miR-137 targets Cdc42 expression, induces cell cycle G1 arrest and inhibits invasion in colorectal cancer cells

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miRNAs have emerged as post-transcriptional regulators that are critically involved in the pathogenesis of a number of human cancers. Cdc42, one of the best characterized members of the Rho GTPase family, is found to be up-regulated in several types of human tumors and has been implicated in cancer initiation and progression. In the present study, we have identified miR-137 as a potential regulator of Cdc42 expression. A bioinformatics search revealed a putative target-site for miR-137 within the Cdc42 3’ UTR at nt 792–798, which is highly conserved across different species. Expression of miR-137 in colorectal cancer cell lines was found inversely correlated with Cdc42 expression. miR-137 could significantly suppress Cdc42 3’ UTR luciferase-reporter activity, and this effect was not detectable when the putative 3’ UTR target-site was mutated. Consistent with the results of the reporter assay, ectopic expression of miR-137 reduced both mRNA and protein expression levels of Cdc42 and mimicked the effect of Cdc42 knockdown in inhibiting proliferation, inducing G1 cell cycle arrest, and blocking invasion of the colorectal cancer cells, whereas anti-miR-137 expression led to the opposite effect. Furthermore, expression of miR-137 suppressed the immediate downstream effector of Cdc42, PAK signaling. Our results suggest that miR-137 may have a tumor suppressor function by directly targeting Cdc42 to inhibit the proliferation and invasion activities of colorectal cancer cells. They raise an interesting possibility that Cdc42 activity and function can be controlled by miRNAs in addition to the classic regulators such as guanine nucleotide exchange factors and GTPase-activating proteins.

Introduction

microRNAs (miRNAs) are a class of naturally occurring small, noncoding RNAs measuring 18–25 nucleotides in length. More than 700 miRNAs have been annotated in the human genome thus far.1 miRNAs in conjunction with the effects of Argonaute (Ago) result in a guide molecule in post-transcriptional gene silencing, which can either suppress translation or degrade miR-bound mRNA by partially complementing with the 3 untranslated region (3’ UTR) of the cognate mRNAs.2–4 Any given miRNA is likely to have multiple gene targets. In fact, it is estimated that as high as 30% of protein-coding genes could be targets of miRNAs. Because miRNAs can target multiple transcripts, they are involved in diverse cell biological processes including cell differentiation, cell proliferation, apoptosis, metabolism, protein secretion and viral infection. Recent studies have also implicated miRNAs in tumorigenesis and cancer progression.4–6 Increasing numbers of miRNAs including miR-21,7 miR-200,8 miR-10,9,10 miR-1511,12 and miR-1611,12 have been reported to display aberrant expressions in cancers and may be involved in modulating cancer cell behaviors.

Rho GTPases form a subfamily of the Ras superfamily of 20–30 kDa GTP-binding proteins that have been shown to mediate a plethora of cellular effects such as cytoskeletal reorganization, membrane trafficking, proliferation, apoptosis/survival, cell polarity, cell adhesion, cell cycle and gene transcription.13,14 Cdc42 (cell division cycle 42), one of the best-characterized members of the Rho GTPase family, has been shown to be up-regulated in several types of human cancers, including testicular cancer,15 colorectal cancer,16 breast cancer,7 head and neck carcinoma18 and melanoma.19 The up-regulated Cdc42 activity may impair c-Cbl-mediated

Key words: miR-137, colorectal cancer, Cdc42, small G protein, cell cycle

Abbreviations: Cdc42: cell division cycle 42; ERK1/2: extracellular signal-regulated kinase 1/2; PAK1: p21 protein (Cdc42/Rac)-activated kinase 1; miRNA: microRNA; MLC: myosin light chain; Si-Cdc42: siRNA targeting cdc42 coding sequences

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EGFR degradation, contribute to EGFR hyperactivity and induce proteasomal degradation of p21CIP1, leading to an increase in cell proliferation and migration.\textsuperscript{20,21} In addition, downregulation of Cdc42 signals can inhibit anchorage-independent growth and induce apoptosis via the PI(3)K-Akt and Erk signaling cascades and the p53 tumor suppressor.\textsuperscript{22} Our previous studies in primary gene targeted mammalian cells have shown that Cdc42 is critically involved in actin filopodia formation, cell motility, directional migration and cell growth. These functional outcomes may be through regulation of PAK1, GSK3β, MLC, ERK1/2, JNK and/or NF-κB pathways.\textsuperscript{23} Given these diverse functions and signaling events dependent on Cdc42, Cdc42 may contribute to multiple stages of tumorigenesis and tumor progression.

To date, biochemical studies have identified a tightly regulated GTP-binding/GTP-hydrolysis cycle that is essential for the regulation of Cdc42 activity. Interestingly, in human tumors, no mutations in Cdc42 coding sequences have been found, suggesting that either the regulation of GTP-binding activity of Cdc42 by the classic regulators such as guanine nucleotide exchange factors, GTPase-activating proteins and GDP-dissociation inhibitors is sufficient in modulating its activity, or additional mode of regulation in the expression level may be involved in controlling its activity and function. The current study is aimed at elucidating the regulatory mechanism of Cdc42 by miRNAs. Our data show that miR-137 can negatively regulate Cdc42 expression and suppress the growth and invasion of colorectal cancer cells.

**Material and Methods**

**Cell culture**

All cell lines were maintained in RPMI 1640 medium (Invitrogen, Gaithersburg, MD, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS)(Gibco, Gaithersburg, USA), 100 U/ml of penicillin G sodium, and 100 μg/ml streptomycin sulfate (Sigma, Saint Louis, MO, USA) in a humidified atmosphere containing 5% CO\textsubscript{2} at 37 °C.

**Transfection**

Control oligo, hsa-miR-137, control anti-miR, anti-miR-137 and Si-Cdc42 (siRNA targeting cdc42 coding sequences, 5’-CCUCUACAUUUGAGAAACU dTdT-3’, 3’-dTdT GGAG AUGAUAACUCUUUGA-5’ were chemically synthesized by Ribobio (Guangzhou, China). DNA oligonucleotides were transfected into cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol.

**Plasmid construct**

The 3’ UTR of the human Cdc42 gene (NM_001791) was PCR amplified from human genomic DNA using primers 5’-GCTCTAGGCGCGTTTCTGCAACGC-3’ and 5’-GCTCTA GAGCTAACCAGCTTTGAATG-3’, and cloned into the Xba1-site of pGL3-control vector (Promega, Madison, WI, USA), which is designated pGL3-Cdc42-wt after sequencing. Site-directed mutagenesis of the miR-137 target-site in the Cdc42 3’ UTR was carried out using site-directed mutagenesis kit (TaKaRa, Dalian, China), with pGL3-Cdc42-wt as a template, and named pGL3-Cdc42-mut (primers: FW, 5’-GCT ACTAGTCTAAATCAAATGAG-3’, RV, 5’-TTAGTAT GATGCCGACACCGA-3’).

**Luciferase assay**

Cells were seeded in 24-well plates 1 day before transfection. For reporter assays, the cells were transiently cotransfected with 0.3 μg wt or mutant reporter plasmid and 60 nM control miRNA or miR-137 using lipofectamine 2000 (Invitrogen). Firefly and Renilla luciferase activities were measured consecutively by using Dual Luciferase Assay (Promega) according to the manufacturer’s instructions, and normalized for transfection efficiency by the control vector containing Renilla luciferase, pRL-TK (promega). Three independent experiments were performed in triplicate.

**Cell growth assay**

SW1116, Lovo and Colo320 colorectal cancer cells were seeded in 96-well plates 1 day before transfection. After transfecting with miR-137, Si-Cdc42, control oligo, anti-miR-137 or control anti-miR, the Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) was used to determine relative cell growth.

**Cell cycle assay**

Cells were harvested by trypsinization 72 hr after transfection, washed three times with ice-cold PBS and fixed with 70% ethanol overnight at 4 °C. The fixed cells were rehydrated in PBS and subjected to PI/RNase staining followed by fluorescence activated cell sorter scan (FACS) analysis (Becton Dickinson, Mountain View, CA, USA). The percentage of cells in each phase of the cell cycle was estimated using ELITE software.

**Matrigel invasion assay**

Twenty-four hours after transfection, 4 × 10\textsuperscript{4} cells were suspended in 0.25 ml of culture medium with 1% FBS and plated in the top chamber with matrigel-coated membrane (24-well insert; pore size, 8 mm; Becton Dickinson). The cells were incubated for 48 hr, after which the cells that did not invade through the pores were removed by a cotton swab. Cells on the lower surface of the membrane were stained with Hematoxylin and Eosine for visualization, and counted.

**Quantitative PCR analysis**

Total RNA was extracted using Trizol (Invitrogen), treated with DNase I (Takara) to eliminate contaminating genomic DNA, and reverse-transcribed into cDNA with the Reverse Transcriptase M-MLV (TaKaRa). Real-time PCR was performed using a SYBR Premix Ex Taq™ kit (TaKaRa) on the iQ5 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). PCR primers used were as follows: Cdc42 FW, 5’-ggtg ggctgtgtgtaaa-3’ and RV, 5’-taatcctagcggctgtaat-3’; β-actin FW,
For analysis of miRNA expression by qRT-PCR, reverse transcription and PCR were carried out using Bulge-Loop™ miRNA qPCR Primer Set for hsa-miR-137 (RiboBio, MQP-0101), and U6 snRNA (RiboBio, MQP-0201) according to the manufacturer’s instructions. Expression of Cdc42, relative to β-actin and miR-137, relative to U6, was determined using the 2−ΔΔCT method.

**Western blot**

Total-cell lysates were prepared using RIPA buffer (150 mM NaCl, 1% NP-40, 50 mM Tris-HCl (pH 7.4), 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 mM deoxycholic acid and 1 mM EDTA) containing a cocktail of protease inhibitors and phosphatase inhibitors (Calbiochem, Darmstadt, Germany). Equal amounts of protein sample (40–60 μg) was separated by 12% SDS-PAGE and transferred to PVDF membrane (Millipore, Bedford, MA, USA) using the Bio-Rad semidry transfer system. The following antibodies were used for Western blotting: Anti-Cdc42 (Becton Dickinson), Anti-β-actin (Santa Cruz, California, USA), anti-PAK1, anti-p44/42, anti-MLC, anti-cyclin D1, anti-Phospho-PAK1 (Thr-423)/PAK2(Thr-402), anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204), anti-phospho-MLC(Ser-473) (Cell Signaling Technology, MA, USA). Blotted proteins were detected and quantified using the ODYSSEY Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA).

**Statistical analysis**

Assays for characterizing phenotypes of cells were analyzed by Student’s test or one-way ANOVA and correlations between groups were calculated with Pearson. P values of <0.05 were deemed statistically significant. Data analysis was achieved using SPSS for windows version 14.0 (SPSS, Chicago, USA).

**Results**

The Cdc42 3’ UTR contains a putative target site for miR-137 that is highly conserved across species

To associate miRNAs with the regulation of Cdc42 expression, we performed a bioinformatic search for putative miRNAs that are predicted to target cdc42 gene using PicTar.24 We found that the 3' UTR of human cdc42 harbors several putative miRNA target sites, including those for hsa-miR-1, hsa-miR-15, hsa-miR-16, hsa-miR-18, hsa-miR-27, hsa-miR-29, hsa-miR-32, hsa-miR-33, hsa-miR-92, hsa-miR-101, hsa-
miR-103, hsa-miR-107, hsa-miR-128, hsa-miR-133, hsa-miR-137, hsa-miR-141, hsa-miR-144, hsa-miR-185, hsa-miR-186, hsa-miR-195, hsa-miR-199, hsa-miR-204, hsa-miR-206, hsa-miR-211, hsa-miR-320 and hsa-miR-342. We initially focused our studies on hsa-miR-101, hsa-miR-133 and hsa-miR-137, since these three miRNAs have been reported to be downregulated in several types of cancer cell lines and tumors. However, in subsequent tests, we soon found that the level of hsa-miR-101 or hsa-miR-133 has no correlation with Cdc42 protein expression, and that hsa-miR-101 and hsa-miR-133 have no effect on Cdc42 3’ UTR luciferase-reporter activity. Additionally, both the Cdc42 protein and mRNA were not affected by the ectopic expression of hsa-miR-101 and hsa-miR-133 (data not shown). Therefore, we focused our studies on hsa-miR-137 exclusively. A sequence analysis revealed that the targeting sequence for miR-137 is located at nt 792–798 of the cdc42 3’ UTR, and this region is highly conserved across different species (Fig. 1).

Figure 2. miR-137 and Cdc42 expressions are inversely correlated in colorectal cancer cell lines. (a) Western-blot analysis for Cdc42 in 7 colorectal cancer cell lines. (b) The band intensities in a were quantified with the ODYSSEY Infrared Imaging System (LI-COR Biosciences). Data represent the mean ± SD, from three independent experiments. (c) qRT-PCR analysis for miR-137 expression in 7 colorectal cancer cell lines. The miR-137 expression was normalized to U6 expression using the 2^−ΔΔCT method. Cdc42 protein expressions are inversely correlated with miR-137 expressions (p = 0.013). Data are representative of three independent experiments performed in triplicate.

Figure 3. The putative miR-137 binding site in cdc42 3’ UTR is functional in a luciferase assay. pGL3-Cdc42-wt or pGL3-Cdc42-mut luciferase constructs containing a wild-type or a mutated Cdc42 3’ UTR were cotransfected into SW1116, Lovo and Hela cells with control miR or miR-137, respectively. Luciferase activity was determined 48 hr after transfection. The ratio of normalized sensor to control luciferase activity is shown. Data are shown as the mean ± SD and were obtained from three independent experiments performed in triplicate. * indicates a significant difference from control miR-transfected cells (p < 0.05).
Figure 4. miR-137 regulates the expression of Cdc42. (a) Western-blot analysis of SW1116 and Lovo cells 72 hr after transfected with miR-137, Si-Cdc42 or control oligo for Cdc42 protein expression. (b) The band intensities in a were quantified with the ODYSSEY Infrared Imaging System (LI-COR Biosciences). Data represent the mean ± SD from three independent experiments. (c) qRT-PCR analysis of Cdc42 mRNA expression in SW1116 and Lovo cells 72 hr after transfected with miR-137, Si-Cdc42 or control oligo. Cdc42 mRNA expression was normalized to β-actin mRNA expression, and data are shown as a ratio of miR-137- and Si-Cdc42-transfected cells to control oligo-transfected cells using the $2^{-\Delta\Delta CT}$ method. (d) qRT-PCR analysis of miR-137 in SW1116 and Lovo cells transfected with miR-137, Si-Cdc42 or control oligo. The miR-137 expression was normalized to U6 expression, and data are shown as a ratio of miR-137- and Si-Cdc42-transfected cells to control oligo-transfected cells using the $2^{-\Delta\Delta CT}$ method. Data are representative of three independent experiments performed in triplicate. (e) Western-blot analysis of Colo320 cells 72 hr after transfected with anti-miR-137 or control anti-miR for Cdc42 protein expression. (f) The intensities of the blots in e were quantified with the ODYSSEY Infrared Imaging System (LI-COR Biosciences). Data represent the mean ± SD from three independent experiments. * indicates a significant difference from control oligo-transfected cells ($p < 0.05$).
The level of miR-137 is inversely correlated with Cdc42 protein expression in colorectal cancer cells

To examine whether miR-137 may functionally affect Cdc42 expression, we first determined expression levels of miR-137 and Cdc42 protein in 7 different colorectal cancer cell lines. In cell lines with low endogenous miR-137 expression, as measured by miRNA real-time PCR (i.e., Lovo, HT-29, COLO205, SW620, SW1116), a relatively high level of Cdc42 protein was observed. Conversely, cell lines with relatively high miR-137 expression (i.e., Colo320 and SW480) showed significantly lower amounts of Cdc42 protein when compared with those containing lower miR-137 (Fig. 2). These results from the available cell lines suggest a reciprocal relationship between levels of miR-137 and Cdc42 protein expression in human colorectal cancer cells ($p = 0.013$), justifying a further examination of the role of miR-137. However, because of the limited number of colorectal cancer cell lines available, a definitive conclusion on this issue needs to wait until a more systemic study is done using larger numbers of primary colorectal cancer samples.

The putative 3' UTR target site of cdc42 is directly regulated by miR-137

From the earlier observations, we hypothesize that cdc42 is a target of miR-137. The 3' UTR of human cdc42 gene was cloned into the Xba1-site of pGL3-luciferase reporter vector (pGL3-control) (Promega) to test whether it might serve as a direct functional target of miR-137. This construct was named pGL3-Cdc42-wt. In parallel, another luciferase reporter construct in which the putative miR-137 targeting region AAGCAAU, located within nt 792–798, was specifically mutated and predicted to abolish miR-137 binding, was designated pGL3-Cdc42-mut. Transient transfection of SW1116 (Fig. 3a), Lovo (Fig. 3b) and HeLa (Fig. 3c) cells with pGL3-Cdc42-wt and miR-137 led to a significant decrease of luciferase activity when compared with the
control. The activity of the mutant reporter construct, however, was unaffected by the cotransfection with miR-137.

Next, to examine whether miR-137 could affect Cdc42 expression in colorectal cell lines, we transfected colorectal cell lines SW1116 and Lovo that show a relatively high Cdc42 expression, with miR-137, a control oligo or Si-Cdc42. The protein and mRNA expression levels of Cdc42 were analyzed by Western blotting and real-time PCR, respectively. As shown in Figure 4, compared with the negative control, transient expression of miR-137 led to a significant decrease in Cdc42 protein (Figs. 4a and 4b) and mRNA expressions (Fig. 4c), similar to that caused by transfection of Si-Cdc42 in both cell lines. Furthermore, to determine whether transfection of cell lines with anti-miR-137 affects Cdc42 protein expression, Colo320 cells with a relatively high miR-137 expression and low Cdc42 expression, were transiently transfected with anti-miR-137 or control-anti-miR. Down-regulation of endogenous miR-137 with anti-miR-137 led to a significant increase in Cdc42 protein expression in Colo320 cells (Figs. 4e and 4f). Taken together, these data suggest that the 3′ UTR of Cdc42 is a functional target site for miR-137 in the colorectal cancer cells.

miR-137 regulates proliferation, invasion and G0/G1 progression of colorectal cancer cells

Cdc42 is known to play a key role in the regulation of cell cycle progression at the G1/S transition. We tested whether the cell growth potential of colorectal cancer cells transfected with miR-137 was inhibited as a consequence of Cdc42 expression suppression. Figure 5 shows the results of CCK-8 assays where the ectopic expression of miR-137 or Si-Cdc42 markedly inhibits the proliferation potentials of SW1116 cells (Fig. 5a) and Lovo cells (Fig. 5b), and anti-miR-137 enhances the proliferation potentials of Colo320 cells (Fig. 5c). Thus, reducing Cdc42 expression in SW1116 and Lovo cells, either by miR-137 or Si-Cdc42, results in similar growth phenotypes of a marked inhibition of cell proliferation, while increasing Cdc42 expression by anti-miR-137 has an opposite effect on Colo320 cells.

To further understand the mechanisms by which cell proliferation is affected, flow cytometry was performed to analyze the cell cycle phase distribution of these cells. The cell cycle progression of SW1116 cells (Fig. 6a) and Lovo cells (Fig. 6b) transfected with miR-137 or Si-Cdc42 was stalled at the G1 phase with a significant decrease in S and G2/M phases compared with cells transfected with control oligo. Furthermore, while the percentage of G0/G1 phase decreased in Colo320 cells after transfection with anti-miR-137, the other phases increased accordingly (Fig. 6c). These results indicate that miR-137 can reduce cell proliferative potential by mimicking Cdc42 reduction and support the role of this miRNAs as a negative regulator of Cdc42 regulated cell growth.

In addition to proliferation regulation, Cdc42 is involved in the invasive phenotype of tumor cells that may contribute to the morbidity and mortality of patients with cancer. To determine whether miR-137 may regulate invasion, SW1116 and Lovo cells were transfected with miR-137, Si-Cdc42 or Control oligo, and Colo320 (a) 72 hr after transfection with anti-miR-137 or Control anti-miR, was determined by FACS analysis. Data are representative of 3 independent experiments. * indicates a significant difference from control oligo-transfected control cells (p < 0.05).
of miR-137 by anti-miRNA (Fig. 7c). These data suggest that miR-137 negatively regulates invasion of the colorectal cancer cells, and this may, at least in part, be due to its targeting effect on Cdc42.

Ectopic expression of miR-137 suppresses Cdc42/PAK signaling
To determine whether the downstream signals of Cdc42 are affected by ectopic expression of miR-137, SW1116 and Lovo cells were transfected with miR-137, Si-Cdc42 or control oligo, and the phosphorylation and/or the expression levels of PAK1 (p21 protein (Cdc42/Rac)-activated kinase 1), myosin light chain (MLC), extracellular signal-regulated kinase 1/2 (ERK1/2) and cyclin D1 were examined by Western blotting. As shown in Figure 8, the phosphorylation levels of PAK1, MLC, ERK1/2 and the expression of Cyclin D1, the known downstream signals of Cdc42, decreased significantly by miR-137 expression. These signaling effects again mimicked those of Cdc42 siRNA knockdown, suggesting that the function of miR-137 in the cancer cells is likely, at least in part, through the Cdc42 regulated PAK signaling pathway. This is consistent with the results of our previous study performed in Cdc42-/- and Cdc42GAP-/- primary mouse embryonic fibroblasts (MEFs) produced by gene targeting as the Cdc42 loss- or gain-of-activity cell model.23

Discussion
Increasing studies in the past few years have shown that miRNAs could serve functionally as “oncogenes” or “tumor suppressor genes” and regulate multiple cellular processes relevant to tumorigenesis and cancer progression. Despite intensive efforts in this area, only a handful of the cases studies have identified the gene targets of specific miRNAs involved in human tumor formation. Since it is important to define the function and mechanism of miRNAs in oncogenesis of different types of human tumors, in this study, we focused on the role of miR-137, which has been found to be down-regulated in colorectal cancer,25 glioblastoma multiforme26 and oral cancer.27

As a key member of the Rho GTPase family, Cdc42 is known to be involved in proliferation, migration, invasion, apoptosis and angiogenesis, in several types of cancers.13,14 Our previous studies suggest that Cdc42 may contribute to the hypoxia-mediated angiogenesis28 and is critically involved
Figure 8. Ectopic expression of miR-137 reduces PAK signaling similarly to that by Cdc42 knockdown. (a) Western-blot analysis of SW1116 and Lovo 72 hr after transfected with miR-137, Si-Cdc42 or control oligo for PAK1, MLC, ERK1/2, cyclin D1, phospho-PAK1, phospho-MLC, phospho-ERK1/2. (b) The band intensities in a were quantified with the ODYSSEY Infrared Imaging System (LI-COR Biosciences). Data represents the mean ± SD from three independent experiments. * indicates a significant difference from control oligo-transfected control cells (p < 0.05).
in actin filopodia formation, cell motility, directional migration and cell growth. Cdc42 is also required for the regulation of multiple signaling pathways, including PAK1, GSK3β, MLC, ERK1/2, JNK and NF-κB, in Cdc42−/− and Cdc42GAP−/− primary mouse embryonic fibroblasts. Unlike Ras, that is found to be constitutively activated by point mutations in its coding sequence in human tumors, to date, no coding region sequence mutation has been detected for the cdc42 gene in human cancers. This suggests that Cdc42 is likely to be regulated by different mechanisms such as overexpression, deregulation of the GTP-binding cycle and/or deregulation of its stability. To understand the contribution of Cdc42 to cancer progression, it is essential to better define the regulatory mechanism of Cdc42.

Since we observed that the 3′ UTR of human Cdc42 harbors one putative site recognized by miR-137, and more importantly, miR-137 and Cdc42 expressions seem to be inversely correlated in several colorectal cancer cells, we hypothesized that Cdc42 may be directly regulated by miR-137 at the posttranscriptional level. By a luciferase-based reporter assay, we show that one predicted miR-137 targeting site in the Cdc42 3′ UTR is functional, and that expression of miR-137 causes a significant decrease in both mRNA and protein of Cdc42. Consistently, the proliferation and invasion activities of the colorectal cancer cells were inhibited by miR-137 similar to that of Cdc42 siRNA, but were enhanced by anti-miR-137 that elevated Cdc42 expression. An examination of the effects by miR-137 expression on downstream signaling of Cdc42 further confirm that miR-137 mimics Cdc42 downregulation in several intracellular signaling events. Together, these functional and signaling findings strongly suggest that miR-137 can act by downregulating Cdc42 expression to impact on cancer cell behavior.

Cell cycle progression involves the sequential activation of different cyclin-dependent kinases (CDKs). The expression of cyclins and other regulators of CDKs vary during cell cycle progression and are often deregulated in cancers. Cdc42 has been shown to contribute to the regulation of cell cycle progression in G1/S-phase transition, but the signal transduction pathways involved are still unclear. Previous studies have indicated miR-137 inhibits proliferation of glioblastoma multiforme cells by an unknown mechanism. Our results may provide a clue for the further study of the mechanism involved. It is tempting to speculate that miR-137 may achieve proliferative inhibition and cell cycle G1 arrest induction via negatively regulating Cdc42 expression, which in turn affects expression and activities of downstream molecules of Cdc42, such as ERK1/2 and cyclin D1.

In addition to effects on the cell cycle, Cdc42 regulates cell cytoskeleton and adhesion, both of which are functions that are important for cell migration and invasion in several types of cancer. To this end, the protein level of Cdc42 is found to be elevated with high incidence (60%) in colorectal cancer. Cdc42 activation promotes lamellipodium formation and concomitantly enhanced cell invasion in human colon cancer cells. In our study, ectopic expression of miR-137 inhibits invasion in colorectal cancer cells, similar to the effects of siRNA downregulation of Cdc42, whereas anti-miR-137 stimulates invasion in Colo320 cells. The phospho-PAK1 and -MLC are also suppressed by miR-137 and Cdc42 siRNA, suggesting that miR-137 may regulate the cancer cell invasion through the Cdc42-PAK1-MLC pathway.

In conclusion, our studies show that miR-137 expression can markedly suppress Cdc42 expression and effectively inhibit Pak-MLC or ERK signaling and the proliferation/invasion of colorectal cancer cells, mimicking the effects of Cdc42 knockdown. Anti-miR-137 shows an opposite effect on Cdc42 expression and cancer cell behaviors. The results suggest that miR-137 is a regulator of Cdc42 activity and may serve as a useful tool in exploring the potential therapeutic benefits of Cdc42 targeted therapy in colorectal cancer.

References