MicroRNA-34a inhibits migration and invasion of colon cancer cells via targeting to Fra-1

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Introduction

Colorectal cancer is the third most common malignancy and the leading cause of cancer-related deaths worldwide (1). Like many other solid tumors, colorectal cancer mortality results from cancer metastasis, a complex process that involves changes in the extracellular matrix to support Invasion, increased cell motility and the ability of cells to initiate and maintain growth at a distant site (2). The molecular mechanisms underlying this process are yet to be elucidated (3). MicroRNAs (miRNAs) are small non-coding regulatory RNA molecules that can influence a wide range of biological processes (4–6). They bind to the 3’-untranslated region (UTR) of target messenger RNA (mRNA) to inhibit translation or to promote RNA degradation and even to regulate mRNA transcription (7,8). A series of studies have revealed that miRNAs can regulate the expression of a variety of genes pivotal for tumor invasion or metastasis (9–11). MicroRNA-34a (miR-34a) was identified as one of the miRNAs that are downregulated in some cancer cells (12). Its expression is low or undetectable in 11 of 15 pancreatic cancer cell lines (13) and significantly reduced in 19 of 25 (76%) human hepatocellular carcinoma (HCC) tissues, which was associated with tumor metastasis and invasion (14). Similar phenomena were observed in human colon cancers (15). Recently, it has been demonstrated that miR-34a is a direct transcriptional target of p53 (13,16–19), and the expression of miR-34a is lower in mutant p53 gliomas than in wild-type (WT) p53 gliomas (20).

miR-34a targets to CDK4/6, cyclin E2 (CCNE2), cyclin D1 (CCND1), E2F3, Bcl-2, MYCN, Notch1/2 and SIRT1 to inhibit cell proliferation, induce cell apoptosis and senescence (16,20–22). It also reduces c-Met expression to inhibit migration and invasion of HCC andveal melanoma cells (14,23). Recently, it was reported that miR-34a suppresses invasion via downregulating Notch1 and Jagged1 in cervical carcinoma and choriocarcinoma cells (24). However, there are other targets of miR-34a that regulate cancer cell migration and invasion needs to be elucidated.

The activator protein-1 (AP-1) transcription factor consists of the family members of Jun (c-Jun, Jun-B and Jun-D) and Fos (c-Fos, Fos-B, Fra-1 and Fra-2) proto-oncogenes (25). Abnormal induction and/or activation of AP1 by a variety of toxicants and mitogens contribute to the development of cancers (25). Overexpression of Fra-1 is a common mechanism of constitutive AP-1 activation in tumors and has a crucial role in AP-1-mediated transformation (26). Fra-1 regulates cell motility and invasion in various malignant epithelial cells, including lung adenocarcinoma (27,28), breast cancer (29,30) and colon carcinomas cells (31–33). However, the precise molecular mechanisms underlying the role of Fra-1 in the cellular malignant and invasive phenotypes are not fully understood.

In this study, we demonstrated that miR-34a regulated Fra-1 expression in HCT116 and RKO cells, two human colon cancer cell lines, and that forced expression of miR-34a led to reduced cell motility and invasion capability, which can be partially rescued by forced Fra-1 expression. In addition, the expression of matrix metalloproteinases (MMPs), in particular MMP-1 and MMP-9, was decreased in miR-34a-transfected cells and Fra-1-knockdown cells. At the end, the expression levels of miR-34a and Fra-1, as well as the DNA-binding activity of p53 in human colon cancer tissues were determined and their possible roles in human colon cancer development were discussed.

Materials and methods

Cell culture and tumor specimens

HEK293T and RKO cells were grown in Dulbecco’s modified Eagle’s medium (HyClone, Logan, UT), supplemented with 10% fetal bovine serum and incubated at 37°C in a humidified incubator with 5% CO2. HCT116 and HCT116 p53—/— cells (kindly provided by Dr Bert Vogelstein, The Johns Hopkins University, Baltimore, Maryland) were grown in McCoy’s 5A medium (Gibco, Grand Island, NY). Actinomycin D (Sigma), when applied, was dissolved in dimethyl sulfoxide and added to the cell medium at indicated concentrations; the treated cells were then harvested 6 h later for RNA and protein analysis. Primary human colon cancer tissues and the adjacent non-tumorous colon tissues were obtained from 40 patients who underwent surgery in Daping Hospital (Chongqing, China). None of the patients received chemotherapy before surgery. Tissue samples were frozen immediately in liquid nitrogen and stored at −80°C before use. All patients gave their informed consent, and the study was approved by the scientific ethics committees of Daping Hospital and Wenzhou Medical College (Wenzhou, China). All colon cancer cases were confirmed by clinical pathology.

miRNAs, small interfering RNAs and transfection

miR-34a mimic, miR-34a inhibitor and the negative control were obtained from Ribobio (Guangzhou, China). For convenience, miR-34a mimic and the negative control were termed miR-34a and miR-con, respectively. Small interfering RNAs (siRNAs), siFRA-1 (sense: CACCAUGAGUGGCAGUGCAAGdTdTand antisense: CUGACUGACCUACUGUGGdTdT) and the negative control siRNA (sciCON) were obtained from Invitrogen (Shanghai, China).

Abbreviations: HCC, human hepatocellular carcinoma; miR-34a, microRNA-34a; mRNA, messenger RNA; MMP, matrix metalloproteinase; PCR, polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction; siRNA, small interfering RNA; WT, wild-type.

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miR-34a and the 263 bp flanking sequence was amplified from human genomic DNA and cloned into the pTarget vector (Promega, Madison, WI). Primer sequences are as follows: (forward: CCTCTGGCAATTTCTTTT, reverse: CCTTGGAAGC). Both pTarget–miR-34a and the empty pTarget plasmids were transfected into HCT116 cells using Lipofectamine 2000 (Invitrogen). The transfected cells were selected in the presence of 1000 μg/ml G418. The resistant clones were further validated for miR-34a expression by real-time RT–PCR and maintained in medium containing 500 μg/ml G418 for subsequent studies.

**RNA extraction and real-time RT–PCR**

Total RNA was extracted from cultured cells and the colon cancer specimens using Trizol (Invitrogen, Carlsbad, CA). The levels of miRNAs and miRNAs were measured by real-time RT–PCR. For miRNA detection, reverse transcription was performed using the M-MLV Reverse Transcriptase System (Promega, Madison, WI), and real-time polymerase chain reaction (PCR) was carried out using SYBR premix Ex Taq (TaKaRa, Dalian, China) with the ABI 7300 Sequence Detection System (Applied Biosystems, Foster, CA). Glyceraldehyde-3-phosphate dehydrogenase was used for normalization. Primer sequences (forward and reverse) are as follows: Fra-1, CATATCTGCAGTGGTGGTGCA and GTAATTTAAGCAATTTTCAGACA; MMP-1, GGGAGATCGTGATCCACACCTGCTGAGTAAAGATTCTCGAGAC; c-Met, TCTGACAGCGAGCTCTGAC; vimentin, ACCCACTCAAACCTTTTCGAGAC; and GAPDH, CCCTCTCGCCTGCTTGGGAAGCTG.

To detect the level of miR-34a, the complementary DNA was synthesized using the TaqMan MicroRNA Reverse Transcription Kit, and real-time PCR analysis was performed with the ABI 7300 Sequence Detection System. A human U6 small nuclear RNA TaqMan probe was used for normalization.

** Luciferase reporter assays**

The 3′-UTR of Fra-1 was ampliﬁed by PCR and inserted into the pGL3-control vector (Promega) at the XbaI site immediately downstream the stop codon of firefly luciferase. To generate the Fra-1 mutant reporter, the seed region of the Fra-1 3′-UTR was mutated to remove all complementarity to nucleotides 1–7 of miR-34a, using the QuikChange II XL mutagenesis kit (Stratagene, La Jolla, CA). HEK293T cells were cotransfected with 0.4 μg firefly luciferase reporter vector, 0.02 μg Renilla luciferase control vector (pRL-CMV) and 25 pmol miR-34a or miR-con, using Lipofectamine 2000 (Invitrogen) in the 24-well plates. Luciferase assays were performed 24 h after transfection, using the dual-luciferase reporter assay system (Promega). Firefly luciferase activity was normalized to Renilla luciferase activity.

**Cell migration and invasion assay**

The 8 mm pore sized matrigel (for migration) or matrigel-coated (for invasion) transwell inserts (Costar, High Wycombe, UK) were placed in the wells of 24-well culture plates; 600 μl transwell inserts (Costar, High Wycombe, UK) were placed in the wells of 24-well culture plates. Luciferase assays were performed 24 h after transfection, using the dual-luciferase reporter assay system (Promega). Firefly luciferase activity was normalized to Renilla luciferase activity.

**Cell adhesion assay**

Cells were plated in the 96-well plates with a series of fetal bovine serum concentrations and transfected with 5 pmol miR-34a, miR-con, siFRA-1 or siCONT as indicated using siPORT NeoFX (Ambion, Austin, TX). Cell proliferation was assessed using the CellTiter 96® AQueous One Solution Cell Proliferation Assay kit (Promega) according to the manufacturer’s instruction.

**Western blotting analysis**

Cells were lysed in RIPA buffer, and total protein was measured using the Bradford protein assay (Bio-Rad, Hercules, CA). Samples were denatured at 100°C for 5 min. Equal amounts of total protein were loaded for electrophoresis in sodium dodecyl sulfate–polyacrylamide gels and then transferred to polyvinylidene ﬂuoride microporous membranes (Millipore, Bedford, MA). Membranes were incubated with horseradish peroxidase-linked secondary antibody for 1 h at room temperature and detected with enhanced chemiluminescence plus reagents (Millipore). The primary antibodies, anti-Fra-1 (C-12) (sc-28310) and anti-β-actin (sc-8178) were from Santa Cruz Biotechnology (Santa Cruz, CA); anti-MMP-1 (10371-2-AP), anti-vimentin (10366-1-AP) and anti-c-Met (19971-1-AP) were from Proteintech Group (Chicago, IL) and anti-MMP-9 (G657) was from Cell Signaling Technology (Beverly, MA).

**Fra-1 rescue experiments**

The pFra-1 plasmid was constructed by inserting the human Fra-1 complementary DNA lacking the 3′-UTR into the pTarget vector (Promega). miR-34a or miR-con was cotransfected with pTFra-1 or the pTarget empty vector. The cells were collected 6 h after transfection and analyzed for migration and invasion as described above. Fra-1 expression was verified by western blotting.

**Chromatin immunoprecipitation**

Chromatin immunoprecipitation assay was carried out using Chromatin Immunoprecipitation Assay Kit (Millipore) according to the manufacturer’s instruction with anti-Fra-1 antibody (sc-28310; Santa Cruz Biotechnology) and the mouse IgG control (Active Motif, Carlsbad, CA). The precipitated DNA was recovered, and real-time PCR was performed as described above. Primer sequences (forward and reverse) are as follows: MMP-1, TCACCGTATCCTTACCTTCCTTGAC; and c-Met, GGCGCCCTCAGGACGCAAGCTTCTCATGCTGGTGCT and TGTGACCCCACCCCTCCTTGA. The precipitated DNA was measured by real-time RT–PCR and maintained in medium containing 500 μg/ml G418 for subsequent studies.

**Statistical analysis**

Each experiment was performed at least three times independently. All data were shown as means ± SDs. Differences between samples were analyzed by independent samples t-test using SPSS V17.0 software. Correlation between two groups was analyzed by bivariate correlation. Statistical significance was accepted at P < 0.05 when compared with the corresponding control or as specifically indicated.
significant reduction of Fra-1 protein (Figure 1D; Supplementary Figure 2A is available at Carcinogenesis Online), and real-time RT–PCR analysis indicated that Fra-1 mRNA levels were also reduced, though to a less extent (Figure 1E). miR-34a is a transcriptional target of p53 (13,16–18); it also increases the acetylation of p53 and induces the transcription of p53 target genes (22). Therefore, a p53-negative HCT116 cell line (HCT116 p53−/−) was used to determine whether p53 is involved in the regulation of Fra-1 by miR-34a. As shown in Figure 1F and G, the expression of Fra-1 was higher, whereas the level of miR-34a was lower in HCT116 p53−/− cells as compared with HCT116 WT cells. Actinomycin D, an anticancer drug known to be able to induce p53 expression and activate p53-dependent transcription (34,35), was used to treat HCT116 p53−/− cells and HCT116 WT cells. We showed that actinomycin D treatment led to a significant increase in the expression of p53 and miR-34a as well as a significant decrease in Fra-1 expression in HCT116 WT cells (Figure 1F and G). However, actinomycin D only slightly affected the expression of Fra-1 and miR-34a in HCT116 p53−/− cells (Figure 1F and G). Transfection of miR-34a inhibitor partially rescued the expression of Fra-1 in actinomycin D-treated HCT116 WT cells, indicating that actinomycin D-induced reduction of Fra-1 is mediated, at least partially, by miR-34a (Supplementary Figure 3 is available at Carcinogenesis Online).

miR-34a decreased the migration and invasion capability of colon cancer cells

Previous studies demonstrated that miR-34a inhibits migration and invasion of HCC cells and uveal melanoma cells (14,23). We
wondered whether miR-34a can regulate the cell motility and invasion capability of colon cancer cells. It is known that miR-34a inhibits the proliferation of glioblastoma and uveal melanoma cells (20,23), and cell proliferation may interfere with the results of cell migration and invasion assays. Therefore, we first investigated the effect of miR-34a on cell proliferation. Although miR-34a caused a dramatic inhibition

Fig. 2. miR-34a inhibits the migration and invasion of colon cancer cells. HCT116 and RKO cells were transfected with miR-34a or miR-con (A-D). (A) Representative micrographs of cell migration assay (top) and the quantification (bottom) from three independent experiments (*P < 0.05). (B) Representative micrographs of cell invasion assay (top) and the quantification (bottom) from three independent experiments (*P < 0.05). (C) The relative mRNA levels of MMP-1, MMP-9, vimentin and c-Met determined by real-time RT–PCR (n = 3; *P < 0.05). (D) The protein levels of MMP-1, MMP-9, vimentin and c-Met analyzed by western blotting, β-actin was used as loading control.

Inhibitory effects of miR-34a on colon cancer cells
of cell proliferation at 48 h after transfection, there was no major difference in proliferation between miR-34a-transfected cells and the control at 24 h (Supplementary Figure 4 is available at Carcinogenesis Online). So, we decided to perform cell migration and invasion assays within 24 h after RNA transfection. As shown in Figure 2A and B, the migration and invasion were reduced in miR-34a-transfected cells as compared with the negative control. In addition, the adhesiveness of cells to fibronectin-coated dishes was not altered by miR-34a transfection (data not shown). Because MMP-1, MMP-9, vimentin and c-Met are involved in cell migration and invasion and are frequently upregulated in cancer cells (14,36,37), we measured the expression of these genes in miR-34a-transfected cells. Our results showed that the mRNA and protein levels of these genes were decreased, except for vimentin (Figure 2C and D). To further investigate the function of miR-34a in cell migration and invasion, we developed two stable miR-34a-overexpressing transfectants from HCT116 cells (Supplementary Figure 5 is available at Carcinogenesis Online). Transwell assay showed that the migration and invasion were significantly reduced in the stable miR-34a overexpressing HCT116 cells, along with decreased protein levels of Fra-1, MMP-1, MMP-9 and c-Met, as compared with the control cells (Figure 2E and F).

Decreased Fra-1 expression reduced the migration and invasion capability of colon cancer cells

Fra-1 is known to regulate cell motility and invasion in various malignant epithelial cells, including lung adenocarcinoma (27,28), breast cancer (29,30) and colon carcinomas cells (31–33). It also regulates the proliferation of breast cancer cells (29). To determine whether these cell characteristics are affected by Fra-1 in colon cancer cells, we knocked down Fra-1 with siRNA in HCT116 and RKO cells (Figure 3A). As shown in Supplementary Figure 6, available at Carcinogenesis Online, although knockdown of Fra-1 significantly reduced cell proliferation after 48 h of transfection, there was no dramatic difference in proliferation between Fra-1-knockdown cells.
miR-34a reduced in vivo binding of Fra-1 to the promoters of MMP-1 and MMP-9

To confirm that miR-34a regulates MMP-1 and MMP-9 through Fra-1, chromatin immunoprecipitation assay was performed to test the association of Fra-1 with the promoters of MMP-1 and MMP-9. Using the anti-Fra-1 precipitated DNA as template, real-time PCR results indicated that the association of endogenous Fra-1 with MMP-1 and MMP-9 promoters was reduced in miR-34a-transfected cells, but significantly increased when Fra-1 was overexpressed (Figure 5A and B). In contrast to the MMP-1 and MMP-9 promoters, PCR amplification signals from the c-Met promoter were extremely weak and no significant difference was observed under all the treatment conditions (data not shown). As a negative control, real-time PCR using IgG precipitated DNA as template detected very low signal.

Expression of miR-34a and Fra-1 and the DNA-binding activity of p53 in human colon cancer tissues

We analyzed the expression of miR-34a and Fra-1 in human colon cancer tissues by real-time RT–PCR. Compared with the adjacent normal tissues, 25 of 40 (62.5%) samples showed reduced miR-34a expression from -24-fold to 1.2-fold (Figure 6A). To determine whether decreased miR-34a expression was associated with colon cancer metastasis and invasion, we compared miR-34a expression in colon cancer tissues from metastatic/invasive samples with lymphatic metastasis and/or local infiltration of the intestinal wall (M; n = 20, samples #1–20) to that from non-metastatic/non-invasive samples (NM; n = 20, samples #21–40) without evident metastasis at the time of surgery. As shown in Figure 6B, miR-34a was significantly downregulated in metastatic/invasive samples. On the contrary, Fra-1 was upregulated in 26 of the same 40 (65%) tumor tissues as compared with the adjacent normal colon tissues (Figure 6C). In addition, Fra-1 mRNA levels were increased in metastatic/invasive samples compared with that in non-metastatic/non-invasive samples (Figure 6D). Although general inverse correlation between miR-34a and Fra-1 expression was not found in 40 tumor tissues (Figure 6E), statistically significant inverse correlation between miR-34a and Fra-1 expression was observed in 14 of 40 samples (Figure 6F). It has been demonstrated that miR-34a is a direct transcriptional target of p53 (13,16–18) and the expression of miR-34a is lower in mutant p53 gliomas than in WT p53 gliomas (20). We then investigated the sequence-specific DNA-binding activity of p53 in human colon cancer tissues. As shown in Supplementary Figure 8A, available at Carcinogenesis Online, the DNA-binding activity of p53 was decreased in 16 of 40 (40%) tumor tissues as compared with the adjacent normal colon tissues, and it was significantly correlated with miR-34a expression (Supplementary Figure 8B is available at Carcinogenesis Online). Unexpectedly, we found that the DNA-binding activity of p53 was not inversely correlated with Fra-1 mRNA level (Supplementary Figure 8C is available at Carcinogenesis Online), even in the 14 tumor tissues that showed significant inverse correlation between miR-34a and Fra-1 expression (Supplementary Figure 8D is available at Carcinogenesis Online). Interestingly, we found that the DNA-binding activity of p53 was lower in metastatic/invasive samples than that in non-metastatic/non-invasive samples (Supplementary Figure 8E is available at Carcinogenesis Online).

Discussion

miR-34a is one of the most prominent miRNAs implicated in the development and progression of human cancers (12–20). It is downregulated in human colon cancers (15). In the present study, we demonstrated that miR-34a expression was significantly decreased in metastatic/invasive colon cancer tissues when compared with the non-metastatic/non-invasive colon cancer tissues (Figure 6B). We also found that miR-34a inhibited the migration and invasion of HCT116 and RKO cells (Figure 2A and B). Several biological targets of miR-34a have been identified recently. For example, miR-34a downregulates c-Met, Notch1 and Jagged1 and eventually inhibits...
cell migration and invasion (14,19,23,24). Here, we showed that miR-34a inhibited Fra-1 expression by directly targeting to its 3’-UTR (Figure 1D and E) and that forced expression of Fra-1 partially rescued the effects of miR-34a on cell migration and invasion (Figure 4B). Therefore, Fra-1 may be one of the targets of miR-34a that promote cell migration and invasion.

Fra-1 is involved in the progression of cancer (25,26) and is upregulated in colon carcinomas (31–33). Consistent with these observations, our study showed higher expression of Fra-1 in 26 of 40 (65%) tumor tissues as compared with the adjacent normal colon tissues (Figure 6C). In addition, statistically significant inverse correlation between miR-34a expression and Fra-1 mRNA levels was only observed in 14 of 40 (35%) human tumor samples (Figure 6E). Unexpectedly, we also found that miR-34a was downregulated in some colon cancer tissue samples (#5, 12, 16, 18, 23, 32 and 37), in which the levels of Fra-1 were not increased compared with the adjacent normal colon tissues (Figure 6A and C). This observation suggests that besides miR-34a, other factors are also involved in the regulation of Fra-1 expression. In fact, Fra-1 expression in colon cells is also dependent on its transcriptional autoregulation and mitogen-activated protein/extracellular signal-regulated kinase-dependent posttranscriptional stabilization (40,41).
miR-34a is a transcriptional target of p53 (13,16–18), and it can induce the activation of p53 (22), indicating a positive feedback loop between p53 and miR-34a. In the present study, we showed that induction of p53 by actinomycin D led to increased expression of miR-34a, which in turn inhibited Fra-1 expression (Figure 1F and G; Supplementary Figure 3 is available at Carcinogenesis Online). In addition, we demonstrated that compared with HCT116 p53−/− cells, HCT116 WT cells express higher level of miR-34a and lower level of Fra-1 (Figure 1F and G). Taken together, these data suggest that p53 can indirectly regulate Fra-1 expression via a miR-34a-dependent manner. p53 is a well-known tumor suppressor gene; inactivation of p53 pathways is a common feature of many cancers (42,43). Some p53 mutants lose their tumor suppressor activity by failing to bind to the consensus DNA sequence (44). We showed here that the DNA-binding activity of p53 was decreased in 16 of 40 (40%) colon cancer tissues, as compared with the adjacent normal colon tissues (Supplementary Figure 8A is available at Carcinogenesis Online). Previously, it has been demonstrated that the average expression level of miR-34a in WT p53 glioblastoma tumors (n = 7) is significantly higher than that in mutant p53 tumors (n = 4) (20). Consistent with this observation, we found that the DNA-binding activity of p53 was significantly correlated with miR-34a expression in colon cancer tissues (Supplementary Figure 8B is available at Carcinogenesis Online). However, we did not observe any correlation between the DNA-binding activity of p53 and the Fra-1 mRNA level in the same specimens, even in the 14 tumor tissues that showed significant inverse correlation between miR-34a and Fra-1 expression (Supplementary Figure 8B and C is available at Carcinogenesis Online). These observations further indicate that p53-miR-34a pathway is not the key regulator of Fra-1 expression in human colon cancer tissues. Interestingly, we found that the DNA-binding activity of p53 and miR-34a expression in metastatic/invasive colon cancer tissues were significantly lower, whereas the Fra-1 expression was obviously higher than those in non-metastatic/non-invasive samples (Figure 6B and D; Supplementary Figure 8E is available at Carcinogenesis Online), suggesting that these factors are all involved in the regulation of tumor metastasis.

Cell migration and invasion occur as normal events in a number of physiological processes. Uncontrolled migration and invasion lead to metastasis, which cause as high as 90% of human cancer deaths (1,45). MMP-1 and MMP-9 are involved in cell migration and invasion and frequently upregulated in cancer cells (26,29). In our study, both miRNA and protein levels of MMP-1 and MMP-9 were decreased in miR-34a-transfected cells (Figure 2C and D), which can be significantly rescued by forced Fra-1 expression (Figure 4D and E). It has been demonstrated that Fra-1 directly induces MMP-1 and MMP-9 promoter activities in breast cancer cells (29) and induces MMP-9 expression to enhance the motility and invasion of lung cancer cells (28). Here, we also demonstrated that inhibition of Fra-1 by miR-34a led to downregulation of MMP-1 and MMP-9 in colon cancer cells (Figures 2–5). c-Met is a well-characterized cell surface receptor tyrosine kinase and commonly upregulated in a variety of tumors, including human colon cancers (46). It has been reported as a direct target of miR-34a in HCC cells and uveal melanoma cells (19,23). c-Met can phosphorylate and activate its downstream signal transduction intermediates, such as extracellular signal-regulated kinase 1/2; the key factors that influence tumor metastasis (47). Activation of extracellular signal-regulated kinase 1/2 results in elevated level of Fra-1 protein (40,41). In addition, c-Met expression is inhibited in Fra-1-silenced mesothelioma cells (39). Our data also showed that inhibition of Fra-1 led to downregulation of c-Met in HCT116 and RKO cells (Figure 3D and E). However, forced Fra-1 expression did not rescue the expression of c-Met in miR-34a-transfected cells (Figure 4D and E), probably due to the fact that c-Met is a direct target of miR-34a in HCT116 cells (19). The molecular mechanism of how Fra-1 regulates c-Met needs further investigation.

Based on these observations, we propose a simple model linking the p53-miR-34a pathway to Fra-1 and cell migration/invasion in colon cancer cells. To summarize, p53 induces the expression of miR-34a, which in turn decreases Fra-1 expression by directly target-

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**Inhibitory effects of miR-34a on colon cancer cells**

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**Supplementary material**

Supplementary Figures 1–8 can be found at http://carcin.oxfordjournals.org/.

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**References**


527

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