Biomarkers, 2012; 17(2): 104–110
© 2012 Informa UK, Ltd.
ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.3109/1354750X.2011.614961

informa
healthcare

Biomarkers Downloaded from informahealthcare.com by Changhua Christian Hospital on 11/14/12
For personal use only.

RESEARCH ARTICLE

MiR-421 is a functional marker of circulating tumor cells in gastric cancer patients

Hui Zhou[1#], Bingxiu Xiao[1], Fei Zhou[1], Hongxia Deng[1], Xinjun Zhang[2], Yanru Lou[3], Zhaohui Gong[1], Caigan Du[4], and Junming Guo[1]

[1]*Institute of Biochemistry and Molecular Biology, Ningbo University School of Medicine, Ningbo, China,* [2]*The Affiliated Hospital, Ningbo University School of Medicine, Ningbo, China,* [3]*Ningbo No. 1 Hospital and the Affiliated Hospital, Ningbo University School of Medicine, Ningbo, China, and* [4]*Vancouver Prostate Centre, University of British Columbia, Vancouver, BC, Canada*

Abstract
The detection of circulating tumor cells (CTCs) has recently received great attention. To evaluate if miR-421 could be used as a specific marker for CTCs, the level of miR-421 in mononuclear cells (MNCs) from peripheral blood were determined by reverse transcription-polymerase chain reaction. Transfection of miR-421 inhibitor significantly suppressed tumor growth in vivo. The level of miR-421 in MNCs from gastric cancer was significantly higher than in those from healthy controls. The area under the receiver operating characteristic curve was 0.773 ± 0.0736. In conclusion, miR-421 may be used as a biomarker for monitoring CTCs in patients with gastric cancer.

Keywords: Tumor markers, microRNA-421, gene diagnosis, circulating tumor cells, gastric cancer

Copyright © 2012 Informa UK Limited
Not for Sale or Commercial Distribution
Unauthorized use prohibited. Authorised users can download,
display, view and print a single copy for personal use

Introduction

Gastric cancer is the fourth most common cancer and the second most common cause of death from cancer worldwide (Parkin et al. 2005). Although surgery and adjuvant chemotherapy have been improved, the survival rate of gastric cancer patients after surgical removal is unsatisfactory because most gastric cancer patients are at an advanced stage when diagnosed (Rohatgi et al. 2006). In clinical practice, most biomarkers are associated with atrophic or inflammatory conditions of gastric mucosa; however, these are not reliable or specific for gastric cancer (di Mario and Cavallaro 2008).

MicroRNAs (miRNAs) are noncoding RNAs that are approximately 22 nucleotides in length and that posttranscriptionally regulate gene expression by translational inhibition and destabilization of mRNAs. Recent studies have discovered that miRNAs are involved in the pathogenesis of many types of cancer, including gastric cancer (Ueda et al. 2010). miRNAs show tumor suppressive activity in the pathogenesis of human gastric cancer. For example, miR-375 is downregulated in gastric carcinomas and negatively regulates tumor cell survival by targeting phosphoinositide-dependent protein kinase-1 (PDK1) (Tsukamoto et al. 2010). miR-9 negatively regulates gastric cancer cell proliferation by targeting nuclear factor-κB1 (NF-κB1) (Wan et al. 2010), and miR-101 is downregulated in gastric cancer and is involved in cell migration and invasion (Wang et al. 2010). Furthermore, the expression of anti-proliferative let-7a is reduced in gastric cancer tissues (Zhu et al. 2010b). However, miRNAs may have also oncogenic functions. For example, overexpression of miR-650 in gastric cancer may promote the proliferation of cancer cells (Zhang et al. 2010). miR-23a promotes the growth of gastric adenocarcinoma cell line MGC-803 (Zhu et al. 2010a), and miR-150 in gastric cancer could promote growth of cancer cells at least partially through directly targeting the tumor suppressor early growth response protein-2 (EGR2) (Wu et al. 2010).

[#]Present address: Department of Laboratory Medicine, The First Hospital of Jiaxing, Jiaxing 314000, China

Address for Correspondence: Junming Guo, Institute of Biochemistry and Molecular Biology, Ningbo University School of Medicine, Ningbo 315211, China, E-mail: junmingguo@yahoo.com; Caigan Du, Vancouver Prostate Centre, University of British Columbia, Vancouver, BC V6H 3Z6, Canada, E-mail: caigan@interchange.ubc.ca

(Received 21 June 2011; revised 10 August 2011; accepted 12 August 2011)

RIGHTSLINK

MiR-421 gene is located at the Xq13.2 region of the X chromosome. Our previous studies have demonstrated that miR-421 is overexpressed in gastric cancer tissues compared with noncancerous tissues (Guo et al. 2009; Jiang et al. 2010), and downregulation of miR-421 expression results in decreasing the proliferation of both MGC-803 and SGC-7901 gastric cancer cells *in vitro* (Jiang et al. 2010). Because the high level of miR-421 in gastric cancer tissues do not correlate with clinicopathological features (Jiang et al. 2010), we propose that miR-421 may be involved in the early stages of stomach carcinogenesis and could be used as an efficient early diagnostic biomarker. From a clinical point of view, the detection of circulating tumor cells (CTCs) is emerging as a useful tool for identifying malignancies, monitoring disease progression, and measuring the outcome of therapy. The objective of this study was to evaluate if miR-421 is a functional biomarker for the detection of CTCs in patients with gastric cancer.

Materials and methods

Cell culture

The human gastric cancer cell line MGC-803 was obtained from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Dulbecco's Modified Eagle Medium (Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) at 37°C in a humidified atmosphere of 5% CO_2. Exponentially growing cells were used for experiments.

Transfection of miR-421 inhibitor

Specific 2′-methoxy-modified RNA oligonucleotide, 5′-GCGCCCAAUUAAUGUCUGUUGAU-3′, was used as a miR-421 inhibitor. Nonspecific scrambled sequence, 5′-CAGUACUUUUGUGUAGUACAA-3′, was used as a control. All RNA oligonucleotides were synthesized and purified by Shanghai GenePharma Co. (Shanghai, China). MGC-803 cells were transfected with the RNA oligonucleotides (0.5 or 1.0 μmol/L) according to the protocol recommended by the manufacturer (Invitrogen Corp., Carlsbad, CA) or as reported previously (Jiang et al. 2010).

Tumorigenicity assays by xenograft in nude mice

Female BALB/c nude mice (5–6 weeks old) were purchased from Slac Laboratory Animal Center (Shanghai, China) and bred in specific pathogen-free (SPF) conditions in the animal care facility at Ningbo University (Ningbo, China). The nude mice were mesh caged under controlled conditions of temperature (23–28°C) and light illumination for 12 h a day. MGC-803 cells were transfected with miR-421 inhibitor or negative control using Lipofectamine 2000 (Invitrogen Corp., Carlsbad, CA) following the manufacturer's instructions. Following incubation of cells for 24 h, a total of 3×10^6 cells were collected in 100 μL phosphate buffered solution (PBS) and subcutaneously injected into the backs of nude mice using sterile 22-gauge needles. The general condition of the mice was observed every day. The sizes of tumors were measured every 3 days from the 26th day after inoculation. The size of the tumors was determined by first measuring length (L, cm) and width (W, cm) and then calculating the volume using the formula $V = W^2 \times L \times 0.5$. Following sacrifice of mice, the blood was collected and used to enrich mononuclear cells (MNCs). Every experimental procedure related to animal use in this study was performed and monitored in accordance with the ethical standards and the care of animal and licensing guidelines, issued by the government administrative, under the protocol approved by the Committee on Animal Welfare of Ningbo University.

Patients and specimens

Peripheral blood samples (2 mL per individual) were collected from 40 preoperative gastric cancer patients (29 male, 11 female; 65.3 ± 9.7 and 63.9 ± 13.1 years old, respectively) after fasting, at Ningbo No. 1, No. 2, and No. 3 Hospitals from May 2008 to December 2009. No chemotherapy or other treatments had been used at the time when the blood samples were obtained. Peripheral blood samples from 17 age- and sex-matched healthy volunteers were used as controls. Informed consent was obtained from all participants, and the Human Research Ethics Committee of Ningbo University approved all aspects of this study. Tumors were staged using the tumor node metastasis (TNM) staging of the International Union Against Cancer (Sobin and Wittekind 1997). Histological grade was assessed according to the World Health Organization criteria (Solcia et al. 2000).

Total RNA preparation

A gradient centrifugation with a Ficoll solution (Shanghai Hengxin Chemical Reagents Company Ltd., Shanghai, China) was used for collecting MNCs, from the peripheral blood of patients as described previously (Guo et al. 2004). Total RNA from MNCs was isolated using Trizol reagent (Invitrogen, Karlsruhe, Germany) following the manufacturer's protocol and was dissolved in 10 μl diethypyrocarbonate-treated water, as described in our previous report (Zhou et al. 2010).

The positive control for detecting CTCs was gastric cancer SGC-7901 cells spiked with blood samples from healthy volunteers. Immunohistochemistry using cytokeratin 18 (CK18) and CK20 as gastric cancer-associated epithelial markers confirmed the effectiveness of the experimental procedure for enriching CTCs from blood samples (Zhou et al. 2010).

Detection of miR-421 levels by real-time RT-PCR

cDNA was generated using the miScript Reverse Transcription (RT) Kit (Qiagen GmbH, Hilden, Germany), as described in our previous study (Zhou et al. 2010). Real-time polymerase chain reaction (PCR) was performed using the miScript SYBR Green PCR Kit (Qiagen) on an Mx3005P QPCR System (Stratagene,

Biomarkers Downloaded from informahealthcare.com by Changhua Christian Hospital on 11/14/12
For personal use only.

© 2012 Informa UK, Ltd.

RIGHTSLINK

Biomarkers Downloaded from informahealthcare.com by Changhua Christian Hospital on 11/14/12
For personal use only.

La Jolla, CA). PCR mixture included 6 µl RT product, 10 µl 2×QuantiTect SYBR Green PCR Master Mix, 1 µl 10×miScript Universal Primer (downstream PCR primer for amplifying small RNAs; Qiagen), 1 µl 10×miScript Primer Assay (upstream PCR primer for amplifying miR-421; Qiagen), and 2 µl autoclaved-distilled water. The reaction mixtures were incubated at 95°C for 15 min, followed by 40 amplification cycles of 94°C for 15 s, 60°C for 30 s, and 70°C for 30 s. The threshold cycle (C_t) was defined as the fractional cycle number at which the fluorescence passed the fixed threshold. We also quantified transcripts of U6 small RNA using the Hs_RNU6B_2 miScript Primer Assay (upstream PCR primer for amplifying U6, Qiagen) for normalizing the levels of miR-421 (Jiang et al. 2010). The specificity of this RT-PCR technique was confirmed by dissociation curve analysis using the Mx3005P QPCR System (Stratagene) according to the manufacturer's instruction (data not shown).

To normalize the levels of miR-421, the ΔC_t method was used for analysis (Xiao et al. 2009; Zhou et al. 2010). The ΔC_t value was the difference between the C_t value of miR-421 and the C_t value of U6. ΔC_t values were used to calculate the concentration of miR-421. The experiment was repeated twice and the data were analyzed blind.

The $\Delta\Delta C_t$ value was the difference in ΔC_t between patients and control and the normalized miR-421 expression levels were calculated with the formula $2^{-\Delta\Delta Ct}$. We used a cutoff value of 2.00, with samples having a $2^{-\Delta\Delta Ct}$ value >2.00 considered positive (Markou et al. 2008), and then caculated the positive detection rate for gastric cancer patients.

Detection of carcinoembryonic antigen levels

Serum carcinoembryonic antigen (CEA) was measured using an Elecsys 2010 machine (Roche Diagnostics, Basel, Switzerland). The cut-off concentration used to distinguish abnormal and normal results was 5 µg/L.

Statistical analysis

Statistical analysis was performed using the Statistical Program for Social Sciences (SPSS) software 18.0 (SPSS Inc., Chicago, IL). The level of significance was set at $p<0.05$. The receiver operating characteristic (ROC) curve was constructed for differentiating gastric cancer cases from healthy volunteers (Bachtiar et al. 2009; Zhou et al. 2010). The diagnostic value of using miR-421 as a maker for detecting CTCs of patients with gastric cancer was determined according to the area under the ROC curve (AUC) value, the sensitivity, and the specificity.

Results

Tumor growth was significantly suppressed by miR-421 inhibitor *in vivo*

We previously demonstrated upregulated expression of miR-421 in cancer tissues (Guo et al. 2009) and its oncogenic activity *in vitro* (Jiang et al. 2010). In this study, its oncogenic activity was further examined *in vivo*. MGC-803 cells were transfected with various concentrations of miR-421 inhibitor (0.5 and 1.0 µmol/L) or scrambled sequence (negative control, 1.0 µmol/L). Transfection of miR-421 inhibitor specifically reduced the level of miR-421 in MGC-803 cells (Jiang et al. 2010). The growth of these transfected cells was examined in the xenograft model in BALB/c nude mice. Three independent experiments were performed, and each group in each experiment consisted of 6 mice. As shown in Figure 1, the tumor growth of MGC-803 cells transfected with miR-421 inhibitor was significantly inhibited compared with that of the control group (Figure 1), as evidenced by the dose-dependent tumor volume reductions in the miR-421 inhibitor-transfected group (Figure 1a; 0.5 µmol/L *vs* control, $p=0.004$; 1.0 µmol/L *vs* control, $p=0.007$). At the early stage (29 days and earlier), the smaller dosage (0.5 µmol/L) seemed to most significantly reduce tumor volume (Figure 1a). However, the difference between the two treatment groups was not statistically significant ($p>0.05$). At the latter stage (32 days and later), the bigger dosage (1.0 µmol/L) appeared to significantly reduce tumor volume compared with the smaller dosage ($p<0.05$). The miR-421 levels from MNCs fractions collected from the host were not measurable (data not shown). Therefore, there were no detectable CTCs in the xenograft mice.

Levels of miR-421 in MNCs from peripheral blood of patients with gastric cancer were higher than those from healthy volunteers

Levels of miR-421 in MNCs from the peripheral blood of patients with gastric cancer were compared with those from healthy volunteers according to their ΔC_t values. As shown in Figure 2, the miR-421 levels in patients' samples were higher than those in controls ($p<0.01$). When a $2^{-\Delta\Delta Ct}$ value >2.00 was considered as positive (Markou et al. 2008), the positive detection rate of patients with gastric cancer was 72.50%. However, when CEA was used as a marker, the positive detection rate was only 23.81%.

Relationship of miR-421 level and clinicopathological factors in gastric cancer patients

The relationship of miR-421 levels in MNCs with clinicopathological factors of gastric cancer patients was investigated. As indicated in Table 1, the level of miR-421 was significantly associated with tumor size ($p=0.007$) but not significantly associated with other clinicopathological features, including age, gender, and differentiation. The average $\triangle C_t$ value of group for tumor size smaller than 2 and above was –0.77 and 4.26, respectively. As a result, the experimental difference in $\triangle C_t$ value was 5.03. It was also interesting to note that the correlation of miR-421 with TNM stages ($p=0.077$) was closer to the significance but not with differentiation ($p=0.932$). The levels of miR-421 in IV stages patients were among the highest (Table 1). However, the linear association between miR-421 level and cancer stage was not found and the standard deviation was

RIGHTSLINK

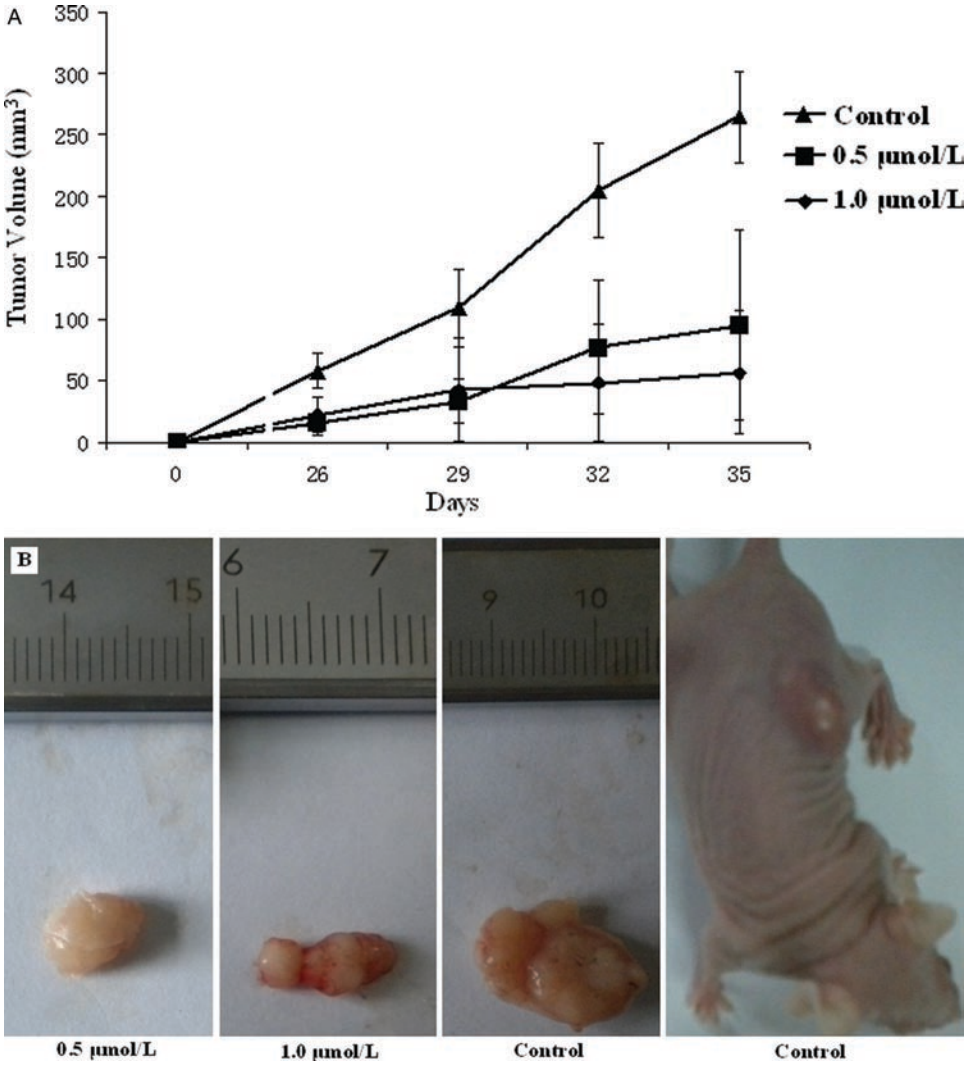

Figure 1. miR-421 inhibitor inhibited tumor growth in nude mice. MGC-803 cells were transfected with miR-421 inhibitor or negative control for 24 h. A total of 3×10^6 cells were subcutaneously injected into the back of nude mice. **A:** The size of the tumors was determined by first measuring length (L, cm) and width (W, cm) and then the volume was calculated using the formula $V = W^2 \times L \times 0.5$. $p = 0.004$ (0.5 μmol/L *vs* control), $p = 0.007$ (1.0 μmol/L *vs* control). **B:** Representative tumor following mouse sacrifice.

extremely large. This may be due to the limited number of patients included in this study.

Diagnostic efficiency of miR-421 in the detection of CTCs in patients with gastric cancer

To evaluate the clinical value of miR-421 as a CTC marker, the AUC value from ROC curve analysis was determined. As shown in Figure 3, the AUC was up to 0.773 ± 0.0736 ($p = 0.0002$), which indicated that it might be useful in clinical diagnosis (Zweig and Campbell 1993). The 95% confidence interval was 0.643 to 0.873. The criterion value (cutoff value) was 7.075 with a sensitivity of 94.12%, and the specificity was 62.50%.

Discussion

To date, the function of miR-421 has not been fully understood. Hu et al. reported that ataxia-telangiectasia mutated (ATM), which plays a central role in the maintenance of genomic integrity, is downregulated by N-Myc-regulated miR-421 (Hu et al. 2010). Ectopic expression of miR-421 results in S-phase cell cycle checkpoint changes and an increase in sensitivity to ionizing radiation (Hu et al. 2010). Our previous studies report that miR-421 is significantly elevated in gastric cancer tissues (Guo et al. 2009; Jiang et al. 2010). Furthermore, *in vitro* research shows that inhibition of miR-421 expression reduces the proliferation of gastric cancer cells by upregulating the expression of its cancer-related target genes *CBX7* (chromobox homolog), and *RBMXL1* (RNA binding motif protein, X-linked-like 1) (Jiang et al. 2010). CBX7 was shown to be associated with several types of cancers (Bernard et al. 2005). RBMXL1 was reported to be associated with apoptosis-related genes such as Bcl-2, Bax, c-erb-B2, and p53 in breast cancer (Martínez-Arribas et al. 2006). A recent study also demonstrated that miR-421 is a potent regulator of deleted in pancreatic carcinoma locus 4

Biomarkers Downloaded from informahealthcare.com by Changhua Christian Hospital on 11/14/12
For personal use only.

© 2012 Informa UK, Ltd.

RIGHTSLINK

(DPC4), a tumor suppressor (Hao et al. 2011). The data presented in this study indicate that the use of miR-421 inhibitor significantly suppresses tumor growth *in vivo* (Figure 1). MiR-421 may be involved in the early stages of stomach carcinogenesis (Jiang et al. 2010). A durable tumor growth inhibitory effect was found in our study (Figure 1). The effectiveness of using miRNA inhibitor in xenograft models and its tumor growth inhibitory effects has been confirmed in several studies (Pichiorri et al. 2008; Su et al. 2009).

Table 1. The relationship of miR-421 level and clinicopathological features of patients with gastric cancer.

Clinicopathological features	No. of patient*	miR-421(average fold-change ± SD)	*p* value
Age (years)			
<60	15	4648.84 ± 5787.59	0.723
≥60	25	3303.81 ± 4703.27	
Gender			
Male	30	4321.81 ± 5635.32	0.636
Female	10	2369.16 ± 2922.71	
Tumor size (cm)			
<2	3	6112.43 ± 3971.21	0.007
≥2	17	4582.64 ± 6067.46	
TNM stage			
I	8	5385.82 ± 5143.98	0.077
II	13	1110.97 ± 1591.43	
III	4	1082.50 ± 2149.46	
IV	5	7295.50 ± 8212.39	
Differentiation			
Poor	16	3654.12 ± 4151.83	0.932
Moderate–Poor	9	5257.38 ± 7177.45	
Moderate	6	4600.72 ± 6482.21	

*Some of clinicopathological features were not indentified.

The *in vivo* and *in vitro* results discussed above clearly imply the feasibility of using miR-421 as a marker for the diagnosis of gastric carcinoma. In the past several years, the important roles of miRNAs in cancer cells have been recognized (Ueda et al. 2010; Wu et al. 2010). MiRNAs may have potential value as diagnostic, prognostic, and predictive biomarkers and may also be considered as therapeutic targets (Kosaka et al. 2010). Specific biomarkers, which can be sampled non-invasively, are mostly welcomed by patients. Recently, it has been discovered that extracellular miRNAs are stably present in body fluids from both healthy people and patients (Gilad et al. 2008; Mitchell et al. 2008). However, their secretory mechanism and biological function, as well as the clinical value of the existence of extracellular miRNAs, remain largely unclear (Kosaka et al. 2010). In another study from our laboratory, we established a new potential way of monitoring gastric carcinoma using the expression pattern of miR-106a and miR-17 in peripheral blood (Zhou et al. 2010). Differing from body fluids, the miRNAs in CTCs are from living cells, and CTCs are major source of mortality in patients with cancer because they can travel in the bloodstream via circulation to distant sites (Lurje et al. 2010).

The transcripts of protein-coding genes such as *CEA* mRNA have been used as biomarkers to detect CTCs of patients with gastric cancer and other cancers (Ikeguchi

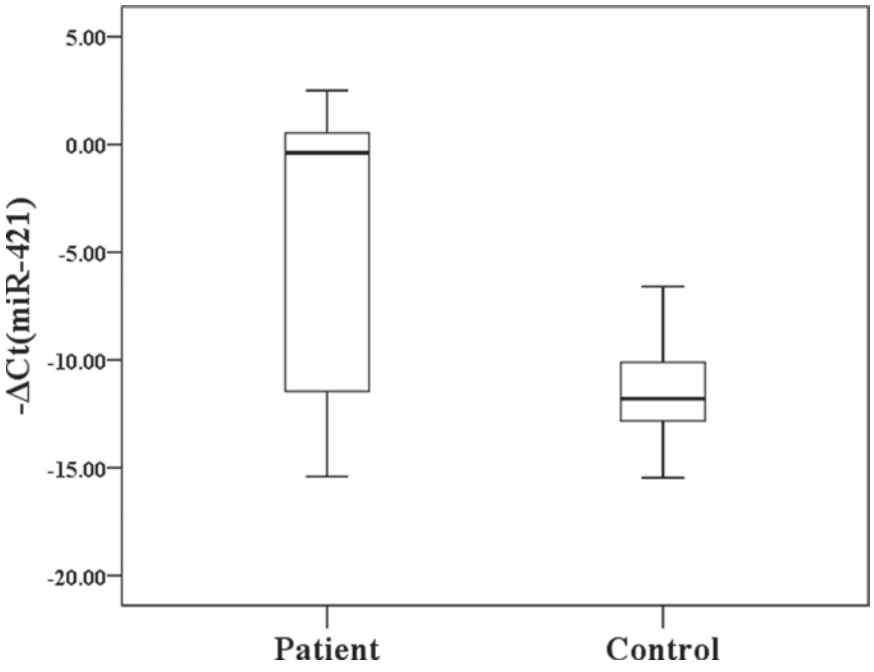

Figure 2. Levels of miR-421 in MNCs from peripheral blood of patients with gastric cancer were higher than that from healthy volunteers. The ΔC_t is equal to the difference between threshold cycles (C_t) for miR-421 (target) and those for U6 RNA (reference). First, the miR-421 and U6 RNA were amplified separately. Then, the C_t for each sample was determined by Mx3005P QPCR System software (Stratagene, La Jolla, CA). Finally, the ΔC_t value was calculated. $\Delta C_t = C_t$ (miR-421) – C_t (U6). Data were expressed as the mean ± standard deviation. The smaller the ΔC_t value or the bigger the $-\Delta C_t$ value indicates higher level of miR-421 expression. Patients ($n = 40$) *vs* control ($n = 17$), $p < 0.01$.

Biomarkers Downloaded from informahealthcare.com by Changhua Christian Hospital on 11/14/12
For personal use only.

RIGHTSLINK

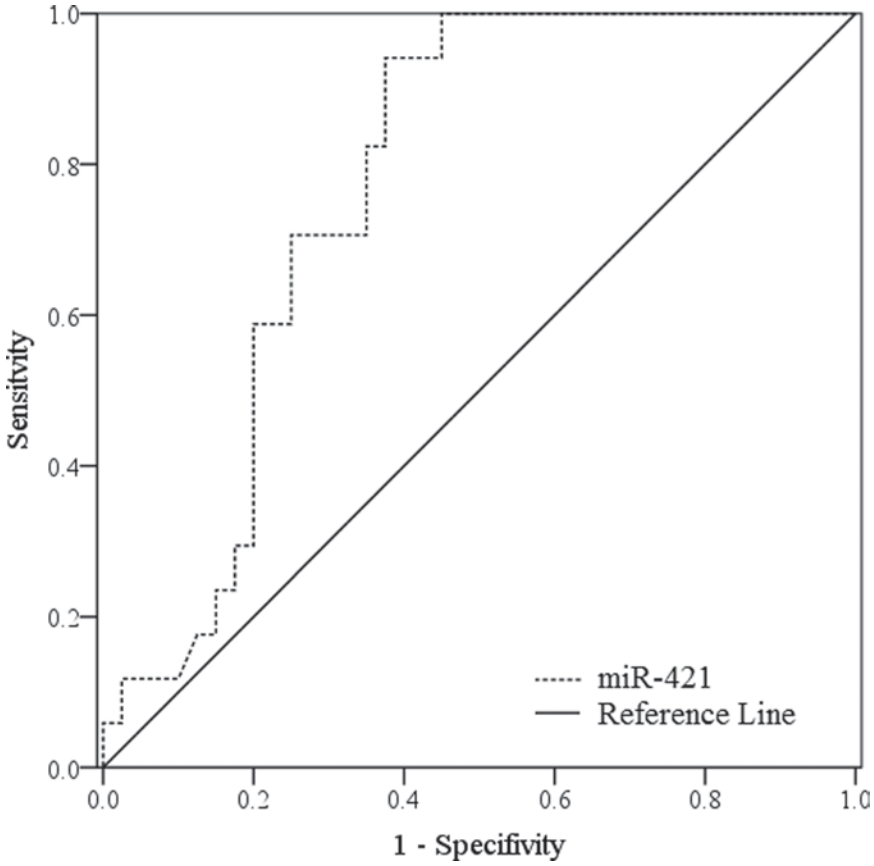

Figure 3. Receiver operation characteristic (ROC) curve. The clinical values were assessed by differentiating 40 preoperative gastric cancer patients from 17 healthy volunteers. The AUC is 0.773 ± 0.0736 ($p = 0.0002$).

Biomarkers Downloaded from informahealthcare.com by Changhua Christian Hospital on 11/14/12
For personal use only.

and Kaibara 2005; Miyazono et al. 2001). However, the specificity of mRNAs as biomarkers remains elusive. *CEA* mRNA was only detected in the blood of 36.8% gastric cancer patients (Miyazono et al. 2001). Unlike mRNA profiling, miRNA expression profiling may hold more promise for discriminating between tumor types (Nelson and Weiss 2008; Zhou et al. 2010). Our results show that the levels of miR-421 in MNCs from peripheral blood of patients with gastric cancer are higher than those from control (Figure 2), and when miR-421 was used as a marker the positive detection rate for patients with gastric cancer was up to 72.50% and the AUC of is 0.773 ± 0.0736 (Figure 3). To date, no data show evidence that miR-421 is upregulated in MNCs, and miR-421 was only found to be highly expressed in cancer cells (Guo et al. 2009; Hao et al. 2011; Jiang et al. 2010). All these data imply that miR-421 might be a potential biomarker for detecting CTCs in patients, including gastric cancer patients. In addition, the levels of miR-421 in MNCs from peripheral blood samples (Table 1) are significantly associated with tumor size, suggesting that miR-421 might play an important role in the early stages of gastric cancer. To demonstrate the relationship between miR-421 levels and early stage carcinogenesis, the stratification of tumor size was performed at 2 cm (Table 1), not at 5 cm as in most reports (Cheng et al. 2011; Xiao et al. 2009). As we found, tumor size seems to reflect the numbers of circulating tumor cells.

In our previous study, we found that the cutoff value for the use of miR-106a or miR-17 as a marker to differentiate gastric cancer patients from health controls was 6.535 or 6.190, respectively (Zhou et al. 2010). Here, we found that the cutoff value for the use of miR-421 as a marker was 7.075. These mean that miR-421 in gastric cancer cells was less expressed than miR-106a and miR-17 did. The differential expression levels among miR-421, miR-106a, and miR-17 have been confirmed in another report (Guo et al. 2009). From the results of recovery experiments (Zhou et al. 2010) and according to the cutoff value, when the quantity of gastric cancer cells in 2 mL of blood reached 10, miR-106a and miR-17 could be detected. Since miR-421 in gastric cancer cells is expressed at lower levels than miR-106a and miR-17, we suppose the detectable number of CTCs should be more than 10 in 2 mL of blood.

In conclusion, a decrease in miR-421 expression in gastric cancer cells significantly suppresses tumor growth *in vivo*, which indicates that miR-421 might play a crucial role in the carcinogenesis. Furthermore, the detection of miR-421 in MNCs from peripheral blood may be a novel method for monitoring CTCs in patients with gastric cancer.

Declaration of interest

This work was supported by Natural Science Foundation of Ningbo (No. 2010A610044), Zhejiang Provincial Research

© 2012 Informa UK, Ltd.

RIGHTSLINK

Project (No. 2010C33112), National Natural Science Foundation of China (No. 30872420), the Scientific Research Foundation of Graduate School of Ningbo University (No. G10JA026), the Excellent Dissertation Foundation of Ningbo University (No. 201014), the Scientific Innovation Team Project of Ningbo (No. 2011B82014), and K. C. Wong Magna Fund in Ningbo University. The authors declare that they have no conflict of interest.

References

Bachtiar I, Santoso JM, Atmanegara B, Gani RA, Hasan I, Lesmana LA, Sulaiman A, Gu J, Tai S. (2009). Combination of alpha-1-acid glycoprotein and alpha-fetoprotein as an improved diagnostic tool for hepatocellular carcinoma. *Clin Chim Acta* 399:97–101.

Bernard D, Martinez-Leal JF, Rizzo S, Martinez D, Hudson D, Visakorpi T, Peters G, Carnero A, Beach D, Gil J. (2005). CBX7 controls the growth of normal and tumor-derived prostate cells by repressing the Ink4a/Arf locus. *Oncogene* 24:5543–5551.

Cheng J, Guo JM, Xiao BX, Miao Y, Jiang Z, Zhou H, Li QN. (2011). piRNA, the new non-coding RNA, is aberrantly expressed in human cancer cells. *Clin Chim Acta* 412:1621–1625.

di Mario F, Cavallaro LG. (2008). Non-invasive tests in gastric diseases. *Dig Liver Dis* 40:523–530.

Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, Benjamin H, Kushnir M, Cholakh H, Melamed N, Bentwich Z, Hod M, Goren Y, Chajut A. (2008). Serum microRNAs are promising novel biomarkers. *Plos ONE* 3:e3148.

Guo J, Miao Y, Xiao B, Huan R, Jiang Z, Meng D, Wang Y. (2009). Differential expression of microRNA species in human gastric cancer versus non-tumorous tissues. *J Gastroenterol Hepatol* 24:652–657.

Guo J, Xiao B, Zhang X, Jin Z, Chen J, Qin L, Mao X, Shen G, Chen H, Liu Z. (2004). Combined use of positive and negative immunomagnetic isolation followed by real-time RT-PCR for detection of the circulating tumor cells in patients with colorectal cancers. *J Mol Med* 82:768–774.

Hao J, Zhang S, Zhou Y, Liu C, Hu X, Shao C. (2011). MicroRNA 421 suppresses DPC4/Smad4 in pancreatic cancer. *Biochem Biophys Res Commun* 406:552–557.

Hu H, Du L, Nagabayashi G, Seeger RC, Gatti RA. (2010). ATM is down-regulated by N-Myc-regulated microRNA-421. *Proc Natl Acad Sci USA* 107:1506–1511.

Ikeguchi M, Kaibara N. (2005). Detection of circulating cancer cells after a gastrectomy for gastric cancer. *Surg Today* 35:436–441.

Jiang Z, Guo J, Xiao B, Miao Y, Huang R, Li D, Zhang Y. (2010). Increased expression of miR-421 in human gastric carcinoma and its clinical association. *J Gastroenterol* 45:17–23.

Kosaka N, Iguchi H, Ochiya T. (2010). Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. *Cancer Sci* 101:2087–2092.

Lurje G, Schiesser M, Claudius A, Schneider PM. (2010). Circulating tumor cells in gastrointestinal malignancies: current techniques and clinical implications. *J Oncol* 2010:392652.

Markou A, Tsaroucha EG, Kaklamanis L, Fotinou M, Georgoulias V, Lianidou ES. (2008). Prognostic value of mature microRNA-21 and microRNA-205 overexpression in non-small cell lung cancer by quantitative real-time RT-PCR. *Clin Chem* 54:1696–1704.

Martínez-Arribas F, Agudo D, Pollán M, Gómez-Esquer F, Díaz-Gil G, Lucas R, Schneider J. (2006). Positive correlation between the expression of X-chromosome RBM genes (RBMX, RBM3, RBM10) and the proapoptotic Bax gene in human breast cancer. *J Cell Biochem* 97:1275–1282.

Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. (2008). Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* 105:10513–10518.

Miyazono F, Natsugoe S, Takao S, Tokuda K, Kijima F, Aridome K, Hokita S, Baba M, Eizuru Y, Aikou T. (2001). Surgical maneuvers enhance molecular detection of circulating tumor cells during gastric cancer surgery. *Ann Surg* 233:189–194.

Nelson KM, Weiss GJ. (2008). MicroRNAs and cancer: past, present, and potential future. *Mol Cancer Ther* 7:3655–3660.

Parkin DM, Bray F, Ferlay J, Pisani P. (2005). Global cancer statistics, 2002. *CA Cancer J Clin* 55:74–108.

Pichiorri F, Suh SS, Ladetto M, Kuehl M, Palumbo T, Drandi D, Taccioli C, Zanesi N, Alder H, Hagan JP, Munker R, Volinia S, Boccadoro M, Garzon R, Palumbo A, Aqeilan RI, Croce CM. (2008). MicroRNAs regulate critical genes associated with multiple myeloma pathogenesis. *Proc Natl Acad Sci USA* 105:12885–12890.

Rohatgi PR, Yao JC, Hess K, Schnirer I, Rashid A, Mansfield PF, Pisters PW, Ajani JA. (2006). Outcome of gastric cancer patients after successful gastrectomy: influence of the type of recurrence and histology on survival. *Cancer* 107:2576–2580.

Sobin L, Wittekind C. (1997). International Union against Cancer. In: Sobin LH, Gospodarowicz MK, Wittekind C, editors. *TNM classification of malignant tumours*. 5th ed. New York: John Wiley, pp. 59–62.

Solcia E, Klöppel G, Sobin L, Williams E. (2000). Endocrine tumours of the gastrointestinal tract. In: Solcia E, editor. *Histological typing of endocrine tumours*. Berlin: Springer, pp. 61–68.

Su H, Yang JR, Xu T, Huang J, Xu L, Yuan Y, Zhuang SM. (2009). MicroRNA-101, down-regulated in hepatocellular carcinoma, promotes apoptosis and suppresses tumorigenicity. *Cancer Res* 69:1135–1142.

Tsukamoto Y, Nakada C, Noguchi T, Tanigawa M, Nguyen LT, Uchida T, Hijiya N, Matsuura K, Fujioka T, Seto M, Moriyama M. (2010). MicroRNA-375 is downregulated in gastric carcinomas and regulates cell survival by targeting PDK1 and 14-3-3zeta. *Cancer Res* 70:2339–2349.

Ueda T, Volinia S, Okumura H, Shimizu M, Taccioli C, Rossi S, Alder H, Liu CG, Oue N, Yasui W, Yoshida K, Sasaki H, Nomura S, Seto Y, Kaminishi M, Calin GA, Croce CM. (2010). Relation between microRNA expression and progression and prognosis of gastric cancer: a microRNA expression analysis. *Lancet Oncol* 11:136–146.

Wan HY, Guo LM, Liu T, Liu M, Li X, Tang H. (2010). Regulation of the transcription factor NF-kappaB1 by microRNA-9 in human gastric adenocarcinoma. *Mol Cancer* 9:16.

Wang HJ, Ruan HJ, He XJ, Ma YY, Jiang XT, Xia YJ, Ye ZY, Tao HQ. (2010). MicroRNA-101 is down-regulated in gastric cancer and involved in cell migration and invasion. *Eur J Cancer* 46:2295–2303.

Wu Q, Jin H, Yang Z, Luo G, Lu Y, Li K, Ren G, Su T, Pan Y, Feng B, Xue Z, Wang X, Fan D. (2010). MiR-150 promotes gastric cancer proliferation by negatively regulating the pro-apoptotic gene EGR2. *Biochem Biophys Res Commun* 392:340–345.

Xiao B, Guo J, Miao Y, Jiang Z, Huan R, Zhang Y, Li D, Zhong J. (2009). Detection of miR-106a in gastric carcinoma and its clinical significance. *Clin Chim Acta* 400:97–102.

Zhang X, Zhu W, Zhang J, Huo S, Zhou L, Gu Z, Zhang M. (2010). MicroRNA-650 targets ING4 to promote gastric cancer tumorigenicity. *Biochem Biophys Res Commun* 395:275–280.

Zhou H, Guo JM, Lou YR, Zhang XJ, Zhong FD, Jiang Z, Cheng J, Xiao BX. (2010). Detection of circulating tumor cells in peripheral blood from patients with gastric cancer using microRNA as a marker. *J Mol Med* 88:709–717.

Zhu LH, Liu T, Tang H, Tian RQ, Su C, Liu M, Li X. (2010a). MicroRNA-23a promotes the growth of gastric adenocarcinoma cell line MGC803 and downregulates interleukin-6 receptor. *Febs J* 277:3726–3734.

Zhu YM, Zhong ZX, Liu ZM. (2010b). Relationship between let-7a and gastric mucosa cancerization and its significance. *World J Gastroenterol* 16:3325–3329.

Zweig MH, Campbell G. (1993). Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* 39:561–577.

Biomarkers Downloaded from informahealthcare.com by Changhua Christian Hospital on 11/14/12
For personal use only.

RIGHTSLINK