MicroRNA-148a Suppresses Tumor Cell Invasion and Metastasis by Downregulating ROCK1 in Gastric Cancer

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Abstract

Purpose: MicroRNAs (miRNA) have been documented playing a critical role in cancer development and progression. In this study, we investigate the role of miR-148a in gastric cancer metastasis.

Experimental Design: We examined miR-148a levels in 90 gastric cancer samples by qRT-PCR and analyzed the clinicopathologic significance of miR-148a expression. The gastric cancer cells stably expressing miRNA-148a were analyzed for migration and invasion assays in vitro and metastasis assays in vivo; the target genes of miR-148a were further explored.

Results: We found that miR-148a expression was suppressed by more than 4-fold in gastric cancer compared with their corresponding nontumorous tissues, and the downregulated miR-148a was significantly associated with tumor-node-metastasis (TNM) stage and lymph node-metastasis. Functional assays showed that overexpression of miR-148a suppressed gastric cancer cell migration and invasion in vitro and lung metastasis formation in vivo. In addition, overexpression of miR-148a in GC cells could reduce the mRNA and protein levels of ROCK1, whereas miR-148a silencing significantly increased ROCK1 expression. Luciferase assays confirmed that miR-148a could directly bind to the 2 sites of 3’ untranslated region of ROCK1. Moreover, in gastric cancer tissues, we observed an inverse correlation between miR-148a and ROCK1 expression. Knockdown of ROCK1 significantly inhibited gastric cancer cell migration and invasion resembling that of miR-148a overexpression. We further found that ROCK1 was involved in miR-148a-induced suppression of gastric cancer cell migration and invasion.

Conclusions: miR-148a functions as a tumor metastasis suppressor in gastric cancer, and downregulation of miR-148a contributes to gastric cancer lymph node-metastasis and progression. miR-148a may have a therapeutic potential to suppress gastric cancer metastasis. Clin Cancer Res; 17(24); 7574–83. ©2011 AACR.

Introduction

Gastric cancer is the second leading cause of cancer-related death worldwide, with an estimated one million new cases per year. Approximately 50% of cases occur in Eastern Asia (mainly in China; ref. 1). In most patients, gastric cancer is diagnosed at advanced stage accompanied by extensive invasion and lymphatic metastasis, successful therapeutic strategies are limited and the mortality is high (2, 3). Therefore, investigations into the molecular mechanisms involving in gastric cancer progression have major importance and may tend to develop novel avenues for targeted therapy.

MicroRNAs (miRNA), a class of small nonprotein-coding RNAs, have been identified as a new kind of gene expression regulators through binding to the 3’ untranslated regions (UTR) of target mRNA, thereby resulting in mRNA degradation or the blockade of mRNA translation (4). Emerging evidence shows that miRNAs are abnormally expressed in various cancers, and deregulated miRNAs are associated with tumor initiation, promotion, and progression through regulating many oncogenes and tumor suppressors (5, 6). Human cancers show a general downregulation of miRNAs, and the miRNAs loss can promote tumorigenesis (7, 8). Gastric cancer has unique dysregulated miRNAs, among them, miR-148a is one of the most downregulated miRNAs (9, 10). In addition, Tchernitsa and colleagues using miRNAs microarray compare the miRNA expression patterns.
Translational Relevance

Gastric cancer is the second leading cause of cancer-related death worldwide. Understanding the molecular mechanisms underlying gastric cancer progression contributes to developing novel avenues for targeted therapy. Previous study shows that miR-148a is specific to be overexpressed in healthy gastric tissues, and the downregulated miR-148a is significantly associated with TNM stage and lymph node metastasis. Functional studies revealed that miR-148a acted as a new tumor metastasis suppressor in gastric cancer. Moreover, we found that miR-148a could target RhoA/ROCK signaling pathway through down-regulating ROCK1. By understanding the function and molecular mechanism of miR-148a in gastric cancer, miR-148a may have a therapeutic potential to suppress gastric cancer metastasis.

Materials and Methods

Human samples

Human gastric cancer and their corresponding nontumorous gastric tissues were collected at the time of surgery resection from 90 patients with gastric adenocarcinoma from 2008 to 2009 at the Department of Gastric Cancer and Soft Tissue Sarcomas, Shanghai Cancer Center of Fudan University. Human tissues were immediately frozen in liquid nitrogen and stored at –80°C refrigerator. Signed informed consent was obtained from all patients and the study was approved by the Clinical Research Ethics Committee of Fudan University Shanghai Cancer Center.

Cell culture

Human gastric cancer cell lines (MGC-803, AGS, HGC-27), and HEK293T cells were purchased from the Cell Resource Center, Shanghai Institute of Biochemistry and Cell Biology at the Chinese Academy of Sciences. Cells were maintained at 37°C in a humidified atmosphere containing 5% carbon dioxide in RPMI1640 (MGC-803, HGC-27), F12 (AGS) or Dulbecco’s Modified Eagle’s Media (HEK293T) supplemented with 10% FBS.

RNA extraction and quantitative real-time PCR

Total RNA from tissue samples and cultured cells was extracted using TRIzol reagent (Invitrogen). Quantitative real-time PCR (qRT-PCR) assays were carried out to detect mRNA expression using the PrimeScript RT Reagent Kit (TaKaRa) and SYBR Premix Ex Taq (TaKaRa) according to the manufacturer’s instructions. The levels of ROCK1 transcript were measured by forward primer: AGGAAGGGCAACATATTAGTCCCT, and reverse primer: AGACGATAGTTGGGTCCCGGC. β-Actin was used as an internal control and amplified with forward primer: AGTGTGACGTGGACATCCGCAAAG, and reverse primer: ATCCACATCTGCTGGAAGGTGGAC. The expression level of mature miR-148a was measured by TaqMan miRNA assays (Applied Biosystems) according to the provided protocol, and U6 small nuclear RNA was used as an internal control.

Vector constructs

The pri-miR-148a sequence was amplified from normal human genomic DNA and constructed into the lentivirus expression vector pWPXL to generate pWPXL-miR-148a. The 3’UTR sequence of ROCK1 was amplified from normal human genomic DNA and subcloned into the region directly downstream of a cytomegalovirus (CMV) promoter-driven firefly luciferase cassette in a pCDNA3.0 vector (pLuc). With appropriate primers, a series of 3’UTR sequences were generated and inserted into the pCDNA3.0 vector. All constructs were verified by sequencing.

Lentivirus production and transduction

A mixture of pWPXL-miR-148a or pWPXL, psPAX2 and pMDG2 were transfected into HEK293T cells using lipofectamine 2000 reagent to generate lentivirus. MGC-803 and AGS cells were infected with the recombinant lentivirus-transducing units plus 6 μg/mL Polybrene (Sigma).

Oligonucleotide transfection

miR-148a inhibitors and siRNA against ROCK1 were synthesized by Ribobio. The miR-148a inhibitors are 2’-O-methyl–modified, single-stranded nucleic acids which can specifically bind to and inhibit endogenous miR-148a. The sequence of siRNA targeting ROCK1 is as follows: CAGC3AAUCUC1AAUGUA1AA. Oligonucleotide transfection was done using Lipofectamine 2000 reagents according to the manufacturer’s protocol.
Cell migration and invasion assays
For the migration assays, 5 × 10^4 cells were added into the upper chamber of the insert (BD Bioscience, 8-μm pore size). For the invasion assays, 1 × 10^5 cells were added into the upper chamber of the insert precoated with Matrigel (BD Bioscience). In both assays, cells were plated in medium without serum, and medium containing 10% FBS in the lower chamber served as chemoattractant. After several hours of incubation, the cells that did not migrate or invade through the pores were carefully wiped out with cotton wool. Then the inserts were stained with 20% methanol and 0.2% crystal violet, imaged, and counted with an IX71 inverted microscope (Olympus).

Luciferase assays
HEK293T cells were seeded in 96-well plates at 8,000 cells per well the day before transfection. A mixture of 100 ng indicated pLuc-3’UTR, 200 ng of pWPXL or pWPXL-miR-148a and 20 ng Renilla plasmid (containing no 3’UTR) was transfected into HEK293T cells with Lipofectamine 2000 in each well. Forty-eight hours later, Firefly and Renilla luciferase activities were measured with a Dual-Luciferase Reporter System (Promega). The Renilla luciferase activities were used as an internal control for transfection efficiency (6).

In vivo metastasis assays
For in vivo metastasis assays, MGC-803 cells infected with either the miR-148a–overexpressing lentivirus or the mock lentivirus were transplanted into nude mice (5-week-old BALB/c-nu/nu, 6 per group, 1 × 10^6 cells for each mouse) through the lateral tail vein. After 7 weeks, mice were sacrificed. Their lungs were removed and subjected to hematoxylin and eosin (H&E) staining. All research involving animal complied with protocols approved by the Shanghai Medical Experimental Animal Care Commission.

Western blot analysis
According to standard Western blot procedures, briefly, proteins were separated by 8% SDS-PAGE and then transferred to nitrocellulose membrane (Bio-Rad). After blocking in 5% nonfat milk, the membranes were incubated with the following primary antibodies: rabbit anti-ROCK1 monoclonal antibody (mAb; 1:500; Novus Biologicals), mouse anti-β-actin mAb (1:10,000; Sigma). The proteins were visualized with enhanced chemiluminescence reagents (Pierce).

Immunohistochemical staining
Immunohistochemical staining was done according to standard procedures. For incubation with primary mAb, Tissue slides were incubated at 4°C overnight with rabbit anti-ROCK1 mAb (1:2000; Novus Biologicals). Negative controls were treated identically, but without the primary antibody. Scoring was measured by the cell cytoplasm staining pattern of tumor or nontumorous tissues as described (13): scored 0, absent cell cytoplasm staining; scored 1, weak cell cytoplasm staining; scored 2, moderate cell cytoplasm staining; scored 3, strong cell cytoplasm staining.

Statistical analysis
Data were shown as mean ± SEM unless otherwise noted, the Student t test was used for statistical analysis, with P < 0.05 considered significant.

Results
miR-148a is downregulated in gastric cancer and associated with advanced clinical stage and lymph node-metastasis
miR-148a has been reported to be downregulated in various cancers including gastric cancer (10, 14, 15). Recently, Tchernitsa and colleagues using miRNA microarray find that miR-148a is associated with lymph node-metastasis in gastric cancer (11). However, Chen and colleagues have not found the relationship between miR-148a expression and lymph node-metastasis in patients with gastric cancer (14). To further understand the relationship between miR-148a expression and gastric

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In gastric cancer, we determined the miR-148a expression levels in 90 pairs of primary gastric cancer and their corresponding nontumorous tissues by qRT-PCR. The relationship between the miR-148a expression levels and clinicopathologic parameters of gastric cancer was summarized in Table 1. The results showed that no significant correlations were observed between the miR-148a expression and age, gender, tumor size, location, differentiation, and local invasion. However, we found that miR-148a expression was suppressed by more than 4-fold in gastric cancer compared with their corresponding nontumorous tissues (median = 0.1997 and 0.8443, respectively; Fig. 1A). Moreover, 79% (71 of 90) of the gastric cancer had at least 2-fold reduced expression of miR-148a compared with their corresponding nontumorous tissues (Fig. 1B). We also found that gastric cancer with advanced stages (stage III and IV) had a lower miR-148a expression than gastric cancer with early stages (stage I and II; Table 1 and Fig. 1C). Interestingly, when 90 gastric cancer samples were stratified on the basis of the status of lymph node-metastasis, we found that miR-148a expression was further significantly downregulated in gastric cancer that had lymph node-metastasis, when compared with those that did not have (P = 0.0003; Fig. 1D). Collectively, the above findings suggest that loss of miR-148a expression may play an important role in gastric cancer metastasis.

**miR-148a suppresses gastric cancer cell invasion in vitro and metastasis in vivo**

Because we observe that the downregulation of miR-148a in gastric cancer is a frequent event and closely associated with gastric cancer metastasis, we postulated that overexpression of miR-148a in gastric cancer cells can exert inhibitory effects of cell invasion and metastasis. Thus, we established miR-148a stably expressing cell lines of MGC-803 and AGS by lentivirus infection, as MGC-803 and AGS cells had low basal levels of miR-148a in gastric cancer cell lines (Supplementary Fig. S1A). Successful overexpression of mature miR-148a was confirmed by qRT-PCR (Supplementary Fig. S1B). We found that miR-148a had no obvious effect on gastric cancer cell proliferation (Supplementary Fig. S1C, S1D, and S1E). Intriguingly, overexpression of miR-148a significantly suppressed the migratory and invasive abilities of gastric cancer cells (Fig. 2A and B). Conversely, we transiently transfected miR-148a inhibitor into HGC-27 cells, which had relatively high endogenous miR-148a expression among gastric cancer cell lines (Supplementary Fig. S1A). We found that knockdown of miR-148a could increase gastric cancer cell migration and invasion.

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**Figure 1.** miR-148a is downregulated in gastric cancer and associated with advanced clinical stage and lymph node-metastasis. A, expression of mature miR-148a was determined by way of qRT-PCR in 90 paired human gastric cancer and their corresponding nontumorous samples (NT) and normalized against an endogenous U6 RNA control. B, the downregulation of miR-148a was found in 79% (71 of 90) of primary gastric cancer compared with corresponding nontumorous tissues, the fold change of relative miR-148a expression (NT/gastric cancer) >2 or <1/2 was defined as significant. C, miR-148a expression in different clinical stages of gastric cancer. Patients were staged in accordance with the 7th Edition of the AJCC Cancer’s TNM Classification. D, downregulation of miR-148a in gastric cancer was associated with lymph node-metastasis; patients were classified into lymph node-metastasis negative group (LN-negative) and positive group (LN-positive). Statistical analysis was conducted by paired t test (A) and the Student t test (C and D).
were selected. We carried out qRT-PCR to screen the genes especially for those that have the abilities to promote tumor invasion and metastasis. Basing on this rationale, 5 candidate genes (ITGA5, MET, ROCK1, SP1, and WNT1) were selected. We carried out qRT-PCR to screen the genes that could be downregulated by miR-148a. The results revealed that the expression of ROCK1 mRNA was most downregulated by miR-148a in the miR-148a stably expressing cell lines of MGC-803 and AGS (Fig. 3A and Supplementary Fig. S2). ROCK1 acts as an essential effector kinase of Rho GTPases, and plays vital roles in regulation of cancer invasion and metastasis (16, 17), so ROCK1 might be one target of miR-148a. In addition, Western blot analysis showed that enforced expression of miR-148a triggered a silencing effect on the endogenous ROCK1 protein expression (Fig. 3B). Furthermore, after transfection with miR-148a inhibitor in HGC-27 cells, the expression of ROCK1 was obviously increased (Fig. 3C). Those results suggest that the ROCK1 expression is regulated by miR-148a in gastric cancer.

miR-148a posttranscriptionally reduces ROCK1 expression by directly targeting its 3’UTR

To explore the molecular mechanism of miR-148a in gastric cancer metastasis, we used TargetScan algorithm to search for putative protein-coding gene targets of miR-148a, especially for those that have the abilities to promote tumor cell invasion and metastasis. Basing on this rationale, 5 candidate genes (ITGA5, MET, ROCK1, SP1, and WNT1) were selected. We carried out qRT-PCR to screen the genes that could be downregulated by miR-148a. The numbers and size of lung metastasis nodules were significantly decreased in MGC-803-miR-148a group when compared with MGC-803-vector group (Fig. 2D). Taken together, our results suggest that miR-148a is a negative regulator for gastric cancer metastasis.

(Fig. 2C). To further explore the role of miR-148a on tumor metastasis in vivo, MGC-803 cells stably expressing miR-148a were transplanted into nude mice through the lateral tail vein. Histologic analysis on the lungs of mice confirmed that miR-148a could suppress lung metastasis formation. The numbers and size of lung metastasis nodules were significantly decreased in MGC-803-miR-148a group when compared with MGC-803-vector group (Fig. 2D). Taken together, our results suggest that miR-148a is a negative regulator for gastric cancer metastasis.

Figure 2. miR-148a suppresses gastric cancer cell invasion in vitro and metastasis in vivo. Overexpression of miR-148a significantly impeded abilities of cell migration (A) and invasion (B) in MGC-803 and AGS cells after infection with miR-148a-expressing or vector lentivirus. C, miR-148a inhibitor enhanced cell migration and invasion in HGC-27. D, representative H&E-stained sections of the lung tissues isolated from mice that injected with MGC-803-Vector or MGC-803-miR-148a cells through the lateral tail vein; arrow head points to the tumor focus formed in the lung. The numbers of metastases in the lungs were counted.
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binding site 1, binding site 2, and their corresponding mutant counterparts were directly fused to the downstream of the firefly luciferase gene (pLuc; Fig. 3D). The luciferase activity was normalized to Renilla luciferase activity. The normalized luciferase activity of pLuc group was set as 1.

Upregulation of ROCK1 is inversely correlated with miR-148a expression in gastric cancer

As miR-148a is downregulated in gastric cancer and targets ROCK1 by binding to its 3'UTR, we next determined whether ROCK1 protein expression is negatively associated with miR-148a levels in the gastric cancer tissue samples.

Analysis of ROCK1 protein expression in gastric cancer and their corresponding nontumorous tissues by immunohistochemical staining showed that ROCK1 was significantly upregulated in gastric cancer (P < 0.0001; Fig. 4A and B). Furthermore, we found that high expression of ROCK1 was more likely to be seen in gastric cancer with low levels of miR-148a (P = 0.0041; Fig. 4C), suggesting that the upregulation of ROCK1 may result from repression of miR-148a in gastric cancer.

ROCK1 is involved in miR-148a–induced suppression of gastric cancer cell migration and invasion

It has been reported that ROCK1 is closely associated with tumor invasion and metastasis (16, 17). However, the effects of ROCK1 on gastric cancer cells have not been characterized. To explore the functions of ROCK1, specific siRNAs against ROCK1 were exploited to knockdown ROCK1 expression. As shown in Fig. 5A and B, si-ROCK1 significantly reduced the expression of ROCK1 protein. Migration and invasion assays showed that si-ROCK1 could inhibit gastric cancer cell migration and invasion (Fig. 5C and D), which resembled the inhibitory effects of miR-148a on the gastric cancer cell migration and invasion (Fig. 2A and B). As miR-148a is downregulated in gastric cancer, and miR-148a silencing enhances ROCK1 expression in gastric cancer.
cancer cells. To determine whether deregulation of ROCK1 is involved in regulation of cell migration and invasion by miR-148a, we cotransfected HGC-27 cells with miR-148a inhibitor and si-ROCK1 as described in Fig. 5E, the expression of ROCK1 was confirmed by Western blotting. Interestingly, we found that the migration- and invasion-promoting effects of anti-miR-148a were partially attenuated by si-ROCK1 (Fig. 5F), suggesting ROCK1 is involved in miR-148a-induced suppression of gastric cancer cell migration and invasion.

Discussion

In this study, we showed that miR-148a was frequently downregulated in human gastric cancer and the downregulated miR-148a was significantly associated with advanced clinical stage and lymph node-metastasis. Further studies showed that overexpression of miR-148a suppressed gastric cancer cell migration and invasion in vitro and metastasis in vivo. ROCK1 was identified as a direct and functional target of miR-148a. The data from the current study suggest that miR-148a acts as a novel metastasis suppressor in gastric cancer and that downregulated miR-148a contributes to lymph node-metastasis and tumor progression in gastric cancer patients.

Downregulation of miR-148a is a frequent event in various cancers (10, 14, 15), suggesting that miR-148a may play an important role in tumorigenesis and tumor progression. Hanoun and colleagues show that miR-148a is repressed not only in pancreatic ductal adenocarcinoma but also in preneoplastic lesions (18), indicating downregulation of miR-148a is an early event in pancreatic carcinogenesis. In this study, we also found that miR-148a was frequently downregulated in gastric cancer, and 79% (71 of 90) of the gastric cancer had at least 2-fold reduced expression of miR-148a compared with their corresponding nontumorous tissues. Intriguingly, we found that lower expression of miR-148a tended to have more advanced TNM stage (stage I/II vs. stage III/IV, \( P = 0.0049 \)), suggesting that low expression of miR-148a is associated with gastric cancer progression. Recently, it is reported that miR-148a is subjected to epigenetic regulation in various tumors including gastric cancer (18–20), which may explain the downregulation of miR-148a in gastric cancer. Interestingly, Ribeiro-dos-Santos and colleagues using next-generation sequencing technology reveal that miR-148a is specific to be overexpressed in healthy gastric tissues (21). Together with our results, these data suggest that miR-148a is likely to play a crucial role in gastric tissue homeostasis, when dysregulated, may contribute to the development of a stomach neoplasia.

Lymph node-metastasis is an initial step of gastric cancer metastasis, and is a crucial factor in the determination of the clinical staging, prognosis, and survival of gastric cancer patients (2). Therefore, identifying metastatic factors and elucidating the molecular mechanisms underlying gastric cancer lymph node-metastasis become critical issues. Recent studies document that miRNAs play important roles in gastric cancer initiation and progression (22–24). It is intriguing that miRNAs are associated with lymph node-metastasis of gastric cancer, such as miR-218 (25), miR-107 (26), miR-146a (12), miR-429 (27), and miR-370 (28), which may provide new insights for designing better therapeutic strategies to treat gastric cancer patients with lymph node-metastasis. In accordance with our results, it has been

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Figure 4. Upregulation of ROCK1 is inversely correlated with miR-148a expression in gastric cancer. A, immunohistochemical staining of ROCK1 protein in 70 paired of gastric cancer and their corresponding nontumorous tissues. The staining intensities were evaluated and represented as follows: (a) nontumorous case 1, score 0; (b) gastric cancer case 1, score 0; (c) gastric cancer case 2, score 1; (d) gastric cancer case 3, score 2; (e) gastric cancer case 4, score 3. Original magnification, 200×. B, statistical analysis of ROCK1 expression according to the scoring (paired t test). C, correlation between ROCK1 expression and miR-148a levels in the 70 gastric cancer tissue samples. The expression levels of ROCK1 were classified into low (scores of 0 and 1) and high groups (scores of 2 and 3) according to the scores of ROCK1 immunohistochemical staining.
reported that miR-148a is one of the most downregulated miRNAs in gastric cancer (9, 10). However, its possible roles in gastric cancer have not been fully elucidated. Although Guo and colleagues have shown that miR-148a promotes gastric cancer cell proliferation by targeting p27 (29), miR-148a seems to act as an oncogene in gastric cancer. Other studies suggest that miR-148a acts as a potential tumor suppressor in cancers by inhibiting cancer cell growth, invasion and multidrug resistance (30–32). Therefore, we speculate that the downregulated miR-148a may have other important functions in gastric cancer. However, the relationship between miR-148a expression and gastric cancer metastasis is largely unknown. Intriguingly, in this study, we showed that miR-148a expression was significantly associated with lymph node-metastasis, as the gastric cancer with lymph node-metastasis (n = 52) had a much lower miR-148a expression than those without lymph node-metastasis (n = 38; P = 0.0003). Supporting this notion, Tchernitsa and colleagues using miRNAs microarray (3 gastric cancer patients with and 3 gastric cancer patients without lymph node-metastases) also find that miR-148a is associated with lymph node-metastasis (11), thus suggesting that miR-148a may have the potential as a risk biomarker for judging gastric cancer metastasis status. In addition, our functional studies further found that miR-148a played an important role in gastric cancer metastasis, overexpression of miR-148a could suppress gastric cancer cell migration and invasion in vitro and metastasis in vivo. Taken together, these data indicate miR-148a has a close relationship with lymph node-metastasis and acts as a metastasis suppressor in gastric cancer.

Cell motility and invasion are required for the spreading of tumor cells from their primary tumor to lymph or blood vessels in the process of metastasis. ROCK1 as an essential effector kinase downstream of Rho GTPases participates in regulation of cytoskeletal reorganization and is crucial for cell motility (17). Increasing evidence have shown that upregulation of Rho-ROCK signaling in cancers contributes to invasive and metastatic behavior (16, 17). It has been reported that activation of RhoA-ROCK signaling pathway is involved in gastric cancer cell migration, invasion and gastric cancer progression (33). Together with this, in this study, we found that miR-148a could target this signaling pathway through downregulating ROCK1, and high ROCK1 expression was associated with low miR-148a levels in gastric cancer, bringing a new insight about the essential mechanisms of regulating the Rho-ROCK pathway in gastric cancer. In addition, miR-146a and miR-584 are also proved to target ROCK1 in prostate cancer and clear cell renal cell carcinoma, respectively (34, 35). miR-139 and miR-124
have shown to modulate hepatocellular carcinoma cell aggressiveness by repressing ROCK2 (36, 37). Valastyan and colleagues have shown that miR-31 inhibits local invasion, extravasation, and metastatic colonization of breast cancer cells by targeting RhoA (38). These evidence suggest that dysregulation of RhoA/ROCK signaling pathway by miRNAs is an important mechanism underlying cancer metastasis, and those miRNAs may serve as potential treatments for modulating this pathway in the metastatic diseases.

In summary, our results show that miR-148a as an important antimetastatic miRNA is downregulated and associated with lymph node-metastasis in gastric cancer. Enforced expression of miR-148a suppresses gastric cancer cell invasion and metastasis through directly targeting ROCK1. These findings suggest that the frequently downregulated miR-148a in gastric cancer contributes to gastric cancer metastasis and progression and that miR-148a may have a therapeutic potential to suppress gastric cancer metastasis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

B. Zheng, X. He, and Y. Shi designed the experiments, interpreted the data, and wrote the manuscript. B. Zheng, J. Liang, S. Huang, J. Liu, D. Jia, Q. Tian, J. Wu, and Y. Ye carried out experiments. R. Zha and Q. Wang conducted the animal experiments. C. Wu, Z. Long, Y. Zhou, X. Cao, and C. Du collected the human samples and clinical data.

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References

Correction: *MicroRNA-148a Suppresses Tumor Cell Invasion and Metastasis by Downregulating ROCK1 in Gastric Cancer*

In this article (Clin Cancer Res 2011;17:7574–83), which was published in the December 15, 2011, issue of *Clinical Cancer Research* (1), there is an error in the description and labeling of ROCK1–3′-UTR in the luciferase reporter assays. The full-length ROCK1–3′-UTR, which is from position 0 to 1,642, is incorrect and should read ROCK1–3′-UTR from position 697 to 1,504, which contains both of the 2 binding sites of miR-148a. The labels for ROCK1–3′-UTR full length in Fig. 3, Fig. 3 legend, and Results should be replaced with ROCK1–3′-UTR site 1 + site 2. The overall conclusions of the study were not affected. The authors regret this error.

Reference


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