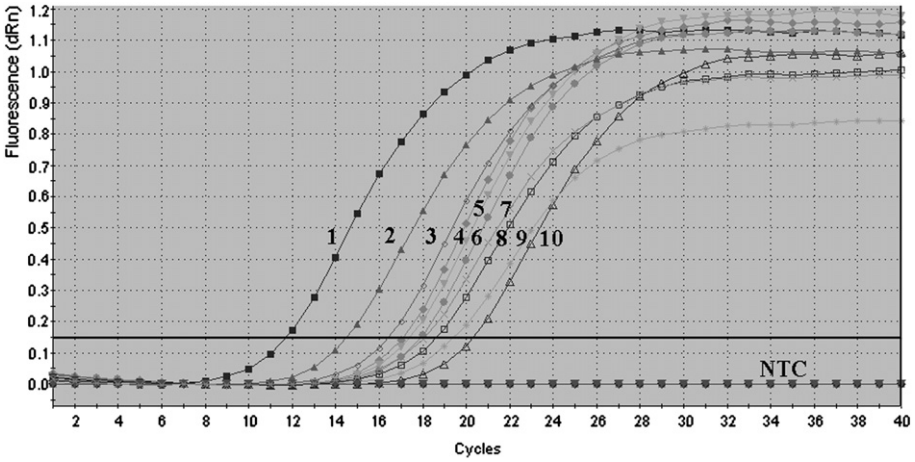

Fig. 1. Representative amplification plots of U6 small RNA and miR-106a in cancer tissues and non-tumor tissues. 1–3, 6, and 8 indicate the RT–PCR results of U6. Their Ct values are 11.73, 14.52, 16.43, 17.87, and 18.64, respectively. 4, 5, 7, 9, and 10 indicate the RT–PCR results of miR-106a. Their Ct values are 17.06, 17.38, 18.05, 19.43, and 20.34, respectively. 1 and 5, 2 and 4, 8 and 9 are from the same patients with gastric carcinoma, respectively. 3 and 7, 6 and 10 are from the same non-tumor tissues, respectively. NTC means no-template control.

been implicated in gastric cancer. As a result, the objective of the present study was to compare the expression profile of miR-106a in gastric cancer and non-tumor tissues. We found that miR-106a expression may be closely related to the development of gastric cancer.

2. Materials and methods

2.1. Patients and specimens

Formaldehyde-fixed, paraffin-embedded (FFPE) tissue samples were from surgical specimens from 55 patients (40 male, 15 female; 61.3±7.6 and 53.6±10.8 y, respectively) with gastric cancer, from May 2005 to December 2007 at Ningbo No. 2 Hospital, China. Non-tumor gastric tissues were randomly selected from 17 of these patients (nine male, eight female; 57.4±3.5 and 55.9±14.9 y, respectively) and used as controls. Informed consent was obtained from all subjects, and the Human Research Ethics Committee from the Ningbo University approved all aspects of the study. Tumors were staged using the tumor–node–metastasis (TNM) staging of the International Union Against Cancer (1997). Histological grade was assessed according to the World Health Organization criteria [16]. The non-tumor tissues were more than 1.5 cm from the tumor and were confirmed as such by an experienced pathologist.

2.2. Cell culture

Human gastric cancer cell line MGC-803 and SGC-7901 were obtained from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Cells were cultured in 24-well plates at 37 °C in a humidified atmosphere of 5% CO_2 with RPMI-1640 Medium (Life Technologies, Grand Island, NY, USA) containing 10% fetal calf serum with 50 U/ml penicillin and 50 μg/ml streptomycin. Exponentially growing cells were used for experiments.

2.3. Total RNA preparation

Total RNA from human FFPE tissues was isolated using RecoverAll™ Total Nucleic Acid Isolation Kit (Ambion, Austin, TX) according to the manufacturer's instructions. Total RNA from fresh cultured cells was extracted using Trizol reagent (Invitrogen, Karlsruhe, Germany) following the manufacturer's protocol. Concentration and purity of total RNA samples were measured using the SmartSpec Plus spectrophotometer (Bio-Rad, Hercules, CA). The ratio of A260:A280 was used to indicate the purity of total RNA.

2.4. Reverse transcription

cDNA was generated using the miScript Reverse Transcription (RT) Kit (Qiagen GmbH, Hilden, Germany). According to the manufacturer's instructions, 1 μg total RNA, 1 μl miScript Reverse Transcriptase Mix, and 4 μl miScript RT buffer were mixed well and incubated for 60 min at 37 °C. All reverse transcriptions and no-template controls were run at the same time.

2.5. Real-time PCR for detection of miR-106a

Real-time polymerase chain reaction (PCR) was performed using miScript SYBR Green PCR Kit (Qiagen) on an Mx3005P QPCR System (Stratagene, La Jolla, CA). The 20 μl PCR mixture included 2 μl reverse transcription product, 10 μl 2× QuantiTect SYBR Green PCR Master Mix, 2 μl 10× miScript Universal Primer, 2 μl 10× miScript Primer Assay (for miR-106a; Qiagen), and 4 μl autoclaved distilled water. The reaction mixtures were incubated at 95 °C for 10 min, followed by 40 amplification cycles of 94 °C for 15 s, 55 °C for 30 s, and 70 °C for 30 s. The threshold cycle (Ct) was defined as the fractional cycle number at which the fluorescence passed the fixed threshold.

We also quantified transcripts of U6 small RNA using Hs_RNU6B_2 miScript Primer Assay (Qiagen) for normalizing the levels of miR-106a. Small RNAs are preferred because they have similar purification and amplification characteristics to miRNAs. Choosing an appropriate control for a miRNA quantification experiment is similar to choosing an appropriate housekeeping gene for normalization when quantifying mRNA. For each sample type or treatment under study, it is necessary to verify that the control RNA is not regulated under the experimental conditions. In addition, a control for a non-coding RNA that shows a constant level of expression and similar abundance to the target miRNA should be chosen. U6 small RNA is a widely used endogenous reference RNA in miRNA quantification studies [6,15].

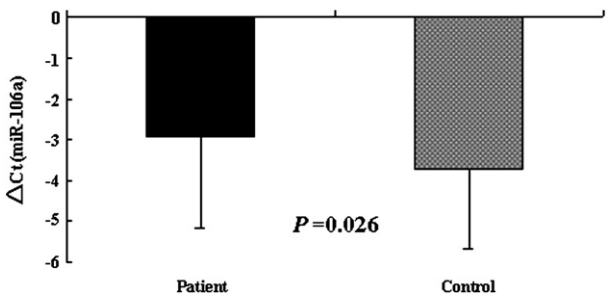

Fig. 2. Level of miR-106a in gastric carcinoma tissues ($n=55$) was higher than in non-tumor tissues ($n=17$). Values shown (ΔCt) are relative to those of U6 small RNA.

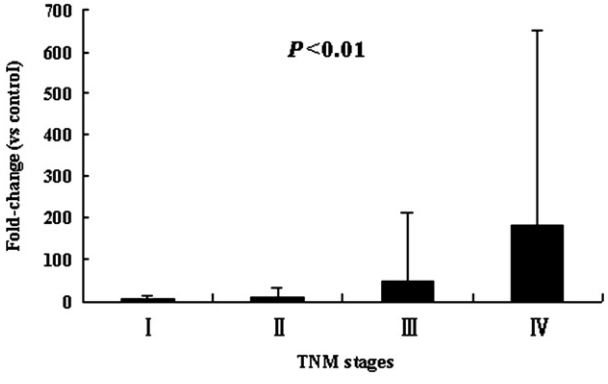

Fig. 3. Expression level of miR-106a was associated with TNM stage. The fold-changes between stages were significantly different.