Int J Clin Exp Pathol 2016;9(10):9993-10001
www.ijcep.com /ISSN:1936-2625/IJCEP0028267

Original Article

microRNA-221 functions as a potential tumor promoter in colon cancer via ERK1/2 signaling pathway

Li Zeng[1], Kai-Xue Li[1], Ruo-Dan Liu[1], Yong-Feng Zhang[2], Jing-Jing Hu[1], Ying Xiong[1]

[1]Department of Gastroenterology, The First Affiliated Hospital of Shenzhen University, The Second People's Hospital of Shenzhen, Shenzhen, China; [2]Shenzhen Clinical Institute of Integrated Traditional Chinese and Western Medicine, The First Affiliated Hospital of Shenzhen University, The Second People's Hospital of Shenzhen, Shenzhen, China

Received March 15, 2016; Accepted May 26, 2016; Epub October 1, 2016; Published October 15, 2016

Abstract: MicroRNAs belong to a class of highly conserved small non-coding RNAs that function as posttranscriptional gene regulators. The aim of this study is to investigate the role of miR-221 in human colon cancer (CC). Total RNA was isolated from 45 stool samples with CC or healthy controls and different CC cell lines. The expression of miR-221 was then detected by Real-time PCR. After miR-221 inhibition in CC cell lines, the cell viability was measured by CCK-8 assay and the cell apoptosis and cell cycle analysis were performed by flow cytometry, respectively. Transwell assay was used to determine the cell invasion of CC cell lines. Expression of apoptosis and invasion-associated factors were measured by Real-time PCR and Western blot. We found that miR-221 was confirmed to be significantly higher in stool of CC patients and LOVO CC cell lines. Inhibition of miR-221 repressed cell viability and invasion of LOVO cells, and cell cycle arrest and apoptosis were also found in LOVO cells with miR-221 inhibition. Apoptotic factors p53, caspase-3 and PTEN were upregulated by inhibition of miR-221, while invasive factors Snail, MMP9 and VEGF were downregulated by inhibition of miR-221 with upregulated E-cadherin exception. Inhibition of miR-221 also induced ERK1/2 signaling inactivation. Taken together, we identified miR-221 that were overexpressed in CC and which may be correlated with colorectal carcinogenesis via ERK1/2 signaling pathway.

Keywords: Colon cancer, miR-221, apoptosis, invasion

Introduction

Colon cancer is the fourth leading cause of cancer-related deaths worldwide, and the third most common cancer, with more than one million cases diagnosed each year [1, 2]. Survival is inversely related to cancer stages, with up to 90% of deaths preventable if detected early; however, it is often asymptomatic in its early stages and remains undiagnosed until advanced stages, where prognosis becomes poor, with the five year survival rate ranges from 40 to 60% [3]. Its high incidence and poor prognosis mean that the mechanisms of CC carcinogenesis and progression have been the subjects of much attention in recent years. For all cancers, the overwhelming cause of death from CC is metastasis, a complex series of multiple sequential steps during which primary tumor translocates to a distant organ and forms a secondary tumor [4]. Several factors have been identified as playing critical roles in its pathogenesis. Oncogenic activation of intracellular signaling pathways, such as EGFR, Wnt, TGF-β and PI3K pathways, have been shown to play substantial roles in maintaining the growth and progression of colon cancer cells [5]. Understanding the molecular mechanism underlying CC carcinogenesis is therefore essential for developing therapies for CC patients.

MicroRNAs (miRNAs) are short non-coding RNAs play important role in almost all biological pathways. It has been reported that miRNAs regulate 30 to 60% of human genes [6], and that are therefore implicated in biological processes such as differentiation [7], cell cycle control [8], apoptosis [9] and metabolism [10]. They have been shown that be also involved in many diseases as well as cancers. They can control several cancer-relevant processes such as proliferation, apoptosis [11], migration and

invasion [12]. MicroRNAs can target up to several hundred mRNAs, which makes them very powerful regulators and an aberrant miRNA expression can disturb a multitude of cell signaling pathways and profoundly influence cancer onset and progression. A number of studies were recently published that focus on the significance of miRNAs in colorectal cancer and several miRNAs participate in the pathogenesis of colon cancer through controlling the expression of key signaling molecules [13, 14]. However, elucidating the roles of miRNAs in cancer biology, especially in colon cancer, remains an ongoing process.

miR-221 belongs to the miR-221/222 family function as a known suppressor of the p27 protein and involved in tumor development by regulating cell proliferation and cell cycle. In support of its role in tumorigenesis, upregulation of miR-221 has been shown to induce cell proliferation and reduce apoptosis in multiple cancers including lung, breast and liver cancer [15]. miR-221 overexpression promotes hepatocellular carcinoma cell proliferation through modulating cyclin dependent kinase inhibitors (CDKIs), including CDKN1C/p57 and CDKN1B/p27 [16]. miR-221 regulates gastric carcinoma cell proliferation and radioresistance by targeting PTEN [17]. ERK signal regulates miR-221 expression, and that these miR-221 might contribute to NGF-dependent cell survival in PC12 cells [18]. miR-221 was also significantly higher in stool as potential biomarkers for diagnosis of colon cancer [19]. However, its role and molecular mechanism involved in cell proliferation, apoptosis and invasion of CC processes remains unexplored.

In this study, we explored the possibility that miR-221 may be involved in CC cell proliferation, apoptosis and invasion and partially elucidated the molecular mechanism underlying its effects. We showed that miR-221 promoted cell proliferation and invasion and suppressed apoptosis may through ERK1/2 signaling pathway, which provides a candidate target for CC treatment.

Materials and methods

Patients and sample collection

Stool samples were collected from 90 subjects including 45 CC and 45 healthy subjects using a 30 mL disposable stool sample container with screw cap. The containers were manufactured under aseptic conditions to eliminate any biological contamination. Stool samples from CC patients were collected 7 days after colonoscopy, whereas stool samples from healthy controls were collected before bowel purgation and colonoscopy. Following collection, all stool samples were immediately stored at 4°C, and transferred to a -80°C freezer within 24 h.

Cell culture

Colon cancer (CC) cell lines including HT-29, SW1116, SW480, LOVO and HCT116 were obtained from the Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China). Cells were cultured in DMEM supplemented with 10% fetal bovine serum, 100 units/mL penicillin and 100 μg/mL streptomycin, and incubated in a humidified atmosphere at 37°C with 5% CO_2.

miRNA transfection

LOVO cells were seeded in antibiotic-free medium the day before transfection. The LOVO cells were transfected with 50 nmol/L of negative control miRNA (Mock), miR-221 inhibitor (Anti-miR-10b) obtained from Beyotime (Shanghai, China) that knockdown of miR-221 by using lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA) according to the instructions provided by the manufacturer. After 48 h, the transfected cells were collected and analyzed as following experiments.

Cell viability assay

Cell viability was assessed by Cell Counting Kit (CCK)-8 kit (Tongren, Shanghai, China). Briefly, 4×10^3 cells were seeded in each 96-well plate, and further incubated for 0, 24, 48 and 72 h, respectively. CCK-8 reagent was added to each well at 1 h before the endpoint of incubation. The optical density (OD) 450 nm values in each well were determined by a microplate reader (Thermo Fisher Scientific Inc., Waltham, MA, USA). Experiments were repeated at least three times each time in triplicate.

Cell cycle and apoptosis assays

After 48 h of transfection, cells were harvested and cell cycle distribution was analyzed using propidium iodide (PI, Sigma-Aldrich) staining on

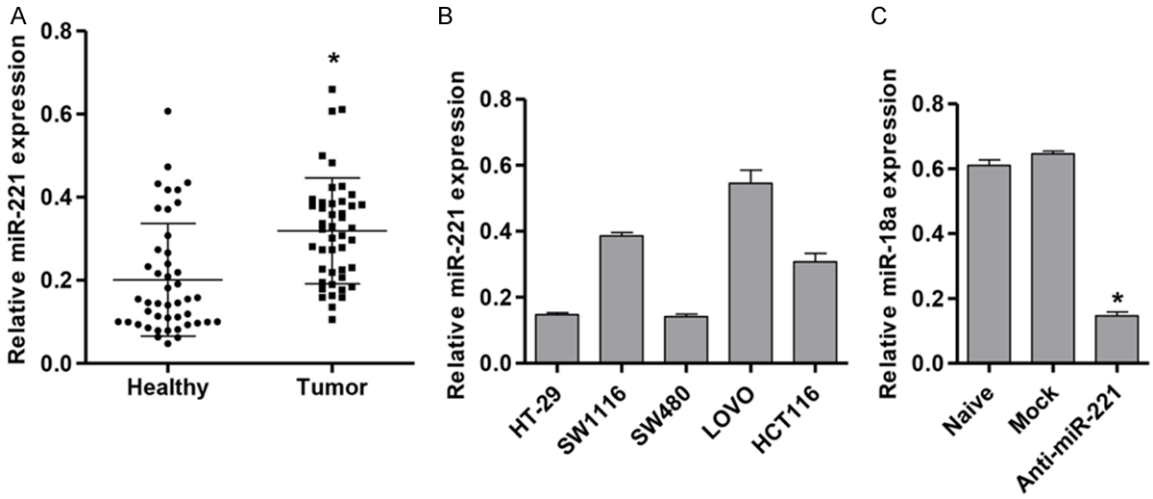

Figure 1. Upregulation of miR-221 expression in stool of CC patients and CC cell lines. A. The expression level of miR-221 was detected by Real-time PCR, and the expression of miR-221 was significantly increased in stool samples than in healthy controls. B. The protein expression of miR-221 in the LOVO CC cell lines was higher than in the four other cell lines, as detected by Western blot. C. 48 h after transfection of anti-miR-221 into LOVO cells, the expression of TRIM27 was significantly decreased than in the untreated Naive cells, as measured by Real-time PCR. GAPDH was used as a loading control. *$P < 0.001$ compared with Healthy control or Naive cells.

a flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) as previously described [20]. After 48 h of transfection, cells were harvested and cell apoptosis was analyzed using Annexin V/PI staining (BD Biosciences) and flow cytometry analysis as previously described [20]. Experiments were repeated at least three times, each time in triplicate.

Transwell assay

The cells were serum-starved for 24 h and subsequently, 1 × 10^5 of cells in 500 μL DMEM were seeded into the upper wells of the Transwell (Corning, Inc., Corning, NY, USA) coated with Matrigel (BD Biosciences). Cell culture medium, supplemented with 10% FBS, was added into the lower well of the chamber. After 48 h of incubation, the cells migrated into the lower well were fixed with methanol and stained with 0.5% Methylrosanilnium Chloride Solution for 30 min. The cells were counted at a magnification of × 200 and the mean number of cells was recorded. Experiments were repeated at least three times, each time in triplicate.

Reverse transcription and real-Time PCR

Total RNA was isolated from CC patients' stool and transfected cells using Trizol reagent (Invitrogen). Reverse transcription reactions were performed as described [21]. miR-221 was reverse transcripted by the looped primer, which binds to six nucleotides at the 3' portion of miR-221 molecules, respectively. Real-time PCR was performed using a standard SYBR Green PCR kit protocol on ABI7300 (Applied Biosystem, Foster City, CA, USA) thermal cycler. The U6 RNA was used as internal controls for miR-221. The ΔΔCt method for relative quantification of gene expression was used to determine miRNA expression levels. The PCR primers for miR-221 were 5'-ACACTCCAGCTGGGAGCTACATTGTCTGCTG-3' (forward) and 5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGAAACC-3' (reverse). The PCR primers for U6 were 5'-CTCGCTTCGGCAGCACA-3' (forward) and 5'-AACGCTTCACGAATTTGCGT-3' (reverse).

Western blot

25 μg of protein from CC cell lines were subjected to SDS-PAGE using a Bio-Rad miniature slab gel apparatus and electrophoretically transferred onto a nitrocellulose sheet. Membranes were washed and incubated with respective secondary antibodies and were visualized by enhanced chemiluminescence (Millipore, Beijing, China) according to the manufacturer's instructions. Antibodies for caspase-3 (1:500), MMP9 (1:500) and VEGF (1:1000) were purchased from Abcam (Cambridge, MA, USA). Antibodies for PTEN (1:1000), E-cadherin (1:1000), Snail (1:1000), p53 (1:1000), p-ERK1/2 (1:

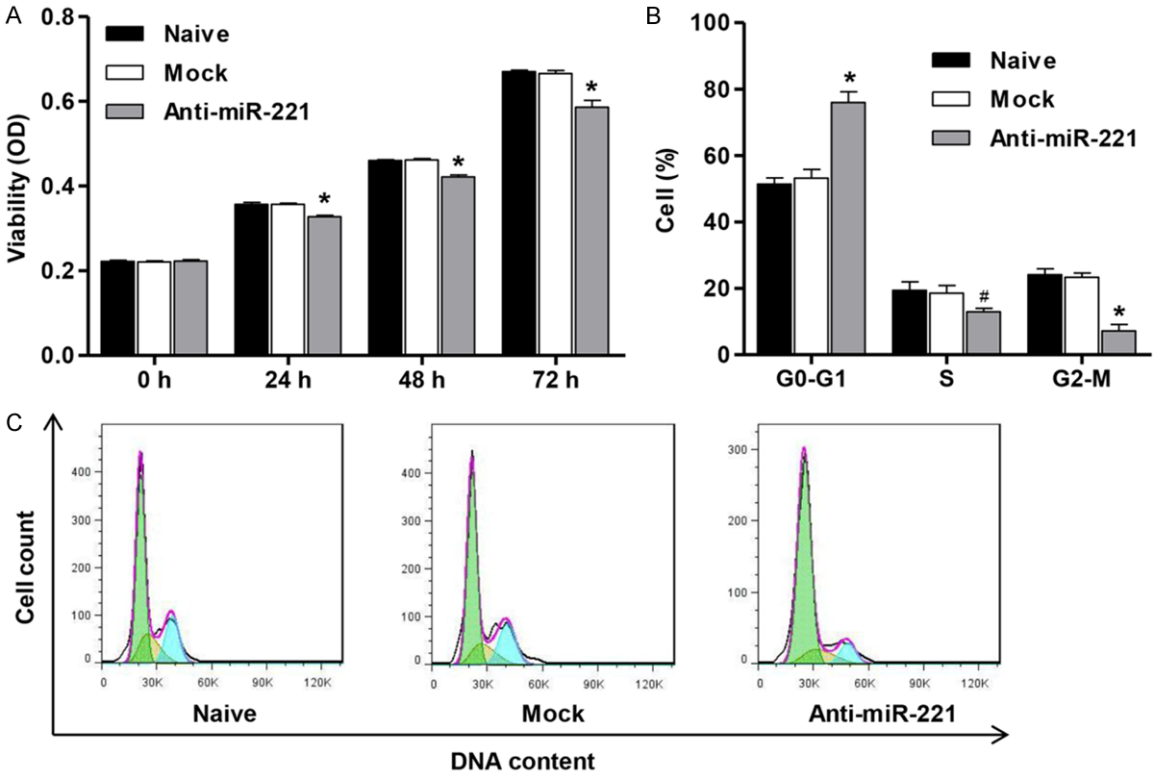

Figure 2. Cell proliferation and cell cycle of LOVO cells after transfection with anti-miR-221. A. Cell proliferation was measured by CCK-8 assay, and transfection of LOVO cells with anti-miR-221 showed significantly decreased proliferation compared with Naive cells. B, C. Cell cycle was assessed using PI staining and flow cytometry. Transfection of LOVO cells with anti-miR-221 showed significantly increased number of G1 cells and decreased number of S and G2-M cells compared with Naive cells. *$P < 0.001$ and #$P < 0.05$ compared with Naive cells.

1000), ERK1/2 (1:1000) and GAPDH (1:1500) were purchased from Cell Signaling Technology, Inc. (Beverley, MA, USA). GAPDH antibody was used as an internal control.

Statistical analysis

Experimental data were presented as mean ± SD of at least three independent replicates through analyzing with SPSS 19.0 and assessing comparisons between different groups by the unpaired, two-tail Student's t test. Differences were considered significant at values of $P < 0.05$.

Results

miR-221 is significantly upregulated in stool samples of CC patients and in CC cell lines

miR-221 has been found to be one of the most upregulated miRNAs in the miRNA expression array in colon cancer [19]. Thus miR-221 was selected for validation in 45 stool samples from CC patients. We found that miR-221 (1.58-fold, $P < 0.0001$) expression was significantly upregulated in stool from CC patients compared with healthy controls (**Figure 1A**). Having documented significantly upregulation of miR-221 in clinical CC patients, we wonder how miR-221 affects CC cell biological behavior. We analyzed miR-221 expression in five CC cell lines, HT-29, SW1116, SW480, LOVO and HCT116 cell lines by Real-time PCR. miR-221 was expressed in higher level in LOVO cell lines compared with another four cell lines (**Figure 1B**). Then, anti-miR-221 was transiently transfected into LOVO cells. As shown in **Figure 1C**, the miRNA-211 was significantly decreased by 76.1% in LOVO cells transfected with anti-miR-221, compared with untransfected LOVO cells (Naive). However, Mock sequence (Mock) had no effect on the expression of miRNA-211.

Inhibition of miR-221 represses cell viability and induces cell cycle arrest in CC cell lines

0, 24, 48 and 72 h after transfection, cell viability was analyzed using CCK-8 assay. As shown

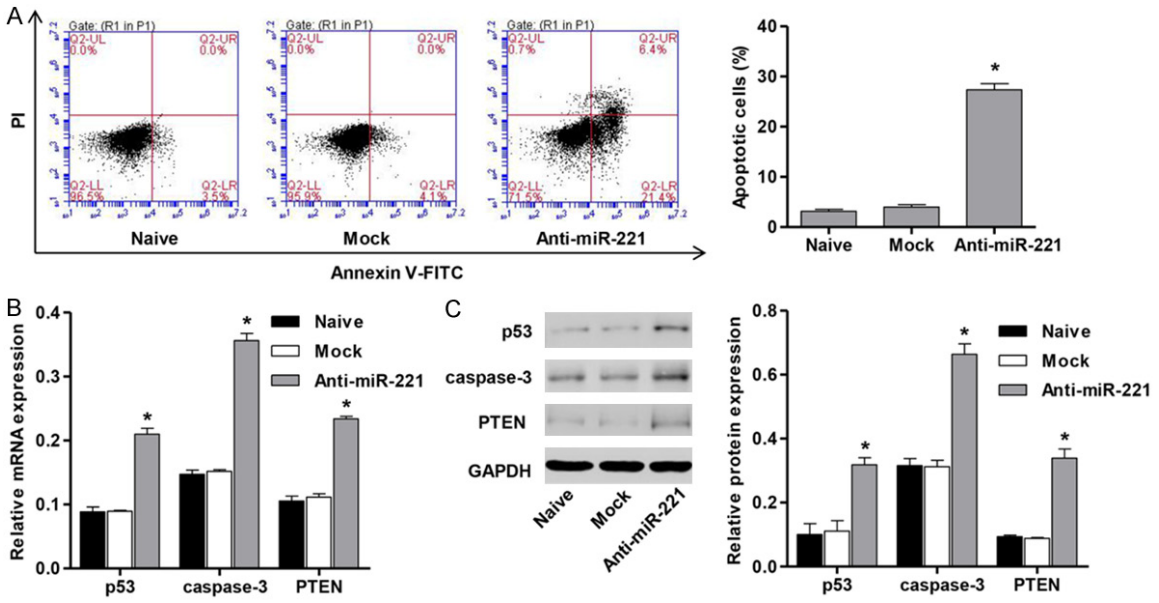

Figure 3. Apoptosis of LOVO cells after transfection with anti-miR-221. A. Cell apoptosis was assessed using annexin V-FITC/PI staining performed staining and flow cytometry. B. The mRNA expression of p53, caspase-3 and PTEN was assessed using Real-time PCR in LOVO cells. C. The production of these proteins was assessed using Western blot in LOVO cells. GAPDH was used as a loading control. *$P < 0.001$ compared with Naive cells.

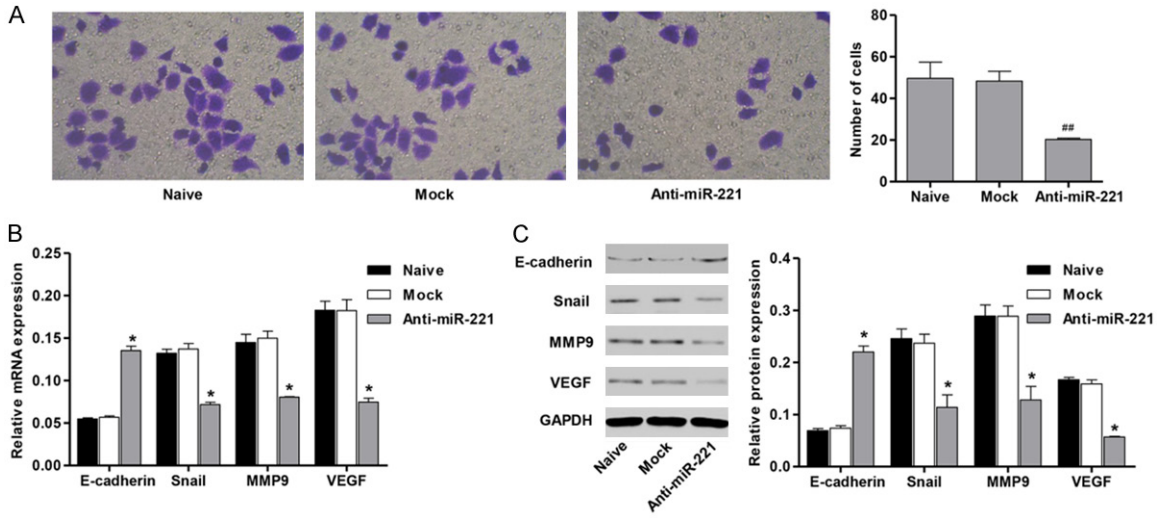

Figure 4. Invasion of LOVO cells after transfection with anti-miR-221. A. Cell invasion was measured by Transwell analysis. B. The mRNA expression of E-cadherin, Snail, MMP9 and VEGF was assessed using Real-time PCR in LOVO cells. C. The production of these proteins was assessed using Western blot in LOVO cells. GAPDH was used as a loading control. *$P < 0.001$ and ##$P < 0.01$ compared with Naive cells.

in **Figure 2A**, inhibition of miRNA-211 induced great inhibition on cell growth compared with Naive cells. However, Mock-transfected cells had no effect on the cell growth of LOVO cells. To further validate the cell proliferation inhibition of anti-miR-221, cell cycle was analyzed in LOVO cells. Cell cycle analysis showed that silencing miR-221 by anti-miR-221 notably increased the population of G0-G1 phase cells and reduced the population of S and G2-M phase cell in LOVO cells (**Figure 2B** and **2C**). However, Mock-transfected cells had no effect on the cell cycle of LOVO cells. These results indicate that silencing miR-221 in CC cell lines

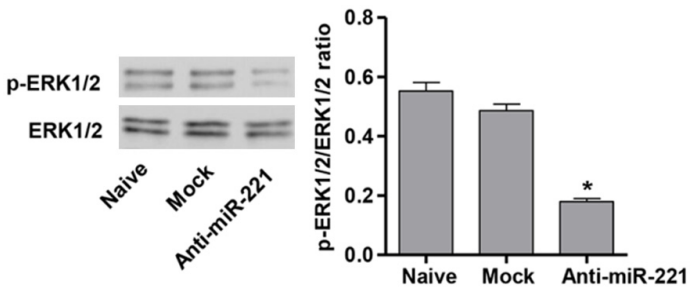

Figure 5. ERK1/2 pathway in LOVO cells after transfection with anti-miR-221. p-ERK1/2 and ERK1/2 protein expression was measured by Western blot in LOVO cells. *$P < 0.001$ compared with Naive cells.

may inhibited cell viability by arresting cell cycle progression in G0/G1 phase.

Inhibition of miR-221 apoptosis in CC cell lines

Then, we evaluated the apoptotic function of miR-221 in LOVO cells by Annexin V-FITC/PI staining assay. As shown in **Figure 3A**, flow cytometry analysis revealed that inhibition of miR-221 in LOVO cells significantly increased cell apoptosis by 7.74-fold compared with Naive cells. However, Mock-transfected cells had no effect on the cell apoptosis of LOVO cells. Having documented significantly effect of miR-221 on apoptosis of CC cell lines, we wonder how miR-221 affects the expression of proteins associated with cell apoptosis. We analyzed three apoptotic factors p53, caspase-3 and PTEN by Real-time PCR (**Figure 3B**) and Western blot (**Figure 3C**). Silence of miR-221 induced upregulation of p53, caspase-3 and PTEN at both mRNA and protein levels. However, Mock-transfected cells had no effect on these protein expressions in LOVO cells. These findings suggest that inhibition of miR-221 affects cell apoptosis via modulating expression of p53/caspase-3/PTEN.

Inhibition of miR-221 suppresses cell invasion in CC cell lines

To investigate the invasion-promoting function of miR-221 in CC cell lines, the invasion capacity of LOVO cells was evaluated by Transwell assay. Knockdown of miR-221 in LOVO cells significantly reduced the cell invasion ability compared with Naive cells (**Figure 4A**). However, Mock-transfected cells had no effect on the cell apoptosis of LOVO cells. Having documented significantly effect of miR-221 on invasion of CC cell lines, we wonder how miR-221 affects the expression of proteins associated with cell invasion. We analyzed four invasion factors E-cadherin, Snail, MMP9 and VEGF by Real-time PCR (**Figure 4B**) and Western blot (**Figure 4C**). Silence of miR-221 induced upregulation of E-cadherin, while induced downregulation of Snail, MMP9 and VEGF at both mRNA and protein levels. However, Mock-transfected cells had no effect on these protein expressions in LOVO cells. These findings suggest that inhibition of miR-221 affects cell apoptosis and invasion via modulating expression of E-cadherin/Snail/MMP9/VEGF in CC cell lines.

Inhibition of miR-221 suppresses ERK1/2 signaling activation

To further investigate the molecular mechanism of miR-221 in CC, p-ERK1/2 and ERK1/2 expression levels were also measured by Western blot. As shown in **Figure 5**, the ratio of p-ERK1/2 and ERK1/2 was significantly decreased in LOVO cells with miR-221 inhibition. However, Mock-transfected cells had no effect on p-ERK1/2 and ERK1/2 expression in LOVO cells. These findings suggest that inhibition of miR-221 affects cell apoptosis and invasion via inactivating ERK1/2 signaling pathway.

Discussion

It has been recognized that tissue miRNAs can serve as accurate diagnosis and prognosis predictors of various tumors. In some situations, obtaining tumor tissues for analysis is challenging. Recent studies detecting aberrantly expressed miRNA in stool has emerged as a promising non-invasive approach to CC screening [22, 23]. Stool miRNAs demonstrate high stability and can be detected with high reproducibility by Real-time PCR. Previous study has investigated the expression profile of 667 miRNAs in a microarray and reported miR-135b and miR-31 as potential biomarkers [24]. In this study, miR-221 was verified in stool samples from 45 CC patients and 45 individuals with normal colonoscopy. miR-221 was significantly upregualted in CC stool compared with controls. Moreover, miR-221 was also upregualted in CC cell lines with highest expression in LOVO cells. Collectively, these results suggest that

the candidate miR-221 regulates key signaling pathways in colorectal tumorigenesis.

miR-221 expression is upregulated in CC, but prior to this study, apart from its ability to suppress cell proliferation inhibitor CDKN1C/p57 *in vitro*, the functional relevance of its upregulation in CC was largely unexplored [16]. miR-221 has been studied for its role in regulating cell proliferation, cell cycle progression and apoptosis in a variety of tumors, including human osteosarcoma [25], melanomas [26] and bladder cancer [27]. In this study we confirm previous reports that miR-221 induced cell proliferation, cell cycle progression and inhibited cell apoptosis in CC LOVO cells. Its invasion and migration promoting effects have been documented for various cancers, including gastric carcinoma [17], melanomas [27] and pancreatic cancer [28]. We subsequently showed that inhibition of miR-221 expression significantly repressed CC cell invasion.

To understand the biological role of miR-221 in CC apoptosis and invasion, we searched for relevant downstream targets. In a number of cancers, miRNAs target cell apoptosis by targeting p53 [29] and PTEN [17]. It has been previously reported that the loss of p53 is one of the most represented genetic abnormalities in cancer, the link between the miR-221 and p53 is important example of microRNA transcriptional regulation [29]. miR-221 targets PTEN, which allows the activation of the PI3K/AKT pathway, resulting in the bypass of the drug induced inhibition of EGFR [30]. In this study, we found that inhibition of miR-221 increased expression of p53, PTEN and caspase-3, suggesting that miR-221 affects cell apoptosis through modulating these three factors. Moreover, we demonstrated that miR-221 knockdown increased E-cadherin but decreased Snail, MMP9 and VEGF expression levels in LOVO cells. Previous studies provide mechanistic evidences that most of the miR-130b-dependent effects are due to PPARγ suppression in CC that in turn deregulates E-cadherin, Snail, and VEGF, key mediators of EMT and angiogenesis involved in cell invasion and migration [31]. Asuthkar et al. found an acute inhibitory effect of miR-211 on glioma cell invasion and migration via suppression of MMP9 [32]. The correlation of miR-221 expression and E-cadherin, Snail, MMP9 and VEGF protein expression in CC cell lines provides further evidence for miR-221's function in invasion as an inhibitor of E-cadherin and a promoter of Snail, MMP9 and VEGF.

We also investigated whether miR-221 regulates MAPK pathways for its pro-proliferative and anti-apoptosis inducing abilities. Since MAPKs are the key components of intracellular signaling for cell fate determination, we examined the phosphorylation status of ERK1/2 in miR-221-downregulated LOVO cells. ERK1/2 is an important candidate protein which assists cell division and proliferation. The inhibitor of ERK1/2 pathways significantly suppressed EMT and cancer stem cell phenotype, indicating that ERK1/2 pathway is required for miR-21 mediating EMT and cancer stem cell phenotype [33]. Furthermore, miR-126 functions as a tumor suppressor in CC cells by targeting CXCR4 via the ERK1/2 signaling pathway [34]. Our results showed that miR-221 inhibition significantly inactivated ERK1/2 pathway, which is in consistent with the previous studies.

In conclusion, this study demonstrates that inhibition of miR-221 effectively inhibited proliferation and invasion of LOVO CC cells, induced G1 cell cycle arrest and apoptosis and caused modulation of apoptosis and invasion-related factors through the inhibition of ERK1/2 pathway. Depletion of miR-221 expression in CC cells may thus provide a new strategy for overcoming CC tumorgenesis.

Acknowledgements

This study was supported by the Science and Technology Project of Shenzhen, China (NO. JCYJ20140414170821182).

Disclosure of conflict of interest

None.

Address correspondence to: Ying Xiong, Department of Gastroenterology, The First Affiliated Hospital of Shenzhen University, The Second People's Hospital of Shenzhen, 3002 Sungang West Road, Futian District, Shenzhen 518035, China. Tel: +86-755-83366388; Fax: +86-755-83366388; E-mail: yingxiongxsz@163.com

References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Weitz J, Koch M, Debus J, Hohler T, Galle PR, Buchler MW. Colorectal cancer. Lancet 2005; 365: 153-165.
[3] Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007; 57: 43-66.
[4] Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. Science 2011; 331: 1559-1564.
[5] Saif MW, Chu E. Biology of colorectal cancer. Cancer J 2010; 16: 196-201.
[6] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136: 215-233.
[7] Harfe BD. MicroRNAs in vertebrate development. Curr Opin Genet Dev 2005; 15: 410-415.
[8] Carleton M, Cleary MA, Linsley PS. MicroRNAs and cell cycle regulation. Cell Cycle 2007; 6: 2127-2132.
[9] Jovanovic M, Hengartner M. miRNAs and apoptosis: RNAs to die for. Oncogene 2006; 25: 6176-6187.
[10] Krützfeldt J, Stoffel M. MicroRNAs: a new class of regulatory genes affecting metabolism. Cell Metab 2006; 4: 9-12.
[11] Hwang H, Mendell J. MicroRNAs in cell proliferation, cell death, and tumorigenesis. Brit J Cancer 2006; 94: 776-780.
[12] Baranwal S, Alahari SK. miRNA control of tumor cell invasion and metastasis. Int J Cancer 2010; 126: 1283-1290.
[13] Valeri N, Braconi C, Gasparini P, Murgia C, Lampis A, Paulus-Hock V, Hart JR, Ueno L, Grivennikov SI, Lovat F. MicroRNA-135b promotes cancer progression by acting as a downstream effector of oncogenic pathways in colon cancer. Cancer Cell 2014; 25: 469-483.
[14] Ogata-Kawata H, Izumiya M, Kurioka D, Honma Y, Yamada Y, Furuta K, Gunji T, Ohta H, Okamoto H, Sonoda H. Circulating exosomal microRNAs as biomarkers of colon cancer. PLoS One 2014; 9: e92921.
[15] Acunzo M, Romano G, Wernicke D, Croce CM. MicroRNA and cancer-a brief overview. Adv Biol Regul 2015; 57: 1-9.
[16] Sun K, Wang W, Zeng JJ, Wu CT, Lei ST, Li GX. MicroRNA-221 inhibits CDKN1C/p57 expression in human colorectal carcinoma. Acta Pharmacol Sin 2011; 32: 375-384.
[17] Chun-Zhi Z, Lei H, An-Ling Z, Yan-Chao F, Xiao Y, Guang-Xiu W, Zhi-Fan J, Pei-Yu P, Qing-Yu Z, Chun-Sheng K. MicroRNA-221 and microRNA-222 regulate gastric carcinoma cell proliferation and radioresistance by targeting PTEN. BMC Cancer 2010; 10: 367.
[18] Terasawa K, Ichimura A, Sato F, Shimizu K, Tsujimoto G. Sustained activation of ERK1/2 by NGF induces microRNA-221 and 222 in PC12 cells. FEBS J 2009; 276: 3269-3276.
[19] Yau T, Wu C, Dong Y, Tang C, Ng S, Chan F, Sung J, Yu J. microRNA-221 and microRNA-18a identification in stool as potential biomarkers for the non-invasive diagnosis of colorectal carcinoma. Brit J Cancer 2014; 111: 1765-1771.
[20] Zhang X, Chen X, Liu J, Dong X, Jin Y, Tian Y, Xue Y, Chen L, Chang Y, Liu Y. Knockdown of WISP1 inhibit proliferation and induce apoptosis in ALL Jurkat cells. Int J Clin Exp Pathol 2015; 8: 15489.
[21] Sun W, Qiu G, Zou Y, Cai Z, Wang P, Lin X, Huang J, Jiang L, Ding X, Hu G. Knockdown of TMEM45A inhibits the proliferation, migration and invasion of glioma cells. Int J Clin Exp Pathol 2015; 8: 12657.
[22] Link A, Balaguer F, Shen Y, Nagasaka T, Lozano JJ, Boland CR, Goel A. Fecal MicroRNAs as novel biomarkers for colon cancer screening. Cancer Epidem Biomark Prev 2010; 19: 1766-1774.
[23] Wu CW, Ng SS, Dong YJ, Ng SC, Leung WW, Lee CW, Wong YN, Chan FK, Yu J, Sung JJ. Detection of miR-92a and miR-21 in stool samples as potential screening biomarkers for colorectal cancer and polyps. Gut 2012; 61: 739-745.
[24] Wu CW, Ng SC, Dong Y, Tian L, Ng SS, Leung WW, Law WT, Yau TO, Chan FK, Sung JJ, Yu J. Identification of microRNA-135b in stool as a potential noninvasive biomarker for colorectal cancer and adenoma. Clin Cancer Res 2014; 20: 2994-3002.
[25] Zhao G, Cai C, Yang T, Qiu X, Liao B, Li W, Ji Z, Zhao J, Zhao H, Guo M. MicroRNA-221 induces cell survival and cisplatin resistance through PI3K/Akt pathway in human osteosarcoma. PLoS One 2013; 8: e53906.
[26] Felicetti F, Errico MC, Bottero L, Segnalini P, Stoppacciaro A, Biffoni M, Felli N, Mattia G, Petrini M, Colombo MP. The promyelocytic leukemia zinc finger-microRNA-221/-222 pathway controls melanoma progression through multiple oncogenic mechanisms. Cancer Res 2008; 68: 2745-2754.
[27] Lu Q, Lu C, Zhou GP, Zhang W, Xiao H, Wang XR. MicroRNA-221 silencing predisposed human bladder cancer cells to undergo apoptosis induced by TRAIL. Urol Oncol Semin Ori Invest 2010; 28: 635-641.
[28] Su A, He S, Tian B, Hu W, Zhang Z. MicroRNA-221 mediates the effects of PDGF-BB on migration, proliferation, and the epithelial-mesenchymal transition in pancreatic cancer cells. PLoS One 2013; 8: e71309.
[29] Pu XX, Huang GL, Guo HQ, Guo CC, Li H, Ye S, Ling S, Jiang L, Tian Y, Lin TY. Circulating miR-221 directly amplified from plasma is a potential diagnostic and prognostic marker of colorectal cancer and is correlated with p53 expression. J Gastroen Hepatol 2010; 25: 1674-1680.

[30] Wang YS, Wang YH, Xia HP, Zhou SW, Schmid-Bindert G, Zhou CC. MicroRNA-214 regulates the acquired resistance to gefitinib via the PTEN/AKT pathway in EGFR-mutant cell lines. Asian Pac J Cancer Prev 2012; 13: 255-260.
[31] Colangelo T, Fucci A, Votino C, Sabatino L, Pancione M, Laudanna C, Binaschi M, Bigioni M, Maggi CA, Parente D. MicroRNA-130b promotes tumor development and is associated with poor prognosis in colorectal cancer. Neoplasia 2013; 15: 1086-1099.
[32] Asuthkar S, Velpula KK, Chetty C, Gorantla B, Rao JS. Epigenetic regulation of miRNA-211 by MMP-9 governs glioma cell apoptosis, chemosensitivity and radiosensitivity. Oncotarget 2012; 3: 1439-1454.
[33] Han M, Liu M, Wang Y, Chen X, Xu J, Sun Y, Zhao L, Qu H, Fan Y, Wu C. Antagonism of miR-21 reverses epithelial-mesenchymal transition and cancer stem cell phenotype through AKT/ERK1/2 inactivation by targeting PTEN. PLoS One 2012; 7: e39520.
[34] Liu Y, Zhou Y, Feng X, An P, Quan X, Wang H, Ye S, Yu C, He Y, Luo H. MicroRNA-126 functions as a tumor suppressor in colorectal cancer cells by targeting CXCR4 via the AKT and ERK1/2 signaling pathways. Int J Oncol 2014; 44: 203-210.