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miR-21 targets the tumor suppressor RhoB and regulates proliferation, invasion and apoptosis in colorectal cancer cells

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ABSTRACT

It has become increasingly clear that microRNAs play an important role in many human diseases including cancer. Here, we show that expression of miR-21 in HEK293 and several colorectal cancer cells was found inversely correlated with ras homolog gene family, member B (RhoB) expression. miR-21 expression significantly suppressed RhoB 3′ UTR luciferase-reporter activity, but the inhibitory effect was lost when the putative target sites were mutated. Exogenous miR-21 over-expression mimicked the effect of RhoB knockdown in promoting proliferation and invasion and inhibiting apoptosis, whereas anti-miR-21 or RhoB expression yielded opposite effects, in colorectal cancer cells. These results suggest that miR-21 is a regulator of RhoB expression and RhoB could be a useful target in exploring the potential therapeutic benefits of miR-21 mediated tumor cell behaviors in colorectal cancer.
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1. Introduction

The RhoA, ras homolog gene family, member B (RhoB), and RhoC proteins form a closely related subgroup of the Rho GTPase family that play crucial roles in the regulation of actin cytoskeleton, cell survival and gene expression [1–3]. Although they share more than 85% sequence identity, RhoA, RhoB and RhoC have different cellular functions and appear to show distinct, sometimes opposite, function in carcinogenesis. RhoA regulates cell contractility and proliferation via the actomyosin machinery and multiple growth related gene expression pathways, RhoC is required for cell–cell adhesion and cell invasion, whereas RhoB, which has a unique endosome localization pattern, is involved in membrane trafficking and cell survival. Whereas RhoA and RhoC are often found over-expressed in tumors, RhoB expression is down-regulated in multiple type of cancers [4–7]. Additionally, in contrast to other Rho family members, RhoB protein is relatively short-lived in cells and its protein expression is readily inducible upon exposure to a wide-variety of biological stimuli including growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and transforming growth factor-β (TGF-β) [8–10]. The induction of RhoB by stress suggests that RhoB is involved in cell growth control and/or DNA damage repair mechanisms, consistent with the finding that overexpression of RhoB, whether in its geranylgeranylated form (RhoB-GG) or farnesylated form (RhoB-F), can inhibit tumor cell growth and transformation [11]. RhoB was shown to be down-regulated in head and neck carcinoma and lung cancer but over-expressed in breast cancer [12–14]. Our previous report has shown that, in gastric cancer tissue and cell lines, RhoB expression is significantly suppressed, and is regulated by histone deacetylation, not by DNA methylation, at the epigenetic level [15,16].

MicroRNAs (miRNAs) are approximately 22-nucleotide, non-coding RNAs that can regulate the expression of multiple genes by targeting mRNAs posttranscriptionally. miRNAs, in conjuction with Argonaute (Ago) protein, function to posttranscriptionally silence genes through either suppressing translation or degrading miR-bound mRNA by complementing the 3 untranslated region (3′ UTR) of the cognate mRNAs [17–19]. It is estimated that as high as 30% of protein-coding genes could serve as miRNA targets. Due to the fact that miRNAs often regulate multiple transcripts, they are involved in diverse biological processes including cell differentiation, proliferation, apoptosis, metabolism, protein secretion, and viral infection. Recently, several studies have described findings of aberrant expression of miRNAs in human tumors and have investigated their potential role as oncogenes or tumor suppressor genes, depending on the targets they regulate

Abbreviations: miRNA, microRNA; RhoB, ras homolog gene family, member B; si-RhoB, siRNA targeting RhoB coding sequences

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[20–22]. Among them, miR-21 has been identified as one of the most highly expressed miRNA in various tumors, including colorectal [23], GBM [24], breast, lung, prostate, pancreas, and stomach cancers [25,26]. Increased miR-21 expression in these tumors is associated with cell proliferation, migration, invasion and metastasis [27], suggesting that miR-21 is a key regulatory molecule in cancer initiation and/or progression.

Our previous studies have shown that among Rho GTPases, RhoA and Cdc42 can be directly regulated by miR-137 and miR-185 that suppress the growth and invasion activities of colorectal cancer cells [28,29]. To date a role for RhoB and its relationship with miRNAs in colorectal cancer has not been reported. The current study is aimed at elucidating the regulatory mechanism of RhoB by miRNAs in colorectal cancer. Our data show that miR-21 can negatively regulate RhoB expression in colorectal cancer cells. Additionally, loss and ectopic RhoB expression induced by miR-21 or RhoB knockdown, and by anti-miR-21 or RhoB expression, respectively, gives opposite effects in several cell phenotypes, including proliferation, invasion and apoptosis, linking miRNA21-RhoB regulatory pathway to colorectal cancer cell functions.

2. Materials and methods

2.1. 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 units/ml of penicillin G sodium, and 100 μg/ml streptomycin sulfate (Sigma, Saint Louis, MO, USA) in a humidified atmosphere containing 5% CO_2 at 37 °C.

2.2. Transfection

Control oligo, hsa-miR-21, control anti-miR, anti-miR-21 and siRNA targeting RhoB coding sequences (si-RhoB, 5′-CCGTCTTCGAGAACTATGT dTdT-3′, 3′-dTdT GGCAGAAGCTCTTGATACA-5′) were chemically synthesized by RiboBio (RiboBio, Guangzhou, China). RNA oligonucleotides were transfected into cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol.

2.3. Plasmid construct

The 3′ UTR of the human RhoB gene (NM_004040) was PCR amplified from human genomic DNA using primers 5′-GCTCTAGATCCCGCCCAAGCATGAAC-3′ and 5′-GCTCTAGACCATTTTATAAATGTCATCATC-3′, and cloned into the Xba1-site of pGL3-control vector (Promega, Madison, WI, USA), which is designated pGL3-RhoB-wt after sequencing. Site-directed mutagenesis of the miR-21 target-site in the RhoB 3′ UTR was carried out using site-directed mutagenesis kit (TaKaRa, Dalian, China), with pGL3-RhoB-wt as a template, and named pGL3-RhoB-mut (primers: FW, 5′-TTCTCGCGATGATGTATACAGTTTTTTATG-3′, RV, 5′-CTTTTATGTAAACATCATAAGCTCA-3′). The pcEFL-GST-RhoB and pcEFL-GST vectors were kindly provided by Professor Zheng (Cincinnati Children's Hospital, Ohio).

2.4. Luciferase assay

Cells were seeded in 24-well plates 1 d before transfection. For reporter assays, the cells were transiently cotransfected in 24-well plates with 0.3 μg wt or mutant reporter plasmid and 60 nM control miRNA or miR-21 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.

2.5. Cell growth assay

SW1116 and Colo320 colorectal cancer cells were seeded in 96-well plates 1 d before transfection. After transfecting with miR-21, si-RhoB, control oligo, anti-miR-21, control anti-miR, pcEFL-GST-RhoB or pcEFL-GST, the Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) was used to determine relative cell growth.

2.6. In vitro apoptosis assay

Forty-eight hours after transfection, cells were incubated in 100 μl binding buffer containing 0.2 μg Annexin V conjugate fluorescein isothiocyanate and then stained with propidium iodide followed by FACS analysis (Fluorescence Activated Cell Sorter Scan) (Becton Dickinson, Mountain View, CA, USA).

2.7. Matrigel invasion assay

Twenty-four hours after transfection, 2×10^4 cells (SW1116 cell) or 4×10^4 cells (Colo320 cell) 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 36 h, 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.

2.8. 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: RhoB FW, 5′-cggactcgctggagaaca-3′ and RV, 5′-gaggtagtcgtaggcttggat-3′; β-actin FW, 5′-tgacgtggacatccgcaaag-3′ and RV, 5′-ctggaaggtggacagcgagg-3′. 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-21 (RiboBio, MQP-0101), and U6 snRNA (RiboBio, MQP-0201) according to the manufacturer's instructions. Expression of RhoB, relative to β-actin and miR-21, relative to U6, was determined using the $2^{-\Delta\Delta CT}$ method.

2.9. 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 (60–80 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-RhoB (Becton Dickinson), Anti-β-actin (Santa Cruz, CA, USA). Blotted proteins were detected and quantified using the ODYSSEY Infrared Imaging System (Li-COR Biosciences, Lincoln, NE, USA).

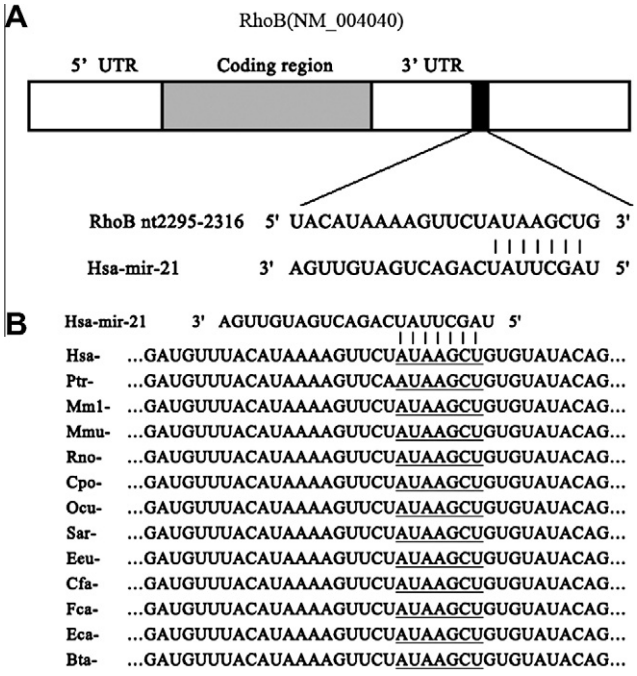

Fig. 1. 3′ UTR of RhoB contains a putative target site for miR-21 that is highly conserved across species. (A) A schematic representation of the RhoB mRNA putative sites targeted by miR-21. (B) Comparison of nucleotide sequences of miR-21 seed sequences with putative target sequence in different species. Sequence analyses indicate that miR-21 target sequence at nt 2309–2315 of the RhoB 3′ UTR is highly conserved across different species. The target-site for miR-21 within the RhoB 3′ UTR at nt 2309–2315 (AUAAGCU) is underlined.

2.10. 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).

3. Results

3.1. miR-21 is a putative regulator targeting RhoB

To identify miRNAs that regulate RhoB expression in colorectal cancer, we designed a miRNA screening assay using a miRNA target database of mammalian cells. We performed a bioinformatic search for putative miRNAs predicted to target RhoB coding or non-coding sequences using TargetScan and PicTar and found that the 3′ UTR of human RhoB gene harbored multiple putative miRNA target sites. miRNAs including hsa-miR-19, hsa-miR-30, hsa-let-7d, hsa-miR-21, hsa-miR-183, hsa-miR-186, hsa-miR-138, hsa-miR-96, hsa-miR-383, hsa-miR-384, hsa-miR-214, hsa-miR-761, hsa-miR-410, hsa-miR-590, hsa-miR-494, hsa-miR-411, hsa-miR-197 and hsa-miR-491 all seem to complement regions of RhoB gene. Considering the position, number, and sequence conservation of miRNA target sites across species, combined with the observation that RhoB protein is down-regulated while miR-21 is up-regulated in multiple cancer cell lines, we chose miR-21 for further characterization. Sequence analysis indicate that miR-21 may complement nt 2309–2315 sequences of the RhoB 3′ UTR that is highly conserved across different species (Fig. 1).

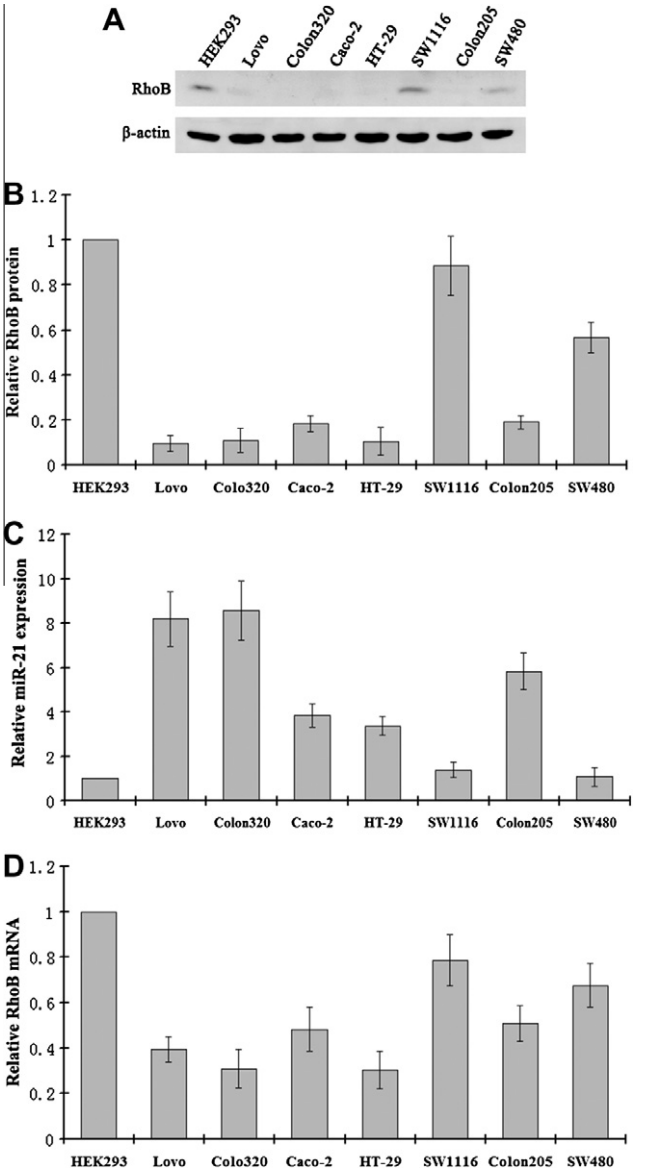

Fig. 2. miR-21 expression is inversely correlated with RhoB expression. (A) Western-blot analysis for RhoB in HEK293 and 7 colorectal cancer cell lines. (B) The intensities of blots in (A) were quantified with the ODYSSEY Infrared Imaging System (Li-COR Biosciences). (C) qRT-PCR analysis for miR-21 expression in HEK293 and 7 colorectal cancer cell lines. The miR-21 expression was normalized to U6 expression using the $2^{-\Delta\Delta CT}$ method. RhoB protein expressions are inversely correlated with miR-21 expressions ($P = 0.012$). (D) qRT-PCR analysis for RhoB mRNA expression in HEK293 and 7 colorectal cancer cell lines. The differences in RhoB protein are higher than for RhoB-mRNA, and RhoB protein and mRNA levels inversely correlate with miR-21. Data are representative of three independent experiments performed in triplicate.

3.2. miR-21 expression is inversely correlated with RhoB protein expression

To examine if miR-21 may functionally regulate RhoB expression, we have examined the expression levels of miR-21 and RhoB (mRNA and protein) in HEK293 and seven different colorectal cancer cell lines (Fig. 2). In cell lines with low endogenous miR-21 expression, as measured by quantitative real-time PCR (i.e., HEK293, SW1116 and SW480), relatively high levels of RhoB protein were detected. Conversely, cell lines with relatively high miR-21 expression

(i.e., Lovo, Colo320 and Colon205) showed relatively lower amounts of RhoB protein compared with those containing lower miR-21 expression. Across all eight cell lines tested, we found a significant inverse correlation between miR-21 and RhoB expressions. However, the differences in RhoB protein are higher than for RhoB-mRNA among the cells, and both show an inverse correlation with miR-21 expression ($P = 0.012$ for RhoB protein, $P = 0.038$ for RhoB mRNA). These data suggest that miR-21 expression is inversely correlated with RhoB expression in human colorectal cancer cells, and it is possible that miR-21 negatively regulates RhoB post-transcriptionally.

3.3. RhoB is directly regulated by miR-21

To obtain direct evidence that RhoB 3′ UTR is a target of miR-21, the 3′ UTR of human RhoB 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-21. This construct was named pGL3-RhoB-wt. In parallel, another luciferase reporter construct in which the putative miR-21 targeting region AUAAGCU, located within nt 2309–2315, was specifically mutated and predicted to abolish miR-21 binding, was designated pGL3-RhoB-mut. Transient transfection of HEK293 (Fig. 3A), SW1116 (Fig. 3B), and SW480 (Fig. 3C) cells with pGL3-RhoB-wt and miR-21 led to a significant decrease of luciferase activity as compared to the control. The activity of the mutant reporter construct, however, was unaffected by co-transfection with miR-21.

Next, to examine if miR-21 could affect RhoB expression, we transfected colorectal cell lines SW1116 and SW480 and HEK293 that show a relatively low miR-21 expression and high RhoB expression, with miR-21 or control miR, and Colo320 cells that show a relatively high miR-21 expression and low RhoB expression, with anti-miR-21 or control-anti-miR. The protein and mRNA expression levels of RhoB were analyzed by Western blotting and real-time PCR, respectively. As shown in Fig. 4, compared with the negative control, transient expression of miR-21 led to a significant decrease in RhoB protein (Fig. 4A) and mRNA expressions (Fig. 4B) in HEK293 and colorectal cell lines SW1116 and SW480. Furthermore, down-regulation of endogenous miR-21 with anti-miR-21 led to a significant increase in RhoB protein expression in Colo320 cells (Fig. 4C and D). Taken together, these results indicate that miR-21 directly targets RhoB expression in colorectal cancer cells.

3.4. miR-21 expression and RhoB knockdown show similar phenotypes in promoting growth and invasion and suppressing apoptosis of colorectal cancer cells

Our previous studies show that RhoB play a key role in the regulation of proliferation of gastric cancer cells [15]. Next we tested whether miR-21 may regulate growth of coloretal cancer cells and if this may be through RhoB expression suppression. Fig. 5A shows the results of CCK-8 assays where ectopic expression of miR-21 or a RhoB siRNA (si-RhoB) markedly enhanced the proliferation potential of SW1116 cells. Thus, miR-21 mimicked RhoB expression knockdown in SW1116 cells, resulting in similar growth phenotypes to that by RhoB-specific siRNA. To understand the mechanism of the proliferative activity of miR-21, we investigated the effect of miR-21 on cell apoptosis, because evasion of apoptosis is a crucial event during malignant transformation. Interestingly, compared with control oligo transfected cells, ectopic expression of miR-21 or si-RhoB resulted in a significant decrease in apoptosis rates as determined by Annexin V staining (Fig. 5C). These results indicate that miR-21 can promote proliferation and inhibit apoptosis, effects that are associated with RhoB downregulation.

In addition to proliferation regulation, our previous studies also show that RhoB is involved in the invasive phenotype of tumor cells that may contribute to the morbidity and mortality of cancer patients [15]. To determine whether miR-21 may regulate invasion through RhoB, SW1116 were transfected with miR-21, si-RhoB or control oligo. In a matrigel invasion assay, we found a significant increase in invasive activity of miR-21-transfected cells, mimicking that of si-RhoB-transfected cells (Fig. 5B). These data suggest that miR-21 positively regulates proliferation and invasion of the colorectal cancer cells, and this may, at least in part, be attributed to its targeting effect on RhoB.

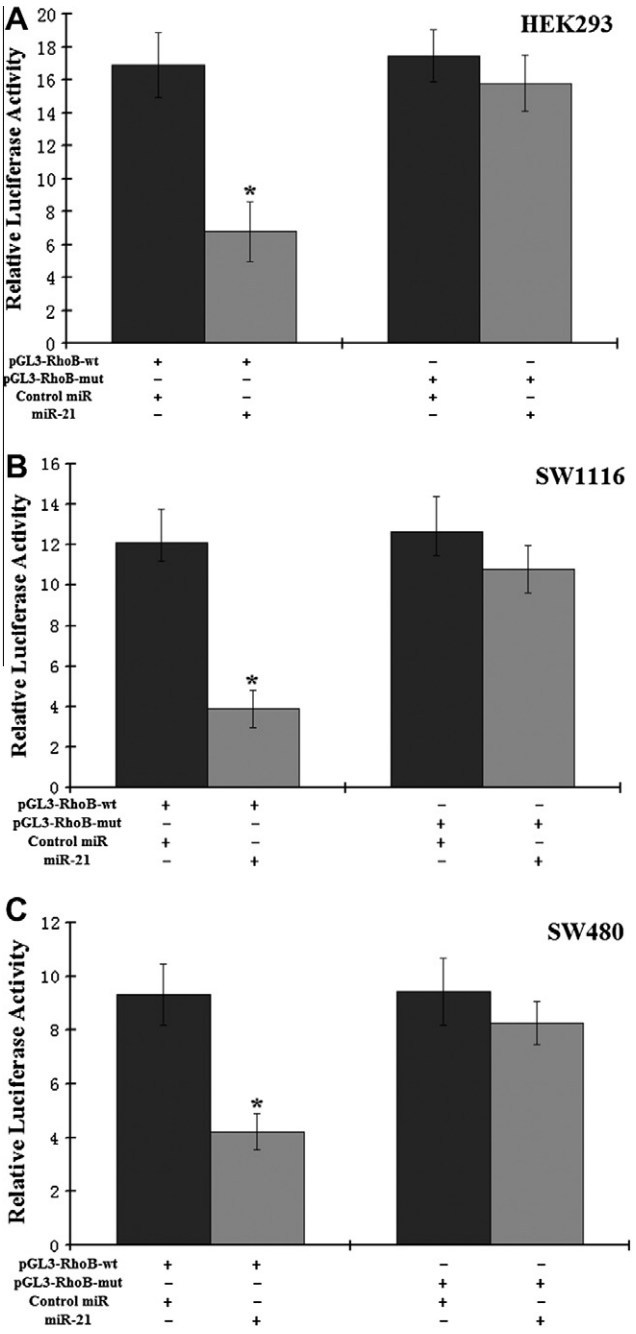

Fig. 3. A putative miR-21 binding site in RhoB 3′ UTR is functional in a luciferase assay. pGL3-RhoB-wt or pGL3-RhoB-mut luciferase constructs containing a wild-type or a mutated RhoB 3′ UTR were cotransfected into HEK293 (A), SW1116 (B) and SW480 (C) with control miR or miR-21, respectively. Luciferase activity was determined 36 h after transfection. The ratio of normalized sensor to control luciferase activity is shown. Data are shown as the mean ± S.D. and were obtained from three independent experiments performed in triplicate. *Significant difference from control miR-transfected cells ($P < 0.05$).

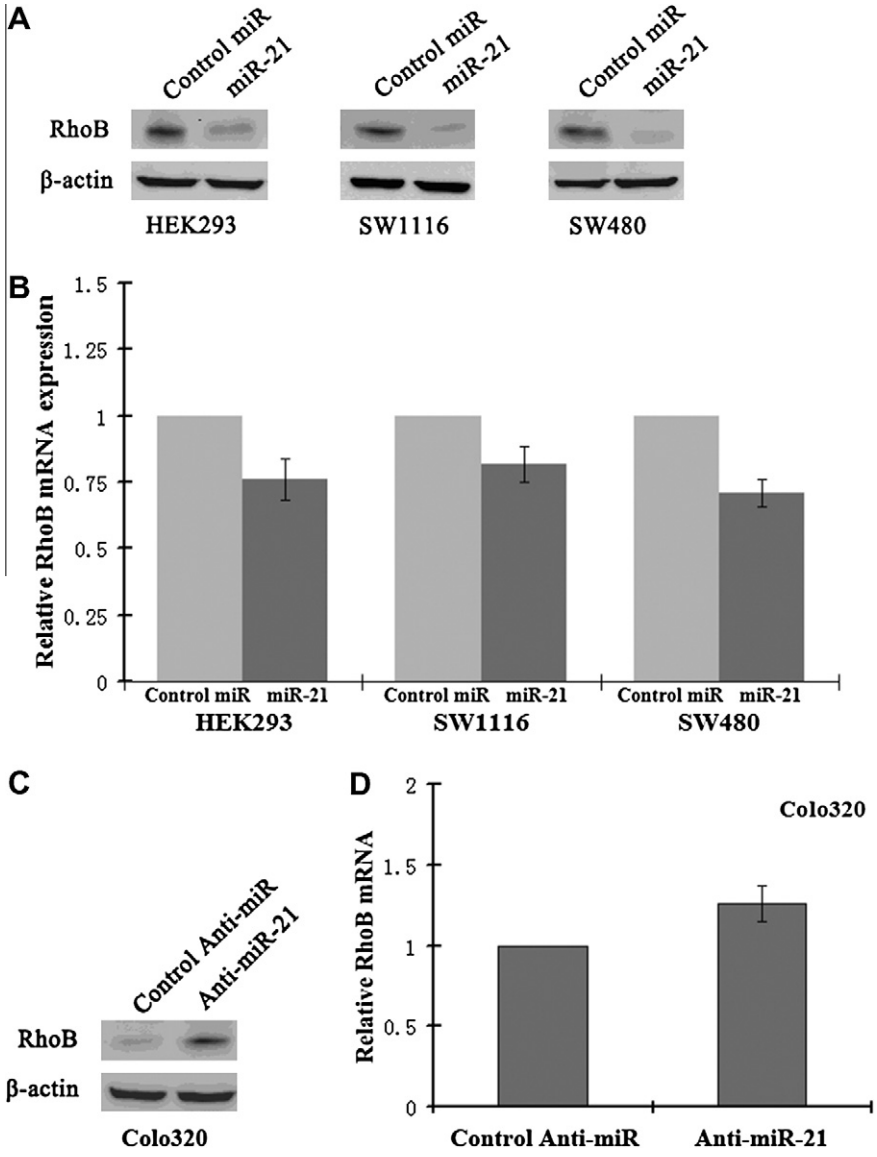

Fig. 4. Ectopic expression of miR-21 or anti-miR-21 regulates both mRNA and protein expression of RhoB. (A) Western-blot analysis of HEK293, SW1116 and SW480 cells 72 h after transfected with miR-21 or control miR for RhoB protein expression. (B) qRT-PCR analysis of RhoB mRNA expression in HEK293, SW1116 and SW480 cells transfected with miR-21 or control miR. (C) Western-blot analysis of Colo320 cells 72 h after transfected with anti-miR-21 or control anti-miR for RhoB protein expression. (D) qRT-PCR analysis of RhoB mRNA expression in Colo320 cells transfected with anti-miR-21 or control anti-miR. RhoB mRNA expression was normalized to β-actin mRNA expression, and data are shown as a ratio of miR-21 or anti-miR-21-transfected cells to control oligo-transfected cells using the $2^{-\Delta\Delta CT}$ method. Data are representative of three independent experiments performed in triplicate. *Significant difference from control oligo-transfected cells ($P < 0.05$).

3.5. Anti-miR-21 mimics RhoB expression in inhibiting cell growth and invasion and inducing apoptosis of colorectal cancer cells

To determine whether anti-miR-21 may have a similar effect to RhoB overexpression on cell proliferation, invasion and apoptosis in colorectal cancer cells, Colo320 cells with a relatively high miR-21 expression and low RhoB expression, were transiently transfected with anti-miR-21 or a control-anti-miR. Opposite to the results of miR-21 expression, inhibition of miR-21 by anti-miR-21 markedly suppressed the proliferation and invasion activities of Colo320 cells (Fig. 6A and B) and enhanced cell apoptosis (Fig. 6C). Furthermore, RhoB ectopic expression in these cells yielded the effect mimicking that of anti-miR-21, i.e., significantly inhibited the proliferation and invasion and promotes apoptosis in colorectal cancer cells. These data further support that miR-21-RhoB signaling axis regulates proliferation and invasion in the colorectal cancer cells.

4. Discussion

Numerous studies in the past few years have shown that miRNAs could serve functionally as "oncogenes" or "tumor suppressor genes" in tumorigenesis and regulate multiple cellular processes in the course of cancer progression. miR-21 is one of the most commonly implicated miRNAs in cancer. Its expression is highly upregulated in a variety of solid tumors and elevated miR-21 expression has been causally linked to proliferation, apoptosis, migration and drug resistance of several cancer cell lines [23–27,30–32], implying that miR-21 is a key regulatory molecule in cancer development that functions by targeting different signaling molecules. However, our knowledge of the molecular mechanisms mediating miR-21 function in cancer, particularly in colorectal cancer, is limited. In the present studies, we have identified miR-21 as a potential regulator of RhoB expression in colorectal cancer. A bioinformatics search revealed a target-site for miR-21 within the RhoB 3′ UTR at nt

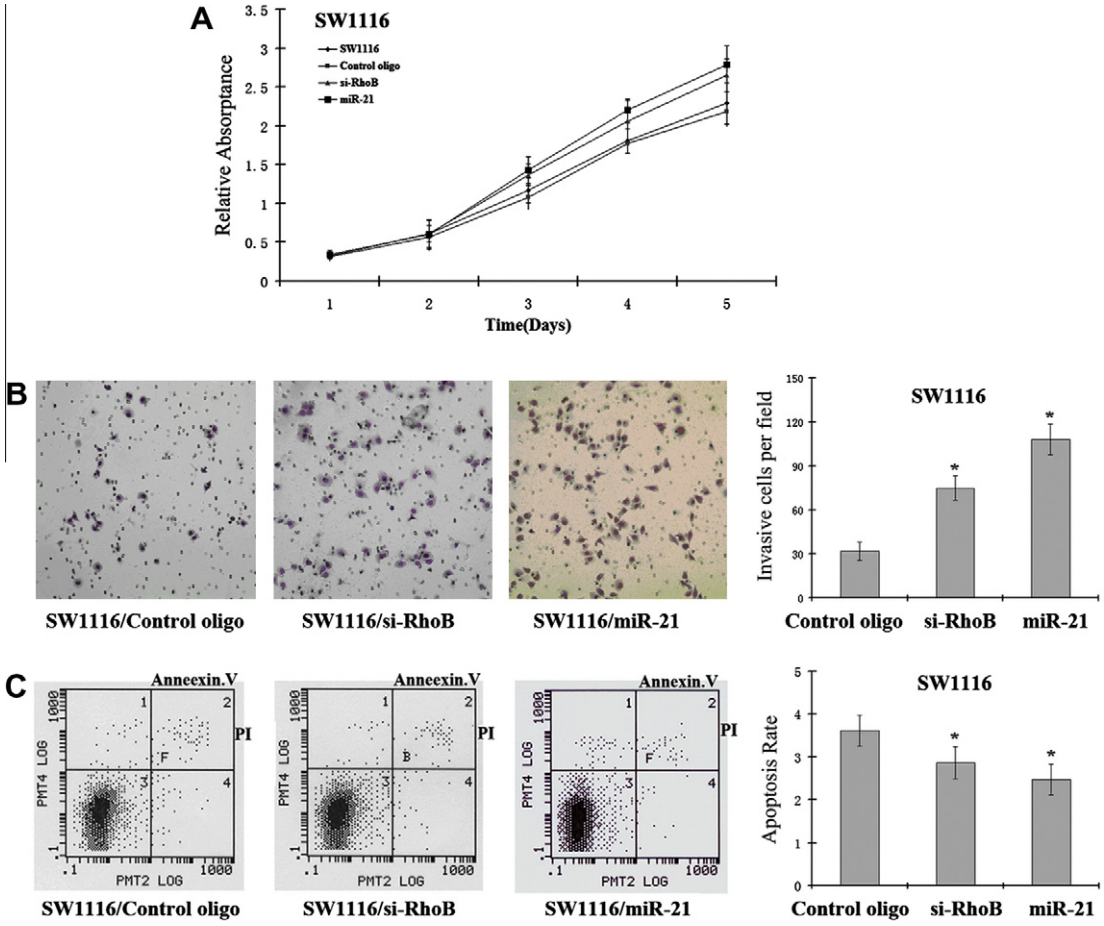

Fig. 5. miR-21 expression and RhoB knockdown show similar phenotypes in promoting growth and invasion and suppressing apoptosis of colorectal cancer cells. (A) Proliferation potential of SW1116 transfected with miR-21, si-RhoB or Control oligo was determined by the CCK-8 Assay. The data represent means ± S.D. from three independent experiments performed in triplicate. (B) The invasive activities of SW1116 transfected with miR-21, si-RhoB or Control oligo were assayed in a matrigel-coated transwell, and the cells that successfully invaded into the matrigel were quantified 36 h after plating. Data are representative of three independent experiments. (C) The apoptosis rates of SW1116 cells 72 h after transfection of miR-21, si-RhoB or Control oligo were determined by FACS analysis. Data are representative of three independent experiments. *Significant difference from control oligo-transfected cells ($P < 0.05$).

2309–2315, which is highly conserved across different species. Expression of miR-21 in colorectal cancer cell lines was found inversely correlated with RhoB expression. miR-21 could significantly suppress RhoB 3′ UTR luciferase-reporter activity. Consistent with the results of a reporter assay, ectopic expression of miR-21 was able to reduce both mRNA and protein expression of RhoB and mimicked the effect of RhoB-specific siRNA knockdown in promoting proliferation and invasion of the colorectal cancer cells. Our results suggest that miR-21 may have a tumor promoting function by directly targeting the Rho GTPase RhoB in regulating the proliferation and invasion activities of colorectal cancer cells.

To date, biochemical studies have identified a tightly regulated GTP-binding/GTP-hydrolysis cycle that is essential for the regulation of Rho family GTPase activities, including RhoB activity. Interestingly, our previous studies show that Rho GTPase activity and function can be controlled by miRNAs in addition to the classic regulators such as guanine nucleotide exchange factors and GTPase-activating proteins. For example, miR-142-3p directly and negatively regulates and suppresses the migration and invasion of hepatocellular carcinoma cells [33], and our previous studies show that miR-137 can negatively regulate Cdc42 expression while miR-185 can directly target both of Cdc42 and RhoA and suppress the growth and invasion of colorectal cancer cells [22,23]. As a key member of the Rho GTPase family, RhoB is known to have tumor suppressor activity, and its down-regulation is associated with more aggressive tumors as well as changes in cell shape, migration, and adhesion. In previous studies we have found that RhoA and RhoC expressions were significantly elevated, while RhoB was reduced or absent, in surgically removed gastric cancer tissues when compared to normal gastric tissues. More importantly, RhoB expression significantly inhibited the proliferation, migration and invasion of the gastric cancer cells and enhanced the chemosensitivity of these cells to anticancer drugs. It appears that RhoB plays an opposing role from that of RhoA and/or RhoC in gastric cancer cell regulation [15]. Several studies have explored the mechanism of decreased RhoB expression in tumor tissues. RhoB gene deletion or mutation was not found in head and neck carcinomas, and the level of RhoB mRNA was barely detectable in many cases. Previous studies showed that RhoB downregulation could be regulated by histone modification. Furthermore, RhoB was reported recently to be targeted by miR-21 in hepatocellular carcinoma cells and endothelial cells, and loss of miR-21 causes a reduction in migration, invasion, and cell elongation [34,35]. Our current work in colorectal cancer cells are consistent with above studies of hepatocellular carcinoma cells, implicating RhoB as an important target for miR-21 and the miR-21-RhoB signaling axis in mediating cell proliferation and invasion in colorectal cancer. The results suggest that RhoB may serve as a useful tool in exploring the potential therapeutic benefits of targeting of miR-21 mediated tumor cell behaviors in colorectal cancer.

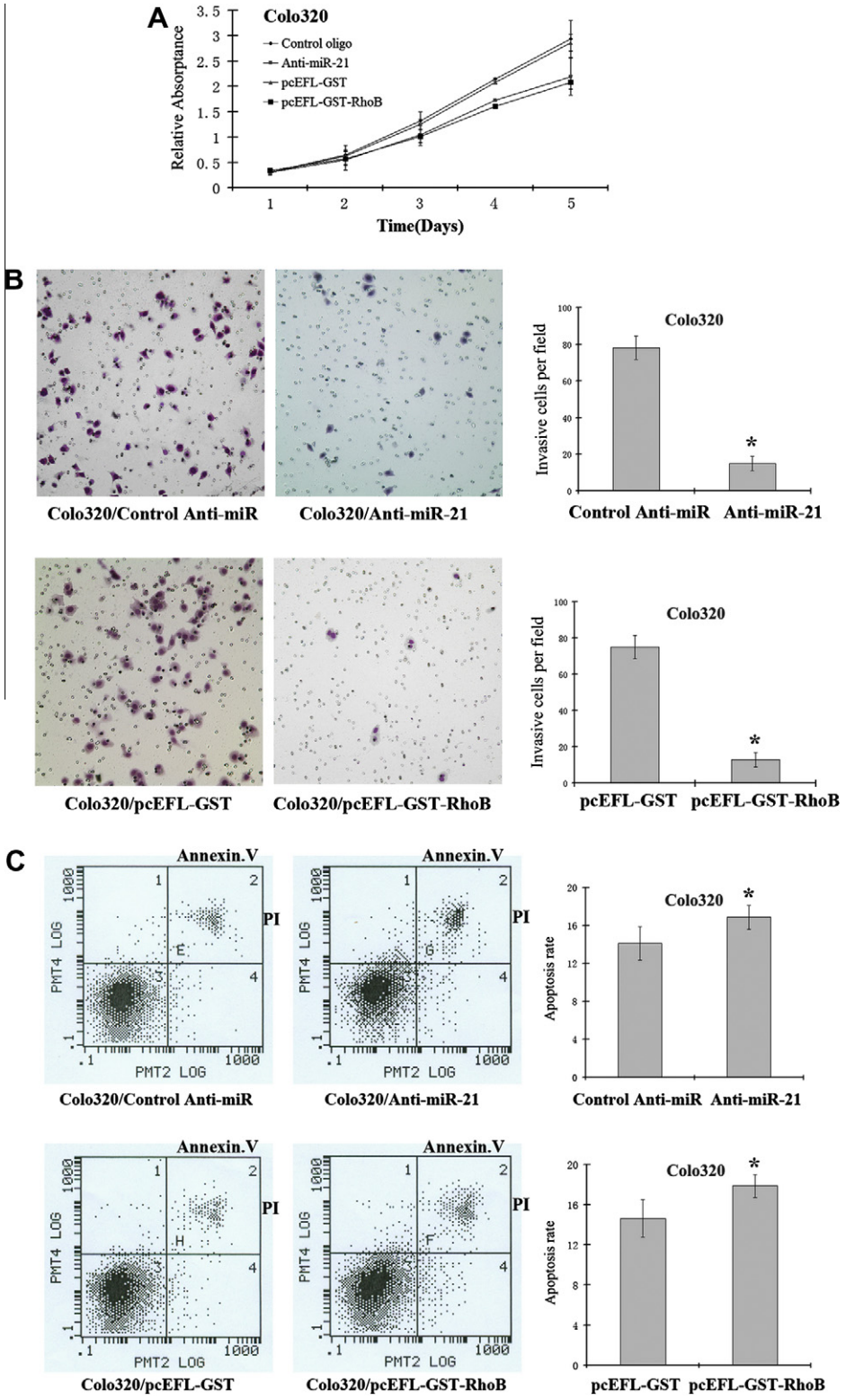

Fig. 6. Anti-miR-21 mimics RhoB expression in inhibiting cell growth and invasion and inducing apoptosis of colorectal cancer cells. (A) Proliferation potential of Colo320 transfected with anti-miR-21, Control anti-miR, pcEFL-GST or pcEFL-GST-RhoB was determined by the CCK-8 Assay. The data represent means ± S.D. from three independent experiments performed in triplicate. (B) The invasive activities of Colo320 transfected anti-miR-21, Control anti-miR, pcEFL-GST or pcEFL-GST-RhoB were assayed in a matrigel-coated transwell, and the cells that successfully invaded into the matrigel were quantified 36 h after plating. Data are representative of three independent experiments. (C) The apoptosis rates of Colo320 cells 72 h after transfection of anti-miR-21, control anti-miR, pcEFL-GST or pcEFL-GST-RhoB were determined by FACS analysis. Data are representative of three independent experiments. *Significant difference from control oligo-transfected cells ($P < 0.05$).

Conflict of interest statement

The authors have no conflict of interests.

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References

[1] Sahai, E. and Marshall, C.J. (2002) Rho-GTPases and cancer. Nat. Rev. Cancer 2, 133–142.
[2] Bar-Sagi, D. and Hall, A. (2000) Ras and Rho GTPases: a family reunion. Cell 103, 227–238.
[3] Aznar, S. and Lacal, J.C. (2001) Searching new targets for anticancer drug design: the families of Ras and RhoGTPases and their effectors. Prog. Nucleic Acid Res. Mol. Biol. 67, 193–234.
[4] Kamai, T., Arai, K., Tsujii, T., Honda, M. and Yoshida, K. (2001) Overexpression of RhoA mRNA is associated with advanced stage in testicular germ cell tumor. BJU Int. 87, 227–231.
[5] Kamai, T., Tsujii, T., Arai, K., Takagi, K., Asami, H., Ito, Y. and Oshima, H. (2003) Significant association of Rho/ROCK pathway with invasion and metastasis of bladder cancer. Clin. Cancer Res. 9, 2632–2641.
[6] Horiuchi, A., Imai, T., Wang, C., Ohira, S., Feng, Y., Nikaido, T. and Konishi, I. (2003) Up-regulation of small GTPases Rho A and Rho C, is associated with tumor progression in ovarian carcinoma. Lab. Invest. 83, 861–870.
[7] Abraham, M.T., Kuriakose, M.A., Sacks, P.G., Yee, H., Chiriboga, L., Bearer, E.L. and Delacure, M.D. (2001) Motility-related proteins as markers for head and neck squamous cell cancer. Laryngoscope 111, 1285–1289.
[8] de Cremoux, P., Gauville, C., Closson, V., Linares, G., Calvo, F., et al. (1994) EGF modulation of the ras-related rhoB gene expression in human breast-cancer cell lines. Int. J. Cancer 59, 408–415.
[9] Jahner, D. and Hunter, T. (1991) The ras-related gene rhoB is an immediate-early gene inducible by v-Fps, epidermal growth factor, and platelet-derived growth factor in rat fibroblasts. Mol. Cell. Biol. 11, 3682–3690.
[10] Engel, M.E., Datta, P.K. and Moses, H.L. (1998) RhoB is stabilized by transforming growth factor beta and antagonizes transcriptional activation. J. Biol. Chem. 273, 9921–9926.
[11] Chen, Z., Sun, J., Pradines, A., Favre, G., Adnane, J. and Sebti, S.M. (2000) Both farnesylated and geranylgeranylated RhoB inhibit malignant transformation and suppress human tumor growth in nude mice. J. Biol. Chem. 275, 17974–17978.
[12] Mazieres, J., Antonia, T., Daste, G., Muro-Cacho, C., Berchery, D., Tillement, V., Pradines, A., Sebti, S. and Favre, G. (2004) Loss of RhoB expression in human lung cancer progression. Clin. Cancer Res. 10, 2742–2750.
[13] Sato, N., Fukui, T., Taniguchi, T., Yokoyama, T., Kondo, M., Nagasaka, T., Goto, Y., Gao, W., Ueda, Y., Yokoi, K., Minna, J.D., Osada, H., et al. (2007) RhoB is frequently downregulated in non-small-cell lung cancer and resides in the 2p24 homozygous deletion region of a lung cancer cell line. Int. J. Cancer 120, 543–551.
[14] Fritz, G., Brachetti, C., Bahlmann, F., Schmidt, M. and Kaina, B. (2002) Rho GTPases in human breast tumours: expression and mutation analyses and correlation with clinical parameters. Br. J. Cancer 87, 635–644.
[15] Zhou, J., Zhu, Y., Zhang, G., Liu, N., Sun, L., Liu, M., Qiu, M., Luo, D., Tang, Q., Liao, Z., Zheng, Y. and Bi, F. (2011) A distinct role of RhoB in gastric cancer suppression. Int. J. Cancer 128, 1057–1068.
[16] Chen, J., Zhou, H., Li, Q., Qiu, M., Li, Z., Tang, Q., Liu, M., Zhu, Y., Huang, J., Lang, N., Liu, Z., Deng, Y., Zhang, S. and Bi, F. (2011) Epigenetic modification of RhoE expression in gastric cancer cells. Oncol. Rep. 25, 173–180.
[17] Griffiths-Jones, S., Saini, H.K., van Dongen, S. and Enright, A.J. (2008) MiRBase: tools for microRNA genomics. Nucleic Acids Res. 36, D154–D158.
[18] Chi, S.W., Zang, J.B., Mele, A. and Darnell, R.B. (2009) Argonaute HITS-CLIP decodes microRNA–mRNA interaction maps. Nature 460, 479–486.
[19] Bartel, D.P. (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116, 281–297.
[20] Lai, E.C. (2002) MicroRNAs are complementary to 3′ UTR sequence motifs that mediate negative post-transcriptional regulation. Nat. Genet. 30, 363–364.
[21] Calin, G.A. and Croce, C.M. (2006) MicroRNA signatures in human cancers. Nat. Rev. Cancer 6, 857–866.
[22] Melo, S.A. and Esteller, M. (2010) Dysregulation of microRNAs in cancer: playing with fire. FEBS Lett. 585, 2087–2099.
[23] Asangani, I.A., Rasheed, S.A., Nikolova, D.A., Leupold, J.H., Colburn, N.H., Post, S. and Allgayer, H. (2008) MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. Oncogene 27, 2128–2136.
[24] Chan, J.A., Krichevsky, A.M. and Kosik, K.S. (2005) MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res. 65, 6029–6033.
[25] Landgraf, P., Rusu, M., Sheridan, R., Sewer, A., Iovino, N., Aravin, A., Pfeffer, S., Rice, A., Kamphorst, A.O., Landthaler, M., Lin, C., Socci, N.D., Hermida, L., Fulci, V., Chiaretti, S., Foa, R., Schliwka, J., Fuchs, U., Novosel, A., Muller, R.U., Schermer, B., Bissels, U., Inman, J., Phan, Q., Chien, M., Weir, D.B., Choksi, R., De Vita, G., Frezzetti, D., Trompeter, H.I., Hornung, V., Teng, G., Hartmann, G., Palkovits, M., Di Lauro, R., Wernet, P., Macino, G., Rogler, C.E., Nagle, J.W., Ju, J., Papavasiliou, F.N., Benzing, T., Lichter, P., Tam, W., Brownstein, M.J., Bosio, A., Borkhardt, A., Russo, J.J., Sander, C., Zavolan, M. and Tuschl, T. (2007) A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 129, 1401–1414.
[26] Volinia, S., Calin, G.A., Liu, C.G., Ambs, S., Cimmino, A., Petrocca, F., Visone, R., Iorio, M., Roldo, C., Ferracin, M., Prueitt, R.L., Yanaihara, N., Lanza, G., Scarpa, A., Vecchione, A., Negrini, M., Harris, C.C. and Croce, C.M. (2006) A microRNA expression signature of human solid tumors defines cancer gene targets. Proc. Natl. Acad. Sci. 103, 2257–2261.
[27] Krichevsky, A.M. and Gabriely, G. (2009) MiR-21: a small multi-faceted RNA. J. Cell. Mol. Med. 13, 39–53.
[28] Liu, M., Lang, N., Qiu, M., Xu, F., Li, Q., Tang, Q., Chen, J., Chen, X., Zhang, S., Liu, Z., Zhou, J., Zhu, Y., Deng, Y., Zheng, Y. and Bi, F. (2011) MiR-137 targets Cdc42 expression, induces cell cycle G1 arrest and inhibits invasion in colorectal cancer cells. Int. J. Cancer 128, 1269–1279.
[29] Liu, M., Lang, N., Chen, X., Tang, Q., Liu, S., Huang, J., Zheng, Y. and Bi, F. (2011) MiR-185 targets RhoA and Cdc42 expression and inhibits the proliferation potential of human colorectal cells. Cancer Lett. 301, 151–160.
[30] Zhu, S., Wu, H., Wu, F., Nie, D., Sheng, S. and Mo, Y.Y. (2008) MicroRNA-21 targets tumor suppressor genes in invasion and metastasis. Cell Res. 18, 350–359.
[31] Meng, F., Henson, R., Wehbe-Janek, H., Ghoshal, K., Jacob, S.T. and Patel, T. (2007) MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology 133, 647–658.
[32] Bai, H., Xu, R., Cao, Z., Wei, D. and Wang, C. (2011) Involvement of miR-21 in resistance to daunorubicin by regulating PTEN expression in the leukaemia K562 cell line. FEBS Lett. 585, 402–408.
[33] Wu, L., Cai, C., Wang, X., Liu, M., Li, X. and Tang, H. (2011) MicroRNA-142-3p, a new regulator of RAC1, suppresses the migration and invasion of hepatocellular carcinoma cells. FEBS Lett. 585, 1322–1330.
[34] Connolly, E.C., Van Doorslaer, K., Rogler, L.E. and Rogler, C.E. (2010) Overexpression of miR-21 promotes an in vitro metastatic phenotype by targeting the tumor suppressor RHOB. Mol Cancer Res. 8, 691–700.
[35] Sabatel, C., Malvaux, L., Bovy, N., Deroanne, C., Lambert, V., Gonzalez, M.L., Colige, A., Rakic, J.M., Noël, A., Martial, J.A. and Struman, I. (2011) MicroRNA-21 exhibits antiangiogenic function by targeting RhoB expression in endothelial cells. PLoS One. 6, e16979.