miR-31 Is a Broad Regulator of β1-Integrin Expression and Function in Cancer Cells

Katarzyna Augoff, Mitali Das, Katarzyna Bialkowska, Brian McCue, Edward F. Plow, and Khalid Sossey-Alaoui

Abstract

Integrins are adhesion receptors involved in bidirectional signaling that are crucial for various cellular responses during normal homeostasis and pathologic conditions such as cancer progression and metastasis. Aberrant expression of noncoding microRNAs (miRNA) has been implicated in the deregulation of integrin expression and activity, leading to the development and progression of cancer tumors, including their acquisition of the metastatic phenotype. miR-31 is a key regulator of several critical genes involved in the invasion-metastasis cascade in cancer. Using diverse cell-based, genetic, biochemical, flow cytometry, and functional analyses, we report that miR-31 is a master regulator of integrins as it targets multiple α and β subunit partners (α2, α5, and αV) of β1 integrins and also β3 integrins. We found that expression of miR-31 in cancer cells resulted in a significant repression of these integrin subunits both at the mRNA and protein levels. Loss of expression of α2, α5, αV, and β3 was a direct consequence of miR-31 targeting conserved seed sequences in the 3’ untranslated region of these integrin subunits leading to their posttranscriptional repression, which was reflected in their diminished surface expression in live cells. The biological consequence of decreased the cell surface of these integrins was a significant inhibition of cell spreading in a ligand-dependent manner. Although different reports have shown that a single integrin can be regulated by several miRNAs, here we show that a single miRNA, miR-31, is able to specifically target several integrin subunits to regulate key aspects of cancer cell invasion and metastasis. Mol Cancer Res; 1–9. ©2011 AACR.

Introduction

As a large family of cell–cell and cell-extracellular matrix (ECM) receptors, integrin α/β heterodimers play prominent roles during embryonic development and postnatal physiology and pathology (1). In addition to their adhesive functions, integrins mediate bidirectional signaling across the cell membrane that regulates numerous cellular responses, including motility, differentiation, growth, and gene expression (2–7). In this context, alterations in integrin expression and function impart phenotypic properties to transformed cells that have significant impact on cancer progression and metastasis (8, 9).

MicroRNAs (miRNA), short noncoding, single-stranded RNAs that regulate protein translation and mRNA degradation of their target genes (10), have been shown to be involved in a large number of biological processes. It is estimated that about one-third of all human mRNAs are regulated by miRNAs (11); and, thus far, more than 1,000 miRNAs have been identified in humans with the potential to control more than 30,000 target genes. The broad spectrum of genes that can be targeted by a single miRNA is attributed to the high level of conserved target motifs, seed sequences, within the 3’ untranslated regions (UTR) of the affected genes, thus making them powerful regulators of gene expression in numerous and complex cellular responses, including cancer cell invasion and metastasis (12–15). miRNAs have also been found to regulate integrin activity in such complex biological processes, such as angiogenesis, cell migration, and invasion (reviewed in ref. 16). Recently, we and others have identified and characterized miR-31 as a master regulator of different steps in the invasion-metastasis-cascade by virtue of its capacity to modulate key metastasis-promoter genes, including WAVE3 and integrin α5 subunit (17, 18). In this study, we show that in addition to α5, miR-31 directly targets seed sequences in the 3’UTRs of 2 other integrin alpha subunits, α2 and αV, and the β3 subunit. We also find that the miR-31–mediated suppression of the integrin alpha subunits indirectly suppresses cell surface expression of their β1 subunit partner and show that suppressed expression of these integrins reduces cell spreading on specific target ECM ligands of these affected integrins.
Materials and Methods

Reagents
All monoclonal antibodies (mAb) to integrins were from Millipore except for anti-β3 integrin, which was from eBioscience and β8 integrin, which was from R&D Systems. UV Live Dead dye and all secondary antibodies were from Molecular Probes. Other reagents were from Sigma.

Flow cytometry
MDA-MB-231 cells expressing GFP-pBABE vector or GFP-miR-31, a kind gift from Dr. Robert Weinberg (18), were propagated in Dulbecco’s modified Eagle’s medium (DMEM) culture medium supplemented with 10% FBS and puromycin (5 μg/mL). Subconfluent cells were detached by trypsinization and washed with Hank’s Balanced Salt Solution with Ca²⁺, Mg²⁺, and 0.1% BSA. Cells were processed for flow cytometry as described (19). Alexa Fluor 647 tagged goat anti-mouse antibody was used to detect all integrin mouse mAbs, including the β1 activation-specific HUTS-4, except for rat anti-β6 integrin mAb, which was detected with Alexa Fluor 647 tagged anti-rat antibody. All primary and secondary antibodies were used at 10 μg/mL. As a positive control for integrin activation, 3.5 mmol/L MnCl₂ was used to treat the cells for 5 minutes at 37°C in a humidified tissue culture incubator. All data were acquired in a BD LSRII instrument and analyzed with FlowJo 7.6.3 (Treestar) software. Binding of only live, GFP-positive cells to extracellular matrix was measured. The cycle threshold (Ct) values were calculated using the 2 uniquermCt method (25) relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The ΔCt was calculated by subtracting the Ct values of GAPDH from the Ct values of the transcript of interest. The ΔΔCt was then calculated by subtracting ΔCt of the parental cells from the control vector- or miR-31-expressing cells. Fold change in the gene expression was calculated according to the equation 2ΔΔCt.

Immunofluorescence
MDA-MB-231 cells expressing miR-31 or the control vector were seeded onto 6-well plates coated with 10 μg/mL laminin, 10 μg/mL fibronectin, 10 μg/mL collagen, or 5 times diluted growth factor-reduced Matrigel for 1 hour, fixed, permeabilized with 0.1% Triton X-100, blocked in horse serum, and stained for 30 minutes with Alexa 568-phalloidin to visualize actin filaments (26).

Cell spreading and integrin blocking
Cell spreading assays were carried out on round coverslips (Fisher Scientific) in 12-well plates (Falcon, Becton Dickinson & Co.). Coverslips were coated 1 hour with Matrigel Matrix (BD Biosciences) diluted 1:6 in PBS, 10 μg/mL collagen I, 10 μg/mL laminin or 2 μg/mL vitronectin (all from Sigma) at 37°C. Following 3 washes with PBS coverslips were blocked for 1 hour with 1% BSA in PBS at 37°C. BSA (1 mg/mL) coated coverslips were used as negative controls.
MDA-MB-231-vector and MDA-MB-231-miR-31 breast cancer cells grown to approximately 80% confluency were detached with trypsin, washed with serum-free-DMEM, resuspended in the same medium and then plated in triplicate at a cell density of $6 \times 10^4$ cells/well. For $\alpha 2\beta 1$ integrin blocking experiments, cells were resuspended in media containing 10 $\mu$g/mL of the $\alpha 2\beta 1$ integrin blocking mAb, LS-C247646, (LS-Bio) and incubated 30 minutes at 37°C before they were seeded on coverslips coated with collagen I or Matrigel. Control cells were resuspended with nonblocking IgG control under the same conditions. Cells were allowed to adhere and spread for 30 minutes and 1 hour in a 37°C CO$_2$ incubator. At the end of the incubation period, unattached cells were removed by careful aspiration and washing 2 times with PBS. Adherent cells were fixed with 4% PFA for 10 minutes and washed twice with PBS. Fixed cells were permeabilized with 0.1% Triton X-100 in PBS for 5 minutes and blocked with 4% bovine serum overnight at 4°C and then stained for 1 hour with Alexa Fluor 568-conjugated phalloidin (Invitrogen, Molecular Probes). After washing, the coverslips were transferred on slides and mounted in immune-fluor mounting medium (MP Biomedicals). Images were acquired with a 20x/0.7 or 40x/1.25 numerical aperture oil objective on a fluorescent microscope (Leica) equipped with digital camera using Image-Pro Plus version 5.1.2.59. Cells were quantified using the ImageJ software version 1.43u and the results are presented as the total surface area covered by cells, in square micrometer, averaged to 300 cells.

Oligonucleotide sequences

Sequences of the oligonucleotide primers used for genomic PCR, RT-PCR, to amplify the entire WAVE3 3’UTR or the individual seed sequences, and the primers used for mutagenesis were from IDT and are listed in Supplementary Table S1.

Statistical analyses

The data are presented as the means ± SE of at least 3 independent experiments. The results were tested for significance using an unpaired Student’s $t$ test and $P$ values of < 0.05 were considered statistically significant.

Results

miR-31 targets the subunits of several integrins

Previous reports have shown that miRNA miR-31 targets the integrin $\alpha 5$ subunit (ITGAV) by interacting with a perfectly matched seed sequence in the 3’UTR of the ITGAV transcript (17, 18). We sought to determine whether miR-31 could also target other integrin subunits. By in silico analyses, we found that the seed sequence of miR-31 was conserved in the 3’UTR of 3 other integrin subunits; $\alpha 2$, $\alpha V$, and $\beta 3$ (ITGA2, ITGAV, and ITGB3, respectively; Fig. 1A). Because binding of miRNAs to their specific seed sequences often represses gene expression, we examined the effect of miR-31 on the expression levels of these candidate target integrin subunits. The analysis was carried out in MDA-MB-231 cells because these breast cancer cells...
have very low levels of endogenous miR-31 (17, 18). Stable expression of miR-31 in these cells had no effect on proliferation compared with control vector transfected cells (Supplementary Fig. S1). Overexpression of miR-31 in MDA-MB-231 cells resulted in approximately 50% decrease in the levels of α2, α5, αV, and β3. This suppression was found both at the mRNA as observed by semiquantitative RT-PCR (Fig. 1B) and qRT-PCR (Fig. 1C) and the cellular protein levels as determined by Western blots (Fig. 1D and Supplementary Fig. S2). All these 4 integrin subunits contain target sites for miR-31 in their respective 3′UTRs (Fig. 1A), suggesting that the silencing of their expression is consequent to miR-31 targeting of the 3′UTR in their transcripts. Integrin subunit β5, which lacks a miR-31 seed sequence, was not affected by miR-31 expression in these cells. We also found that miR-31 overexpression resulted in approximately 2-fold decrease in β1 subunit (ITGB1) mRNA and protein expression levels compared with the parental cells or the vector-transfected cells (Fig. 1, all panels), even though β1 does not contain a target sequence for miR-31 in its 3′UTR. This result could be a secondary to effect of miR-31 on the expression levels of α2, α5, and αV, alpha subunits partners of β1; which could lead to degradation of surplus β1 subunit. The effect of miR-31 on the expression of these specific integrin subunits, α2, α5 and αV, and β1, was also confirmed in LNCaP prostate cancer cells, and β5, which lacks the miR-31 target sequence, was again unaffected (Supplementary Fig. S3).

**Surface expression of α-subunits partners of β1 integrins are reduced due to miR-31**

Because integrin-mediated cellular responses depend upon the expression at the cell surface, as a next step, we monitored the surface expression patterns of the different α-subunits that commonly form heterodimers with β1 subunit in cells stably expressing either the empty control vector or miR-31 by flow cytometry. In accord with our qRT-PCR, semiquantitative RT-PCR, and Western blot results (Fig. 1), flow cytometry analyses revealed that miR-31 significantly reduced the surface expression of α2 (P < 0.05), α5 (P < 0.05), and αV (P < 0.001) subunits (Fig. 2). Consistent with the observed reduction in expression of β1 integrin subunit detected at the mRNA and protein levels, there was also a reduction in surface expression of the β1 subunit partner of these α subunits on the surface of the miR-31 expressing cells.

**β3 integrin is a target of miR-31**

αV integrin subunit is known to complex with several β integrins, including β1, β3, β5, and β6 integrins. Because our results show miR-31 can affect β1 indirectly, we evaluated whether other common partners of αV, β3, β5, β6, and β8, were also targeted by miR-31. Only one of these 4 integrin β subunits, the 3′UTR of β3, was found to harbor the seed sequence that could be targeted by miR-31 (Fig. 1). Consistent with this prediction, we found β3 surface levels were significantly reduced (P < 0.05) in cells expressing high levels of miR-31 compared with vector control (Fig. 3A), which is concordant with the RT-PCR and Western blotting analyses (Fig. 1). Surface expression of β5 subunit was also reduced in the presence of miR-31, probably as an indirect effect because there is no seed sequence of miR-31 in the 3′UTR of β5 (Fig. 3A). Reduced expression levels of either αV or β3 subunits resulted in a significant reduction of cell surface expression of the αVβ3 and the αVβ5 heterodimers using antibodies that specifically recognize these integrins heterodimers (Fig. 3B). Thus, miR-31 can simultaneously regulate the expression of different β integrins.

**Figure 2.** Surface expression of integrin α-subunits and their β1 subunit partner is suppressed by miR-31. Expression profiles of different integrin α subunits in MDA-MB-231 cells overexpressing GFP-pBABE vector control or GFP-miR-31. Surface expression of different α subunit partners of the β1 integrin subunit is quantitated as the relative fluorescence intensity (RFI) with subunit-specific mAbs by flow cytometry. Values are means ± S.E. and are representative of 3 independent experiments. Representative histograms are provided below in which the empty histograms are for the vector control and the filled histograms are for miR-31 overexpressing cells.
of multiple α and β integrin subunits in a cellular environment.

Reduced expression of α2, α5, and αV subunits by miR-31 results in decreased expression of activated β1 integrin on the cell surface

On the basis of the above results, we assessed whether miR-31–mediated suppression also affected the activation state of integrins expressed on the surface of the MDA-MB-231 cells. This analysis was done using HUTS-4, a mAb that recognizes the active conformation of β1 integrins upon dimerization with one of its α subunit partner (27). miR-31 transfectants showed a significantly reduction in their capacity to spontaneously bind HUTS-4 compared with vector control (P < 0.001), and this difference remained significant in the presence of Mn2+ (P < 0.05), which activates integrins upon binding to their extracellular domain (Fig 4A). However, this decrease in HUTS-4 binding was proportional to the decrease in the surface expression of β1 integrins in these cells (Fig. 4B). Thus, when the activation status of these integrins is expressed as a ratio of the total β1 surface expression, measured in HUTS-4 binding, no significant differences were found between the vector or miR-31 expressants in either unstimulated (resting) cells or cells in which integrins were activated with Mn2+ (Fig. 4B).

miR-31 directly targets the 3’UTR of ITGA2, ITGA5, ITGAV, and ITGB3 integrins and represses their expression

We used the Firefly–Renilla dual luciferase reporter gene assay to show that the posttranscriptional repression of the α2, α5, αV, and β3 integrin subunits is a consequence of direct binding of miR-31 to target seed sequences within the 3’UTR of the transcripts for these integrin subunits. A DNA fragment, approximately 350 bp-long from the 3’UTR sequence of these integrins encompassing the miR-31 seed sequence, was subcloned within the 3’UTR of the firefly luciferase gene into the pmirGlo vector, and luciferase activity was measured in MDA-MB-231 breast cancer cells, which express very low or no levels of miR-31. For each construct, firefly luciferase activity was normalized to Renilla luciferase activity and the ratio of miR-31 compared with control miR treatment was calculated. We found no difference in luciferase activity between MDA-MB-231 cells transfected with control vector (Glo) alone or with control vector cotransfected with either miR-31 or control miRNA (Supplementary Fig. S4), showing the lack of a miR-31 effect in the absence of its specific target sequence. In contrast, in cells transfected with the individual integrin 3’UTR-containing vector, overexpression of miR-31 resulted in a significant reduction in luciferase activity, ranging from 30% to 55% depending on the integrin

![Figure 3](https://www.aacrjournals.org/mcr/2011/OF5/images/Figure3.png)

**Figure 3.** Expression profile of different integrin β subunits in MDA-MB-231 cells expressing and GFP-miR-31 or control GFP-pBABE. A, surface expression of different β integrin subunits (other than β1) as determined by flow cytometry with representative histograms given below. Empty histogram is for vector and filled histogram is for miR-31 overexpressing cells. Values are means ± S.E. and are representative of 3 independent experiments. B, surface levels of αVβ3, αVβ5, and αVβ6 reveal differences between vector and miR-31 cells for αVβ3 and αVβ5 integrins. The integrin αV level has been used as the control. Empty histogram is for vector and filled histogram is for miR-31 overexpressing cells. Values are means ± S.E. and are representative of 3 independent experiments.
Our in silico analyses also found a second potential seed sequence of miR-31 in the 3'UTR of ITGAV (ITGAV-2, Fig. 5), which only partially aligned to the miR-31 target sequence. However, this second seed sequence was insufficient to be regulated by miR-31; its presence had no effect on luciferase activity. As a negative control for the effect of miR-31 on the luciferase activity, the 3'UTR of ITGB5 which does not contain a seed sequence for miR-31, showed no different in Renilla luciferase activity in the low or high miR-31 expressing cells. The luciferase assay for ITGB1 could not be done because of the large size (more than 1,200 bp) of its 3'UTR. Similar results were obtained when these experiments were repeated in the LNCaP prostate cancer cells (Supplementary Fig. S5); namely, miR-31 repressed expression of the genes encoding for α2, α5, αV, and β3 integrin subunits but did not affect β5 expression. These results show that the effect of miR-31 is not restricted to a single cell line but seems to extend to other cancers and show that miR-31 specifically targets and represses several integrin subunits simultaneously.

**miR-31 affects cell spreading in a ligand-dependent manner**

Because miR-31 overexpression leads to diminished expression of β1 integrin and several of its α subunits partners on the cell surface (Fig. 2), we anticipated that specific integrin-mediated cellular responses might be suppressed. Accordingly, we plated control or miR-31-MDA-MB-231 cells on different ECM ligands that are recognized by various β1-containing integrins and examined cell spreading. The cells were allowed to spread for 30 or 60 minutes on collagen (an α2β1 ligand), fibronectin (an α5β1 ligand) or laminin (an α6β1 ligand) or Matrigel (containing mostly laminin and collagen IV, ligands for α1β1, α2β1, α3β1, and α6β1 integrins [28]). At the 2 selected time points, cells were fixed and stained with phalloidin to visualize actin (Fig. 6A). We found a striking difference in cell size and actin organization between control cells and miR-31–expressing cells on collagen and Matrigel after 1 hour (Fig. 6, top 2 panels). The effect of miR-31 on the ability of cells to spread on these 2 substrates was also observed after 30 minutes (Supplementary Fig. S6) but became more pronounced after 1 hour. While the control cells became large and fully spread, and developed extensive stress fibers and lamellipodia at the cell periphery, the miR-31 expressing cells remained small and round on these substrata. In control experiments, a blocking mAb (LS-C247646) to α2β1 completely inhibited spreading of both the parental and the miR-31 expressing MDA-MB-231 cells on collagen (Fig. 7), but not on Matrigel (not shown), suggesting the roles of not only α2β1 but also other integrins that were suppressed by miR-31 expression. The difference in cell spreading between the control cells and the miR-31 expressing cells was also evident on laminin and vitronectin, but no difference was observed when cells were plated on fibronectin (Fig. 6, bottom panels).

**Discussion**

Cell adhesion, spreading, and migration on ECM proteins are critical processes for both physiologic responses, such as organ development, cell-mediated immunity, and wound healing, and pathologic responses, such as cancer

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**Figure 4.** Targeting of α2, α5, and αV subunits by miR-31 decreases β1 integrin activation. MDA-MB-231 cells stably overexpressing GFP-miR-31 show reduced β1 integrin activation due to a quantitative reduction in β1 integrin surface expression. A, activation of β1 integrins was monitored with HUTS-4 antibody, specific for activated β1 integrins, by flow cytometry. Spontaneous HUTS-4 binding or that induced by MnCl2 are shown. Cells were either left untreated or treated with 3.5 mmol/L MnCl2 and incubated with HUTS-4 antibody followed by anti-mouse Alexa Fluor 647 IgG to monitor HUTS-4 binding normalized for β1 integrin expression (RFI) for the parental and the miR-31 expressing MDA-MB-231 cells on collagen (Fig. 7), but not on Matrigel (not shown), suggesting the roles of not only α2β1 but also other integrins that were suppressed by miR-31 expression. The difference in cell spreading between the control cells and the miR-31 expressing cells was also evident on laminin and vitronectin, but no difference was observed when cells were plated on fibronectin (Fig. 6, bottom panels).
miR-31 Regulates Several Integrins

Figure 5. miR-31 directly targets the 3’UTR of ITGA2, ITGA5, ITGAV, and ITGB3 integrins and represses their expression in MDA-MB-231 cells. MDA-MB-231 cells were transfected with the luciferase reporter plasmids containing the 3’UTR of the indicated integrin subunit or with deletion of the miR31 seed sequences, and cotransfected with or without miR31. Luciferase activities were measured after 24 hours. For all luciferase activity assays, Renilla luciferase activity was used for normalization. The normalized firefly luciferase activity of each construct was plotted as a ratio of the control miRNA over the miR-31 treatment. The data are the mean ± S.D. of at least 3 independent transfections.

progression and metastasis. Cell adhesion and spreading are triggered by cell contact with ECM proteins such as collagen, laminin, fibronectin, vitronectin, and fibrinogen. Physiologic regulation of cell-matrix interactions is mediated by cell-to-ECM (inside out) and ECM-to-cell (outside in) signals that are themselves transmitted by various adhesion receptors on the cell surface, including integrins (28–31). The β1 integrin subunit, in association with several different α integrin subunits, serves as the principal receptors for type I collagen, the most abundant extracellular matrix protein in mammals (28). Binding of β1 integrin to collagen results in downstream rearrangements of the actin cytoskeleton by providing a scaffold for cytoskeletal proteins and multiple signaling molecules that are involved in the regulation of cell adhesion and spreading (32). In this report, we identify the regulation of expression and activity of multiple integrins by miR-31 as a mechanism for the modulation of cancer cell spreading. We and others have previously reported the targeting of integrin α5 subunit by miR-31 (17, 18). In this report, we show that, in addition to α5, miR-31 directly targets and represses the mRNAs and proteins of 2 other integrin alpha subunits (α2 and αV). This repression is mediated by recognition of a conserved seed sequence of miR-31 in the 3’UTRs of the respective target transcripts. The suppression of these integrin alpha subunits by miR-31 was observed in a series of in vitro molecular and cellular assays, as well as independent experiments to verify that miR-31 regulates the expression and activity of these integrins subunits. First, we showed that overexpression of miR-31 in MDA-MB-231 breast cancer cells, which has low endogenous levels of miR-31, resulted in a significant decrease in the expression levels of multiple alpha integrins subunits along with the β1 subunit, using both real-time qRT-PCR and Western blotting analyses. Second, we used a luciferase reporter gene assay to show that the miR-31–mediated suppression of these integrins subunits arises from direct and specific targeting of a conserved miR-31 seed sequence in their 3’UTRs, and that mutation of the seed sequence abrogates the effect of miR-31. Targeting of these α subunits by miR-31 is consistent with indirect repression of the β1 integrin subunit which associates with α2, α5, and αV partners to form functional integrin heterodimers. The expression of α1, α3, α4, and α6 integrins remained similar between the vector and miR-31 expressing cells, showing that the effect of miR-31 is selective for specific α integrins. Interestingly, although analysis of β1 integrin 3’UTR revealed it lacks a seed sequence for miR-31, it was significantly (P < 0.05) reduced at the mRNA and protein levels, which further resulted in a significant reduction in its cell surface expression. Because there was no compensatory increase in the levels of the α1, α3, α4, and α6 integrins in the miR-31 transfectants, it can be expected that there is a net decrease in the number of available α integrin subunits for β1 to complex within these cells. Third, we showed that loss of mRNA and protein expression of these integrins downstream of miR-31 resulted in loss of both surface expression and activation as shown by flow cytometric analyses with integrin subunit specific mAbs and the β1 activation-specific mAb, HUTS4. Fourth, and importantly, we provided evidence that the miR-31-mediated loss of α2, α5, and αV integrins, and their β1 partner, and β3 integrins resulted in a significant inhibition of cell spreading in a ligand-dependent manner. The inhibition in cell spreading could reflect reduction in surface expression of total or activated integrins.

This study, together with the previously published reports from both our group (17) and others (16, 18) establish the important role of miR-31 in regulating the different aspect of cell adhesion, spreading and cancer cell invasion, and metastasis; and solidifies its role a master...
metastasis suppressor gene (17, 18). Natural regulation of cancer metastasis is mediated by cancer metastasis suppressor genes which either prevent the progression of tumor cells to a metastatic phenotype or reverse the metastatic phenotype. miR-31 is one of these metastasis suppressor genes, whose pleiotropic functions regulate multiple steps of the invasion-metastasis cascade by tar-

getting a cohort of prometastatic targets genes all of which are known to play an important role in metastasis, including integrins. The pleiotropic effect of miR-31 is of potential interest from a translational perspective because modulation of a single miRNA can affect the function of several genes involved in cancer metastasis. Thus, these data reinforce the important role of miR-31 in the regulation of the cancer invasion-metastasis cascade and identify miR-31 as an attractive therapeutic target for blunting cancer metastasis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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