Tumor Suppressor Functions of miR-133a in Colorectal Cancer

Yujuan Dong1,2, Junhong Zhao1, Chung-Wah Wu1, Lijing Zhang3, Xiaodong Liu4, Wei Kang5, Wing-Wah Leung6, Ning Zhang7, Francis K.L. Chan1, Joseph J.Y. Sung1, Simon S.M. Ng2, and Jun Yu1

Abstract

Dysregulated microRNA (miRNA) expression was profiled through a miRNA array comparison between human colorectal cancer tumors and their adjacent normal tissues. Specifically, using laser capture microdissection, miR-133a was shown to be significantly downregulated in primary colorectal cancer specimens compared with matched adjacent normal tissue. Ectopic expression of miR-133a significantly suppressed colorectal cancer cell growth in vitro and in vivo. Cell-cycle analysis revealed that miR-133a induced a G0/G1-phase arrest, concomitant with the upregulation of the key G1-phase regulator p21<sup>Cip1</sup>. We further revealed that miR-133a markedly increased p53 protein and induced p21<sup>Cip1</sup> transcription. Studies in silico revealed that the 3′UTR of the ring finger and FYVE-like domain containing E3-ubiquitin protein ligase (RFFL), which regulates p53 protein, contains an evolutionarily conserved miR-133a binding site. miR-133a repressed RFFL-3′UTR reporter activity and reduced RFFL protein levels, indicating that miR-133a directly bound to RFFL mRNA and inhibited RFFL translation. Moreover, miR-133a sensitized colon cancer cells to doxorubicin and oxaliplatin by enhancing apoptosis and inhibiting cell proliferation. These data add weight to the significance of miR-133a in the development of CRC.

Implications: miR-133a serves as a potential tumor suppressor upstream of p53 in colorectal cancer and may sensitize cells to therapeutics. Mol Cancer Res; 11(9); 1051–60. ©2013 AACR.

Introduction

Colorectal cancer (CRC) is the third most common cancer and the third leading cause of cancer-related death in the world, with an estimated incidence of 1.2 million new cases and a mortality of more than 600,000 deaths annually (1). While CRC carcinogenesis involving multistep progression has been extensively studied at the genetic level, recent data highlight that microRNAs (miRNAs) are closely associated with CRC tumorigenesis. miRNAs belong to a class of highly conserved 22-nucleotide single-stranded RNAs that suppress target genes translation or induce messenger RNA (mRNA) degradation through binding to the 3′-untranslated region (UTR; ref. 2). It is estimated that miRNAs can regulate up to 50% human genes translation (3). By targeting multiple transcripts, miRNAs epigenetically regulate fundamental cellular processes such as cell proliferation, apoptosis, differentiation, and migration, which strongly indicates that they may function as potential oncogenes or tumor suppressors in cancer development. Indeed, a global impairment of miRNA was described in various human cancers including CRCs (4). However, the precise mechanisms of these aberrantly expressed miRNAs in CRC genesis, progression, and therapeutic response are still largely unknown. An investigation into the mechanism of the dysregulated miRNAs in pathogenesis of CRCs can help identify novel molecular events and signal pathways for the development of anticancer therapy. We recently identified dysregulated miRNAs in human CRC through comparison between tumors and their adjacent normal tissues by miRNA array (GSE45349). In this study, we aim to clarify miR-133a biologic function, molecular basis, and target gene in CRCs.

Materials and Methods

Tissue samples

Two cohorts of totally 95 patients with histologically confirmed CRC from 1999 to 2009 who underwent surgery at the Prince of Wales Hospital, Hong Kong, were included.
in this study, including 4 patients for building of the miRNA expression profiles and 91 patients for miRNA validation (Supplementary Tables S1 and 2). CRC tissues and paired adjacent normal tissues were obtained from the resected surgical specimens. The adjacent normal tissue is composed of normal colonic mucosa located approximately 10 cm away from the cancer tissue. In addition, 9 biopsy specimens of normal rectal mucosa from healthy controls and 11 pairs of CRC paraffin-embedded tissue samples were obtained at the Prince of Wales Hospital. All subjects provided informed consent before specimen collection. The study protocol was approved by the Ethics Committee of The Chinese University of Hong Kong.

**Cell culture**

Cell lines included in the present study (Caco2, colo205, DLD1, HCT116, HT29, LoVo, LS180, SW480, SW620) were obtained from American Type Culture Collection and propagated at 37°C in the presence of 5% CO2. LoVo was cultured in Dulbecco’s Modified Eagle’s Medium (Gibco BRL) supplemented with 10% (v/v) FBS (HyClone, Thermo Fisher Scientific). HCT116 and HT29 were cultured in McCoy’s 5A medium (Sigma-Aldrich) containing 10% (v/v) FBS. When required, media were supplemented with 2 μg/mL puromycin (Invitrogen).

**miRNA array**

Reverse transcription for miRNA microarray was carried out using Megaplex Primer pools (Human Pools A v2.1 and B v3.0) (Applied Biosystems). The cDNA product was used to conduct miRNA array. miRNA profiling of 754 human miRNAs was conducted using TaqMan Human MiRNA Array Set v3.0. Quantitative real-time PCR (qRT-PCR) was carried out using Applied Biosystems 7900HT Real-Time PCR System (Applied Biosystems). Results were analyzed by the SDS RQ Manager 1.2 software (Applied Biosystems).

**miRNA and mRNA expression analyses**

Total RNA was extracted from cell pellets or tissues using Quizol reagent (Qiagen). TaqMan miRNA assays were used to quantify levels of mature miR-133a and RNU6B (KIT 002246 and 001093, Applied Biosystems). mRNA levels were determined with qRT-PCR using SYBR Green master mixture on HT7500 system (Applied Biosystems) with the housekeeping gene ACTB as an internal control. Cycle threshold (Ct) values >35 were set as the detection limit. Primers used in this study are listed in Supplementary Table S3.

**Microdissection and RNA extraction from paraffin sections**

Paraffin-embedded tissue samples used for laser capture microdissection were randomly selected from validation cohort II. Samples were cut into 10 μm thick sections using a microtome. Tissue slides were reviewed by a pathologist, and the muscularis mucosae in normal tissues were then excluded using laser capture microdissection under microscope. Total RNA was extracted from the tissues using RecoverAll Total Nucleic Acid Isolation Kit (Ambion).

**miRNA transfection and RNA interference**

Human miR-133a precursor (PM10413) was purchased from Applied Biosystems. A control miRNA (miR-Ctrl; AM17110, Applied Biosystems) did not target any human genes was used as a negative control. Cells were transfected with miRNAs using Lipofectamine 2000 (Invitrogen). Short hairpin RNAs (shRNA) against human RFFL (Origene) were delivered into cells using Lipofectamine 2000, and stable cell lines were created from puromycin-resistant colonies. siRNAs against p53 (Santa Cruz Biotechnology) were delivered into cell using Lipofectamine 2000. Cells transfected with miRNA or siRNA were harvested 12 to 48 hours after transfection.

**Colonies formation and MTT cell viability assay**

After 24 hours of transfection, cells were collected and seeded (500–1,000 per well) in a fresh 6-well plate for 7 to 9 days. Colonies were counted after staining with crystal violet solution. Cell viability was determined using the conversion of MTT to formazan via mitochondrial oxidation. MTT solution was then added to each well at a final concentration of 0.2 mg/mL per well and the plates were incubated at 37°C for another 1 hour. After incubation, 200 μL of dimethyl sulfoxide was added to each well to dissolve the formazan formed, and the absorbance was measured at 570 nm using a spectrophotometer. All the experiments were repeated in 3 independent experiments in triplicate.

**Flow cytometry**

At 24 hours posttransfection, cells were treated with nocodazole (25 ng/mL, Sigma-Aldrich) for an additional 14 hours, or doxorubicin (100 nmol/L, Sigma-Aldrich), or oxaliplatin (1 μmol/L, Sigma-Aldrich) for additional 48 hours. Cells were fixed and stained with propidium iodide (BD Biosciences). Cells undergoing apoptosis were detected by Annexin V/7-aminoactinomycin D (AAD) staining (BD Biosciences). A total of 30,000 events were counted for each sample and cell-cycle profiles were analyzed by ModFit 3.0 software (BD Biosciences). All the experiments were repeated in 3 independent experiments in triplicate.

**Western blot analysis**

Protein concentration was measured by the Bradford DC protein assay (Bio-Rad). About 5 to 20 μg of protein from each sample was separated on 12% Bis–Tris PAGE through electrophoresis and blotted onto nitrocellulose membranes (GE Healthcare). Blots were immunostained with primary antibodies at 4°C overnight and secondary antibody at room temperature for 1 hour. Protein was visualized using ECL Plus Western Blotting Detection Reagents (GE Healthcare). Antibodies against p53 (sc-126; Santa Cruz, dilution 1:1,000), CDKN1A (p21) (sc-6246; Santa Cruz, dilution 1:1,000), and RFFL (Rockland, dilution 1:2,000) were used.
in Western blot analysis. Anti-GAPDH antibodies (sc-25778; Santa Cruz, dilution 1:2,000) were used to test for equal loading.

**Plasmids and dual-luciferase reporter assay**

The full-length open reading sequence of human RFFL (NM_001017368.1) was PCR-amplified from HCT116 cDNA. The PCR aliquots were subcloned into pcDNA 3.1 (Invitrogen) to generated pRFFL plasmid and then verified by DNA sequence. Human miR-133a expression plasmid pmiR-133a (SC400158) and control vector pCMVMIR were purchased from Origene. To generate the reporter plasmid pMIR-RFFL-3’UTR and pMIR-RFPLmut-3’UTR, the annealed oligonucleotides containing wild-type or mutated human RFFL 3’-UTR were cloned into pMIR-REPORT vector (Ambion) at the SacI and SpeI sites, respectively. Primer sequences are listed in Supplementary Table S3. pGL13 plasmid (containing 14 copies of the wild-type p53 response elements that drives the luciferase reporter gene: 5’-CCAGGCGAGGTTCCAGGGCAGG-3’) and p21-luc plasmid (containing 2.1-kb p21 promoter region that drives the luciferase reporter gene) were used to investigate the p53 signaling pathways modulated by miR-133a. Cotransfection of reporter plasmids and miRNA in 24-well plates were carried out with Lipofectamine 2000 as described by the manufacture. Per well, 195 ng luciferase reporter plasmid, 5 ng pRL-cytomegalovirus (CMV) vector, and 15 pmol miRNA were applied. Cells were harvested 48 hours posttransfection and luciferase activities were analyzed by the dual-luciferase reporter assay system (Promega).

**Tumor xenografts in nude mouse model**

In the transient miR-133a expression model, approximately 2 \times 10^6 HCT116 cells were subcutaneously implanted into the right flank of the female BALB/c nude mice (4 weeks old, 4 mice per group). miR-133a or miR-Ctrl were injected into the tumor using syringes and 30G needles when tumor size reached to 3 mm (length) \times 3 mm (width). miRNAs were prepared by preincubating miRNA (0.3 nmol) with Lipofectamine 2000 (2.5 μL) (Invitrogen) for 15 minutes, and injection was made in a final volume of 100 μL in McCoy’s 5A medium (Sigma-Aldrich) per mouse. The injection was repeated every 3 days and consisted of 4 consecutive injections as described before (5). In the stable miRNA expression model, 2 \times 10^6 HCT116 cells stably expressing miR-133a or control vector were injected subcutaneously into the left flank of the female BALB/c nude mice (4 weeks old, 4 mice per group), respectively. Tumor sizes were measured starting from the first injection and the tumor volume was calculated as (length \times width^2)/2. The experiments were approved by Animal Experimentation Ethics Committee, The Chinese University of Hong Kong.

**Statistical analysis**

The results were expressed as mean ± SD. The difference in miRNA levels between paired tissue samples was determined by the Wilcoxon matched-pairs test. P < 0.05 was taken as statistical significance. All the tests were conducted by GraphPad Prism 5.0.

**Results**

**Downregulation of miR-133a in primary human CRC and colon cancer cell lines**

To identify candidate miRNAs associated with CRCs, we compared miRNA expression profiles of 4 pairs of CRC and adjacent normal tissues using miRNA microarray covering 754 miRNAs. We found that miR-133a was significantly downregulated in CRCs based on the array data (GSE45349). We then verified the downregulation of miR-133a in 91 pairs of primary CRCs and paired normal tissues from 2 cohorts of patients with CRC by qRT-PCR analysis. U6 small nuclear 2 (RNU6B) was used as an internal control. Two pairs of tissues were excluded from this study due to undetectable miR-133a or RNU6B. Among the 89 pairs of CRC tissues specimens examined, 80 (89.9%) tumor tissues showed significantly lower miR-133a expression when compared with matched adjacent normal tissues (36 of 44, 81.8%, P < 0.001 for cohort I; and 44 of 45, 97.8%, P < 0.001 for cohort II). In addition, 44 of 45, 97.8%, P < 0.001 for cohort II, with a median difference of 0.15-fold [interquartile range (IQR), 0.46–0.95] in cohort I and 0.05-fold (IQR, 0.13–0.02) in cohort II, respectively. miR-133a was first identified as a muscle-specific miRNA (6). The presence of smooth muscle in the adjacent normal tissues might affect the results in our qRT-PCR assay. To clarify the issue, we eliminated the muscularis mucosae with microdissection in randomly selected 11 pairs of CRCs and adjacent normal tissues. The level of miR-133a was significantly decreased in tumor tissues compared with normal epithelial cells (Fig. 1A3, P < 0.01). These data further strengthened the significance of Fig. 1A1 and 2. In addition, we evaluated the expression of miR-133a in 9 colon cancer cell lines and 9 normal rectal tissues from healthy subjects. miR-133a was dramatically underexpressed in all 9 colon cancer cell lines (100%) examined, suggesting aberrant downregulated expression of miR-133a in CRCs (Fig. 1B).

**Ectopic expression of miR-133a inhibits tumorigenic properties of CRC cells**

The frequent silence of miR-133a in CRC primary cancers and cell lines suggests that miR-133a might be a putative tumor suppressor. In this regard, we examined the effect of ectopic expression of miR-133a on cell growth in colon cancer cell lines. We noticed that the miR-133a levels in HCT116 and LoVo were about 10^5-fold less than those in the normal tissues (Fig. 1B). To eliminate the nonspecific effects generated by overtransfection, we adjusted the transfection amount of miRNA and restored miR-133a level approximately equivalent to the physiologic level (Fig. 2A). Ectopic expression of miR-133a significantly suppressed colony formation with 20% reduction in HCT116 and 26% reduction in LoVo (P < 0.01) when compared with miR-Ctrl–transfected cells (Fig. 2B). This effect was further confirmed by MTT assay. Overexpression of miR-133a
caused a significant decrease in cell viability in both p53 wild-type cell lines HCT116 \((P < 0.01)\) and LoVo \((P < 0.05;\) Fig. 2C and D). miR-133a also exerted inhibitory effects on p53-mutant cell lines DLD1 and HT29 (Supplementary Fig. S1). These results indicated that miR-133a exerts growth-inhibitory ability in colon cancer cells and functions as a potential tumor suppressor.

**miR-133a inhibits tumor growth in nude mice**

Because miR-133a can suppress CRC cell growth in vitro, we next tested whether miR-133a could suppress tumorigenesis in vivo. We used 2 different miRNA expression models to study the miR-133a function in vivo: (i) miR-133a precursor transient expression model and (ii) vector-based miR-133a constitutive expression model. In the transient expression model, the BALB/c nude mice intratumoral injected with miR-133a precursor showed a reduced tumor growth rate compared with the miR-Ctrl–injected cells \((P < 0.05;\) Fig. 2E). In the constitutive expression model, we generated the miR-133a and control vector stable transfectants (designated as HCT116-miR-133a and HCT116-miR-Ctrl). HCT116-miR-133a exhibited markedly reduced growth rate in nude mice compared with the HCT116-miR-Ctrl cells \((P < 0.01;\) Fig. 2F), supporting that miR-133a suppressed the colon cancer growth in vivo. We also checked the miR-133a expression levels in both xenograft models. In the transient expression model, we found that the miR-133a levels showed a 10\(^5\)-fold increase compared with those in the control group 24 hours after the final injection at day 13, and the miR-133a in the tumors still showed a 10\(^2\)-fold increase at the end of the experiment at day 36 (23 days following the final injection). In the stable transfectants group, the amounts of miR-133a were approximately 10\(^2\)-fold higher in HCT116-miR-133a than those in the control group at the beginning (day 4). No significant miR-133a reduction was observed at the end of the experiment (day 34; Supplementary Fig. S2).

**miR-133a induces G\(_0\)–G\(_1\) phase arrest**

To elucidate the mechanism of miR-133a function in the control of CRC cell growth, we next examined the effects of miR-133a on cell-cycle regulation. Cells were synchronized with an antimitotic drug nocodazole (7). Transfection of miR-133a significantly increased the fraction of cells in G\(_0\)–G\(_1\) phase compared with the miR-Ctrl–transfected cells in both HCT116 \((P < 0.01)\) and LoVo \((P < 0.01;\) Fig. 3A)
and B). The role of miR-133a in apoptosis was also evaluated, and no significant proapoptotic effect was observed.

**miR-133a upregulates p53/p21 pathway**

The G0–G1 cell-cycle arrest phenomenon promoted us to examine the regulation role of miR-133a on the CDK inhibitor CDKN1A (p21) and tumor suppressor p53. Ectopic expression of miR-133a dramatically increased p21 mRNA and protein expressions (Fig. 3C and D). The increased p21 localized in the nucleus (Supplementary Fig. S3). We found that the p53 protein but not mRNA was significantly upregulated after treatment with miR-133a in both HCT116 and LoVo cells (Fig. 3C and E). On the other hand, c-myc, an oncprotein that could suppress p21 expression, was unchanged after transfection with miR-133a in both cell lines (Fig. 3C), favoring the hypothesis that miR-133a positively regulated p53/p21 pathway. To confirm the interaction between miR-133a and p53/p21 pathway, we further conducted the promoter luciferase activity assays using pGL13 and p21-Luc luciferase reporters in HCT116 cells. As expected, cotransfection of miR-133a and pGL13 significantly increased p53 luciferase reporter activities by 2.6-fold in HCT116 (Fig. 3F; P < 0.001) and 1.4-fold in LoVo (Fig. 3G; P < 0.05). Cotransfection of
miR-133a and p21-Luc also increased p21 luciferase reporter activities by 1.9-fold in HCT116 (Fig. 3F; \(P < 0.001\)) and 1.8-fold in LoVo (Fig. 3G; \(P < 0.05\)). Moreover, we depleted p53 by siRNA (si-p53) and found that the enhanced p21 expression in miR-133a-transfected cells was completely abrogated in both HCT116 and LoVo cells (Fig. 3H), confirming that p53 is required for the miR-133a-mediated induction of p21. In addition, knockdown of p53 also antagonized the growth-inhibitory effect of miR-133a (Fig. 3I and J). These data suggested that miR-133a suppresses cell growth, at least in part, by upregulation of p53 and its downstream effector p21.

miR-133a inhibits RFFL by direct interaction with its 3'UTR

We then searched for the direct target of miR-133a based on the following criteria: the target should show oncogenic property and regulate the p53/p21 signaling pathway.
Among the top 200 targets of miR-133a predicted by online algorithms (Targetscan, mirSVR, Ring finger, and FYVE-like domain-containing E3 ubiquitin protein ligase (RFFL)) shows a high prediction score and also fit our hypothesis. RFFL contains an evolutionarily conserved binding site for miR-133a and was also reported to attenuate p53 signaling transduction (8–10). To test whether RFFL is targeted by miR-133a in vitro, we attached the predicted RFFL 3'UTR-binding site, as well as its mutant form to firefly luciferase reporter gene, respectively (Fig. 4A). HCT116 cells transfected with miR-133a repressed wild-type RFFL-3'UTR reporter activity for about 49% compared with the cells cotransfected with empty vector (P < 0.01). On the other hand, miR-133a had no inhibition effect on the mutant RFFL-3'UTR reporter activity (Fig. 4B), indicating the direct regulation of miR-133a in the 3'UTR of RFFL mRNA.

To determine whether these findings reflect the regulation of endogenous RFFL by miR-133a, we transiently reintroduced miR-133a precursor into 3 colon cancer cell lines HCT116, HT29, and LoVo. Ectopic expression of RFFL in HCT116 significantly suppressed p53 protein expression when compared with the control vector (Fig. 5A). The effect of RFFL on p53 was further verified by RFFL knockdown experiment. Knockdown of RFFL led to a 48% to 60% decrease of RFFL mRNA expression in HCT116. Silencing of RFFL induced p53 and its downstream target p21 protein expression and caused about 5% increase in G0–G1 phase arrest (Fig. 5B and E). Moreover, RFFL-depleted cells exhibited impaired cell growth compared with control cells as revealed by MTT assay and colony formation assay (Fig. 5C). The colonies formed by RFFL knockdown in HCT116 cells were fewer than the control cells (P < 0.05; Fig. 5D). We also investigated the level of RFFL protein in 18 randomly selected patients from the validation cohort. Our data showed that RFFL protein was increased in 7 of 18 (38.9%) tumors. Exon sequencing of p53 gene on these 18 CRC tumor samples was conducted, and the results showed that all 7 RFFL-upregulated CRC tumor samples carrying wild-type p53 (data not shown).

miR-133a sensitizes CRC cells to chemotherapeutic drugs treatment

p53 status was associated with the cell responses to chemotherapeutic agents. It is thus possible that restoration of miR-133a would render cancer cell sensitivity to chemotherapeutic agents. To test this hypothesis, we exposed cells to doxorubicin (a commonly used DNA damage agent) or oxaliplatin (a first-line drug for advanced CRC treatment),
respectively, for 48 hours after miR-133a transfection. As expected, cell viability was significantly reduced in miR-133a–transfected HCT116 upon exposure to doxorubicin ($P < 0.001$) or oxaliplatin ($P < 0.05$; Fig. 6A). Colony formation was significantly suppressed when HCT116 cells transfected with miR-133a after exposure to doxorubicin ($P < 0.001$) or oxaliplatin ($P < 0.001$), respectively (Fig. 6B).

We further quantified the apoptotic cell by Annexin V/7-AAD double stain and revealed that overexpression of miR-133a sensitized HCT116 to chemotherapeutic drug–induced apoptosis, with about 23% increase in early apoptotic population for doxorubicin (40% vs. 63%, $P < 0.01$) and about 6% increase for oxaliplatin (17% vs. 23%, $P < 0.01$), respectively (Fig. 6C). No significant differences were found in the late apoptosis. These data suggested that the overexpression of miR-133a had proapoptotic effects which sensitized colon cancer cell to chemotherapeutic treatments.

**Discussion**

Human miR-133a is encoded by 2 distinct genes, miR-133a-1 and miR-133a-2, which are processed into an identical mature sequence (http://www.mirbase.org). miR-133a-1 and miR-133a-2 are embedded in the MIB1 gene on chromosome 18 and C20orf166 on chromosome 20, respectively. The sequence of miR-133b differs from miR-133a with a single nucleotide on the 3′ flank. However, the $C_i$ values of miR-133b in normal tissues were significantly lower than that of miR-133a (data not shown), suggesting that miR-133a is the dominant form of miR-133 in normal colorectal tissue. Epigenetic silencing of miRNAs with tumor suppressor features by CpG island hypermethylation is a common hallmark of human tumors (11). Most recently, it was documented that hypermethylation of the CpG islands upstream of miR-1-133a might be involved in the downregulation of miR-133a in CRCs (12). In the present study, we showed that miR-133a was significantly downregulated in primary CRC tissues using microdissection technique.

The effect of miR-133a in colon cancer was therefore examined in vitro and in vivo. To avoid the artificial interference, we restored miR-133a in those cell lines closer to the physiologic level. Ectopic expression of miR-133a in these silenced colon cancer cells significantly inhibited cell viability and clonogenic survival. Increase in G0–G1 phase arrest was shown to contribute to the cell growth inhibition by miR-133a. To assure the physiologic relevance, we used 2 xenograft models to study the function of miR-133a. Our study clearly showed that intratumoral injection of either miR-133a or miR-133a stable expression in HCT116 cells significantly inhibited
CRC xenograft tumor growth. Collectively, these data indicated for the first time that miR-133a function as tumor suppressor in CRCs and mediated the growth-suppressing effects by negatively governing cell-cycle progression. Therefore, miR-133a would contribute to the depressed cell proliferation in colon cancer cells.

G0–G1 arrest by miR-133a re-expression was shown to be associated with the induction of p53 and its downstream targets p21. p53 is essential for the miR-133a–mediated induction of p21 based on the following evidences: p21 was found to be markedly upregulated in HCT116 and LoVo at both protein and mRNA level by miR-133a. Moreover, we showed that the re-expression of miR-133a strongly increased p53 protein level, enhanced p53 response element binding activity, and the p21 promoter reporter activity, which suggests that miR-133a positively modulates the p53/p21 signaling pathway. Finally, silencing of p53 abrogated miR-133a–mediated induction of p21 and reversed the growth inhibition property of miR-133a. Taken together, these findings indicate that miR-133a is a novel component in the p53 tumor suppressor network and positively regulates p53/p21 signaling pathway in colon cancer. Inhibition of miR-133a will impair the p53 safeguard function and allow tumor growth in a wild-type p53 background.

Having observed substantial suppression of colon cancer cell growth by miR-133a through mediating p53/p21 signaling, we carried out experiments to test the potential target for miR-133a. Among the different putative targets for miR-133a predicted using miRNA target prediction algorithms, RFFL is recognized as the most promising target as others have reported that p53 and phospho-p53 were the main targets of RFFL (9). In the present work, we revealed a direct interaction that miR-133a negatively regulating RFFL protein in colon cancer cell lines as evidenced by luciferase activity assay and by Western blotting. Re-expression of miR-133a in the miR-133a–low-expressed tumor cells induces activated p53/p21 signaling and G0–G1 phase arrest; the same phenotypes can also be exerted by knockdown of RFFL in the cells. Moreover, we showed that silencing of RFFL suppressed CRC cell growth. These findings supported the idea that miR-133a suppressed CRC growth through directly targeting the potential oncoprotein RFFL and activating the p53/p21 pathway.

Recent researches have shed light on the potential use of miRNAs as noninvasive markers for CRC early diagnosis, as well as their association with CRC clinical phenotypes. Chen and colleagues mentioned that patients with CRC with liver metastasis were associated with lower expression of miR-133a (12). Unfortunately, we did not observe such.
could have a benefit on colon cancer cells to doxorubicin and oxaliplatin.

Restoration of miR-133a in colon cancer cells resulted in downregulated in CRC tissues and colon cancer lines. The tumor growth inhibition by miR-133a could be attributed, at least in part, to miR-133a directly reducing RFFL translation and activating p53/p21 pathway. Besides, miR-133a could sensitize cancer cell to chemotherapeutic agents, suggesting its potential clinical therapeutic implication.

Disclosure of Potential Conflicts of Interest

F.K.L. Chan has honoraria from speakers’ bureau from Eisai. No potential conflicts of interest were disclosed by the other authors.

References
