MicroRNA-145 Suppresses Cell Invasion and Metastasis by Directly Targeting Mucin 1

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Abstract

MicroRNAs are important gene regulators that could play a profound role in tumorigenesis. Our previous studies indicate that miR-145 is a tumor suppressor capable of inhibiting tumor cell growth both in vitro and in vivo. In this study, we show that miR-145 exerts its function in a cell-specific manner. Although miR-145 inhibits cell growth in MCF-7 and HCT-116 cells, it has no significant effect on cell growth in metastatic breast cancer cell lines. However, miR-145 significantly suppresses cell invasion in these cells; in contrast, the antisense oligo against miR-145 increases cell invasion. miR-145 is also able to suppress lung metastasis in an experimental metastasis animal model. This miR-145–mediated suppression of cell invasion is in part due to the silencing of the metastasis gene mucin 1 (MUC1). Using luciferase reporters carrying the 3′-untranslated region of MUC1 combined with Western blot and immunofluorescence staining, we identify MUC1 as a direct target of miR-145. Moreover, ectopic expression of MUC1 enhances cell invasion, which can be blocked by miR-145. Of interest, suppression of MUC1 by miR-145 causes a reduction of β-catenin as well as the oncogenic cadherin 11. Finally, suppression of MUC1 by RNAi mimics the miR-145 action in suppression of invasion, which is associated with downregulation of β-catenin and cadherin 11. Taken together, these results suggest that as a tumor suppressor, miR-145 inhibits not only tumor growth but also cell invasion and metastasis.

Introduction

Cell migration and invasion are the major features of metastatic tumor cells that are responsible for most cancer-related deaths. It is well known that the potential of a tumor cell to metastasize depends on numerous factors. Accumulating evidence suggests that microRNAs (miRNA) could be key players in regulation of tumor cell invasion and metastasis (1). miRNAs are small noncoding RNAs that serve as negative regulators in the regulation of diverse cellular functions, and deregulation of miRNA expression is often associated with a variety of disorders, including human malignancy.

Increasing evidence indicates that miRNAs may function as either oncogenes or tumor suppressors (8). For example, miR-145 is a putative tumor-suppressive miRNA that is underexpressed in several types of tumors (9–11) and causes cell growth inhibition by targeting c-Myc (12) and IRS-1 (13). In addition, miR-145 is able to target the pluripotency factors OCT4, SOX2, and KLF4 and functions as a key regulator of human stem cells (14) or promotes differentiation and represses proliferation of smooth muscle cells (15). We have previously shown that miR-145 plays an important role in p33-mediated repression of c-Myc (12). During the further characterization of miR-145 in different cancer cell lines, we found that miR-145 functions as a tumor suppressor in a cell type–specific manner. We showed that miR-145 is a tumor suppressor affecting invasion and metastasis in part by targeting mucin 1 (MUC1).

Materials and Methods

Reagents. Primary antibodies were purchased from the following vendors: MUC1 (small isoform), β-catenin, and cyclin D1 from Epitomics; cadherin 11 from Invitrogen; Myc-tag from Applied Biological Materials; and the antibody specific to large isoforms of MUC1 from Santa Cruz Biotechnology. Secondary antibodies conjugated with IRDye 800CW or IRDye 680 were purchased from LI-COR Biosciences. PCR primers and anti–miR-145 LNA oligo were purchased from IDT. MUC1 siRNA was purchased from Open Biosystems. Freshly frozen breast tumor specimens and their matching normal breast specimens were obtained from Cooperative Human Tissue Network (Midwestern Division).

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**Cell culture.** All cell lines were purchased from the American Tissue Culture Collection except for LM2-4142 (16), which was a generous gift from Dr. Joan Massagué (Sloan-Kettering Institute, New York, NY). Breast cancer cell lines BT-549, MDA-MB-231, and LM2-4142 were grown in RPMI 1640 (Cambrex) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich). HEK-293T cells were cultured in DMEM (Cambrex) supplemented with 10% FBS. All media contained 2 mmol/L glutamine, 100 units of penicillin/mL, and 100 μg streptomycin/mL. Cells were incubated at 37°C and supplemented with 5% CO2 in the humidified chamber.

**Transfection.** MDA-MB-231, LM2-4142, or BT-549 cells were transfected with anti-miR-145 using RNAfectin reagent (Applied Biological Materials) following the manufacturer’s protocol.

**Plasmids.** The plasmid expressing miR-145 in pCMV or in lentiviral vector pCDH-CMV-MCS-EF1-copGFP (System Biosciences) or a mutant miR-145 expression vector has been described previously (12). Expression of the mature miR-145 was verified by TaqMan real-time reverse transcription-PCR (RT-PCR; 17).

To ectopically express MUC1, we cloned the MUC1 coding region in pCMV-Myc. The PCR product for MUC1 without UTR was obtained by primers MUC1-R1-5.1 (5′-GAATTC-TTCGGCATGAGGTG) and MUC1-Not1-3.1 (5′-GGGCGGCTCTACAGTTGGGAAGTGC; for MUC1 with UTR, we used primers MUC1-R1-5.1 and MUC1-UTR-Not1-3.1 (see below)). The PCR product was first cloned into a PCR cloning vector (pCR8) and then subcloned into pCMV-Myc at EcoR1 and NotI sites.

The luciferase-UTR reporter constructs were generated by introducing the MUC1 3′-UTR carrying a putative miR-145 binding site into pGL3 control vector (Promega). We first amplified the MUC1 3′-UTR sequence by PCR using primers MUC1-UTR-5.1 (5′-TGCGAGCGCTCTACAGTTGGGAAGTGC) and MUC1-UTR-Not1-3.1 (5′-GGGCGGCTCTACAGTTGGGAAGTGC; for MUC1 with UTR, we used primers MUC1-R1-5.1 and MUC1-UTR-Not1-3.1 (see below)). The PCR product was first cloned into a PCR cloning vector (pCR8) and then subcloned into pCMV-Myc at EcoR1 and NotI sites.

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Luciferase assays were performed using a luciferase assay kit (Promega) according to the manufacturer’s protocol. β-Galactosidase or renilla luciferase was used for normalization.

**PCR/RT-PCR and real-time RT-PCR.** PCR reactions were performed to amplify the MUC1 with or without 3′-UTR according to the standard three-step procedure. To detect MUC1 mRNA, we used the SYBR green method with primers MUC1-5.1 (5′-ACAGCTACACAGCCGCCCTAA) and MUC1-3.1 (5′-CATGGCCGAGGTCGGTTTCTTCC); average levels of 5s RNA and β-actin were used as an internal control.

**Cell proliferation assay.** Cell growth assays were carried out by MTT assays according to standard methods, as previously described (18).

**Invasion assay.** Matrigel chambers (BD Biosciences) were used to determine the effect of miR-145 or MUC1 on invasiveness per the manufacturer’s protocol. In brief, infected cells were harvested, resuspended in serum-free medium, and then transfected to the hydrated Matrigel chambers (~25,000 cells per well). The chambers were then incubated for 24 h in culture medium with 10% FBS in the bottom chambers before examination. The cells on the upper surface were scraped and washed away, whereas the invaded cells on the lower surface were fixed and stained with 0.05% crystal violet for 2 h. Finally, invaded cells were counted under a microscope and the relative number was calculated.

**Western blot.** Cells were harvested and protein was extracted from transfected cells as previously described (12, 18).

**Immunofluorescence microscopy.** To determine the effect of miR-145 on the protein level of MUC1, we also performed immunofluorescence staining using the MUC1 antibody using the previously described procedure (19).

**Immunohistochemistry.** Immunohistochemistry (IHC) was used to detect MUC1 in paraffin-embedded breast tumor tissue or to detect cadherin 11 in cell culture. For paraffin-embedded breast tumor tissue, slides were pretreated at 65°C for 2 h, followed by deparaffinization using standard procedures. After antigen retrieval, MUC1 antibody was applied to slides, followed by the secondary antibody conjugated with horseradish peroxidase. Signals were revealed by using the Histostain Plus kit (Invitrogen) according to the manufacturer’s instruction. To detect cadherin 11 expression in stably transduced MDA-MB-231 cells, the cells were grown on the coated coverslips overnight before IHC staining.

**Experimental metastasis assay.** Female athymic nude (nu/nu) mice (ages 4–5 wk) were purchased from Harlan Sprague-Dawley and were maintained in the Southern Illinois University School of Medicine’s accredited animal facility. All animal studies were conducted in accordance with NIH animal use guidelines and a protocol approved by Southern Illinois University Animal Care Committee. In brief, 1.5 × 10^6 exponentially growing MDA-MB-231 or LM2-4142 cells with ectopic expression of appropriate genes were injected into nude mice through tail vein. Four weeks after injection, the animals were sacrificed. The lungs were harvested and fixed in Bouin’s solution, and tumor nodules were counted.

**Statistical analyses.** Statistical significance of the studies was analyzed by Student’s t test. Differences with P values of <0.05 are considered significant. The linear correlation coefficient (Pearson’s r) was calculated to estimate the correlation between miR-145 values and MUC1 levels in the matched breast tumor specimens.

**Results**

miR-145 suppresses cell invasion in metastatic breast cancer cell lines. We have previously shown that as a
miR-145 tumor suppressor inhibited cell growth in MCF-7 and HCT-116 cells (12). However, further characterization indicated that miR-145 had no significant effect on cell growth in other cell lines such as MDA-MB-231 and LM2-4142 (Supplementary Fig. S1), suggesting a cell type–specific function of miR-145. Of note, we observed changes in morphology of the miR-145–infected cells that revealed a less invasive morphologic phenotype, i.e., round and flat compared with vector control. These results suggest that miR-145 may play a suppressive role in cell invasion in these cells because both MDA-MB-231 and LM2-4142 are metastatic breast cancer cell lines.

Therefore, we determined whether miR-145 affects cell invasion by Matrigel chamber assay. As expected, miR-145 significantly impaired invasion (Fig. 1A). For example, in MDA-MB-231 cells, miR-145 caused a reduction of invasive cells by more than 50%. In contrast, anti-miR-145 enhanced cell invasion up to 50%, suggesting that this suppression is specific to miR-145. We found more reduction of invasion by miR-145 (by 75%) in LM2-4142, whereas anti-miR-145 increased their invasiveness (Fig. 1C). To determine whether miR-145 suppresses cell invasion in vivo, we used an experimental metastasis model by tail vein injection. As shown in Fig. 1B, whereas the average lung tumor nodules for the vector control were 32, there were only 9 nodules for miR-145. A similar result was obtained for LM2-4142 cells (Fig. 1D). These results further support the role of miR-145 in suppression of invasion and metastasis.

miR-145 directly targets MUC1 by interaction with the 3′-UTR. To understand the mechanism of the miR-145–mediated inhibition of cell invasion, we first examined the effect of c-Myc on cell invasion because we previously showed that miR-145 directly targets c-Myc (12). As a well-known oncogene, c-Myc has been implicated in the involvement of cell invasion indirectly (20). However, we found that ectopic expression of c-Myc gave rise to a modest increase in cell
invasion (data not shown), which is not comparable with the suppressive effect of miR-145, suggesting that some other miR-145 targets may play a more important role in this aspect.

Hence, we searched for additional miR-145 targets using computer-aided miRNA target prediction programs, such as TargetScan4 (21) and miRBase targets, and found several putative miR-145 target genes that might play a role in cell invasion, including ATF1, ATF3, MMP11, ANGPT2, and MUC1. Luciferase reporter assays showed that two of them, MUC1 and MMP11, met the arbitrary 35% reduction cutoff. We were particularly interested in MUC1 because MUC1 is a well-known metastasis-promoting gene that is upregulated in several types of tumors (22, 23). Therefore, we further characterized how miR-145 suppresses MUC1.

As shown in Fig. 2A, miR-145 suppressed more than 55% activity for Luc-MUC1-UTR compared with vector control; neither miR-206 nor miR-224 suppressed Luc-MUC1-UTR. Furthermore, the mutant miR-145 had no suppressive effect. We then deleted this site from the MUC1-UTR or made a site-directed mutant (Fig. 2A, top), and this suppression was abolished in the construct without this miR-145 binding site (Luc-MUC1-UTR-d) or with a mutant site (Luc-MUC1-UTR-mt; Fig. 2A, bottom).

Next, we determined whether ectopic expression of miR-145 can suppress the endogenous MUC1 at the protein level by Western blot. Several MUC1 isoforms in the lysates of MUC1-positive cultured cancer cells have been reported: high molecular weight isoforms (150–300 kDa), presumably due to glycosylation, and a low molecular weight isoform (20–35 kDa; ref. 24). As shown in Fig. 2B (top), miR-145 suppressed both large and small isoforms of MUC1. Because the small isoform is the predominant form in cancer cell lines and tumor specimens, and because it mediates tumor cell growth (24), this study focused on this small isoform. We further showed that the level of small isoform of MUC1 in the miR-145 cells was <40% of vector control cells, whereas anti–miR-145 increased its protein level by more than 200% (Supplementary Fig. S2). In contrast, miR-224 or anti–miR-224 had no effect on MUC1 expression (Fig. 2B, bottom).

**Figure 2.** miR-145 directly targets MUC1. A, effect of miR-145 on MUC1 3′-UTR luciferase reporters. Top, alignment of MUC1 3′-UTR and miR-145, along with a mutant miR-145 or mutant MUC1 3′-UTR in which the sequences in red were deleted or mutagenized. Bottom, a luciferase reporter carrying the 3′-UTR of MUC1 (Luc-MUC1-UTR), deletion of miR-145 binding site at the 3′-UTR (Luc-MUC1-UTR-d), or mutant 3′-UTR in which the miR-145 binding site was mutated (Luc-MUC1-UTR-mt) was introduced into 293T cells along with miR-145 (145), miR-206 (206), miR-224 (224), mutant miR-145 (145-mt), or vector control (V). The cells were harvested 24 h later for luciferase assays. miR-206 and miR-224 serve as a negative control. B, miR-145 suppresses, whereas anti–miR-145 enhances, the endogenous protein levels of MUC1, as detected by Western blot. Stably transduced MDA-MB-231 cells ectopically expressing miR-145 or transiently transfected with anti–miR-145 were used for Western blot analysis. Top, effect of miR-145 or anti–miR-145 on large (L) and small (S) isoforms of MUC1. Bottom, stably transduced MDA-MB-231 cells with miR-224 reveal no suppression of MUC1. Topoisomerase I (topo I) and β-actin serve as loading controls. C, immunofluorescence staining further confirms suppression of MUC1 by miR-145 in stably transduced MDA-MB-231 cells. The cells were grown on coverslips overnight and then subjected to immunostaining with anti-MUC1 antibody, as described in Materials and Methods. All images with red signals (MUC1) were taken at the same fixed time. Merged pictures are overlays of both MUC1 red signals and nuclear staining by Hoechst dye (blue). Columns, mean of three independent experiments; bars, SEM. **, P < 0.01; SC, scrambled oligo; anti-145, anti–miR-145.
We also tested two additional metastatic breast cancer cell lines, LM2-4142 and BT-549. The relative expression levels of miR-145 in these cells were shown in Supplementary Fig. S3. Similar to the result in MDA-MB-231 cells, whereas miR-145 suppressed the level of MUC1, anti–miR-145 increased the level of MUC1 in these cells (Supplementary Fig. S4). Finally, we confirmed this miR-145–mediated repression of MUC1 by immunofluorescence staining. The red signal of (MUC1) in the miR-145–transduced MDA-MB-231 (Fig. 2C) and LM2-4142 cells (Supplementary Fig. S5) were visibly low compared with that of the cells infected with vector control, especially compared with the cells transfected with...
anti–miR-145 (Supplementary Fig. S5; Fig. 2C, middle of right panel), supporting the suppressive role of miR-145 in MUC1 expression.

Real-time RT-PCR analysis detected a slight reduction of MUC1 mRNA in miR-145 cells compared with the vector control in MDA-MB-231 or LM2-4142 cells, but this difference was not significant (Supplementary Fig. S6), suggesting that miR-145 silences MUC1 mainly at the translational level.

**Ectopic expression of MUC1 promotes invasiveness.** To confirm the role of miR-145–mediated MUC1 suppression in invasion, we cloned the coding region of MUC1 with or without the 3′-UTR and tagged them with the Myc epitope. We first verified the ectopic expression for these two constructs. About the same amount of the exogenous MUC1 was expressed with or without the 3′-UTR (Fig. 3A, left). Cotransfection with miR-145 caused a marked reduction of MUC1 protein level for the clone carrying the 3′-UTR, but not with the construct carrying no 3′-UTR (Fig. 3A, right).

Having shown that MUC1 is functionally expressed, we next determined the invasive potential of MUC1. We found notable morphology changes in these cells; they appeared more elongated, a more invasive phenotype that can be reversed by miR-145 (Fig. 3B, top right). In contrast, ~30% miR-145 cells were round and with more epithelial characteristics compared with <5% round cells for vector control (Fig. 3B, top left). Consistent with the morphologic changes, MUC1 enhanced cell invasiveness (Fig. 3C), which was inhibited by miR-145. Experimental metastasis assays further supported the role of miR-145 in suppressing invasion and metastasis by targeting MUC1. For example, MUC1 plus vector produced an average of 85 tumor nodules compared with...
miR-145 was repressed in MDA-MB-231 cells expressing miR-145 because this may suggest that β-catenin also interacts with these proteins (Fig. 4D). Therefore, suppression of cadherin 11 by miR-145 through targeting MUC1 is likely to contribute to the miR-145-mediated reduction of cell invasion.

Effect of miR-145 on cadherin 11. MUC1 promotes cell invasion and metastasis possibly through interactions with different cell signaling molecules, causing stabilization of β-catenin (25). Thus, suppression of MUC1 by miR-145 leading to the reduction of cell invasion is of particular interest because this may suggest that miR-145 also affects the level of β-catenin. Therefore, we first determined the effect of miR-145 on β-catenin. As expected, suppression of MUC1 by miR-145 decreased the level of β-catenin and its downstream gene cyclin D1 (Fig. 4A, left). In contrast, anti-miR-145 increased β-catenin and cyclin D1 (Fig. 4A, right). In silico analysis did not find any miR-145 binding site in the 3' UTRs of β-catenin and cyclin D1; furthermore, luciferase reporters carrying the corresponding UTR revealed no effect by miR-145 (data not shown). Thus, the suppression of β-catenin and cyclin D1 by miR-145 is possibly an indirect effect through MUC1. It has been reported that β-catenin forms a complex with E-cadherin and downregulation of MUC1 in cancer cells inhibits cell migration by promoting the E-cadherin/catenin complex formation (26). However, there is no detectable E-cadherin in MDA-MB-231 cells, which instead express a high level of cadherin 11 (27). Evidence further suggests that cadherin 11 plays an oncogenic role and it also interacts with β-catenin (27–29). Therefore, we examined the effect of miR-145 on cadherin 11. Whereas miR-145 suppressed cadherin 11, anti-miR-145 increased cadherin 11 (Fig. 4B). We further confirmed by IHC that cadherin 11 was repressed in MDA-MB-231 cells expressing miR-145 (Fig. 4C). Therefore, suppression of cadherin 11 by miR-145 could derepress MUC1 as tumor cells progress and metastasize; thus, modulation of miR-145 may provide a therapeutic strategy for metastatic breast cancer.

MUC1 is upregulated in breast cancer specimens. Finally, we examined MUC1 expression in matched breast tumor specimens to determine its clinical relevance. As shown in Fig. 6A, MUC1 level was visibly elevated in four of six cases of advanced breast cancer with an average value of 2.4 compared with the matched normal tissue with a value of 1.0 (Fig. 6B), which inversely correlated with miR-145 expression (Fig. 6C). We then determined whether metastasis status affects MUC1 expression by IHC staining breast cancer tissue microarrays and found that, in general, MUC1 level was higher in metastatic tumors than in nonmetastatic tumors (Supplementary Table S1; Fig. 6D), which is consistent with the previous reports that MUC1 correlates with poor survival in colorectal cancer (30) and breast cancer (31). Downregulation of miR-145 could derepress MUC1 as tumor cells progress and metastasize; thus, modulation of miR-145 may provide a therapeutic strategy for metastatic breast cancer.
Discussion

We have previously shown that miR-145 is able to suppress tumor cell growth both in vitro and in vivo in part through targeting the oncogene c-Myc (12). Although c-Myc has been implicated in indirect regulation of invasion and metastasis, our study suggests that factors other than c-Myc may play a more important role in miR-145–mediated suppression of cell invasion. The present study shows that MUC1 is a direct target for miR-145. Ectopic expression of MUC1 and siRNA knockdown confirmed its invasion-promoting activity. Moreover, miR-145–mediated suppression of MUC1 is dependent on the 3′-UTR. Finally, MUC1-induced cell invasion can be reversed by miR-145. Therefore, these results highlight the significance of miR-145 as a tumor suppressor in cell invasion and metastasis by targeting MUC1.

Increasing evidence suggests that MUC1 plays a role in invasion and metastasis (30). For example, MUC1 promotes invasion in breast cancer by interacting with β-catenin (29); moreover, MUC1 and β-catenin are coexpressed at the invasion front of colorectal carcinomas, which correlates with poor prognosis in colorectal cancer (30). However, the precise role of MUC1 in invasion and metastasis as well as its regulation is not well understood. MUC1 is a member of a large mucin family, which are characterized by a variable number of tandem repeats (VNTR) responsible for its glycosylation. MUC1 belongs to type I membrane glycoprotein subfamily and possess a single membrane–spanning domain and a short cytoplasmic tail in addition to the extensive extracellular domain (22). MUC1 has seven variants that differ mainly in the VNTR region. Because all variants share the same 3′-UTR, miR-145 is expected to be able to suppress all of the variants. Our Western blot data support this notion (Fig. 2B).

The role of MUC1 in invasion and metastasis has been shown in different models. For example, the cytoplasmic tail of MUC1 was reported to enhance the invasion of MDA-MB-468 breast cancer cells expressing wild-type GSK-3β and β-catenin (32), suggesting possible interactions between these proteins. Moreover, MUC1 expression is associated with increased steady-state levels of β-catenin in the cytoplasm and nucleus of breast carcinoma cells by blocking the GSK-3β–mediated phosphorylation of β-catenin, thus preventing proteosomal degradation (29). It is possible that the cytoplasmic tail of MUC1 serves as a scaffold protein, enabling interaction between different regulators or alternatively might compete for or sequester β-catenin. In some cell types, the MUC1 cytoplasmic tail is also involved in the transcriptional activation of β-catenin–TCF binding sites and transcriptional activation of cyclin D1 (25). Consistent with this finding, we detected decreased levels of β-catenin and cyclin D1 in the miR-145 cells (Fig. 4A). Moreover, MUC1 may play an antiapoptotic role in response to cellular stresses by stimulating Akt and the antiapoptotic protein Bcl-X to attenuate genotoxin-induced apoptosis (33). A recent report suggests that this MUC1-mediated tumorigenesis is likely through the transforming growth factor-α signaling pathway (34).

![Figure 6](image-url)

**Figure 6.** Expression of MUC1 in breast tumor specimens. A and B, Western blot reveals upregulation of MUC1 in breast tumors (T) compared with matched normal breast tissue (N). C, a negative correlation between MUC1 and miR-145 in breast tumor specimens after normalization with normal tissue. D, expression of MUC1 in breast tumor specimens with different metastasis status by IHC. Top, infiltrating ductal carcinoma without metastasis; Bottom, infiltrating ductal carcinoma with metastasis.
Although MUC1 is often deregulated in tumors, information about its regulation is limited. In one report, MUC1 was shown to be induced by hypoxia in a lung adenocarcinoma cell line (35); a recent report indicates that MUC1 and galecin-3 oncoproteins function in a miRNA-dependent regulatory loop (36). N-glycosylated MUC1 COOH-terminal subunit increases galecin-3 mRNA levels by suppressing expression of miR-322 and thereby stabilizing galecin-3. However, it is not clear whether MUC1 itself is subject to miRNA regulation. Our study establishes the posttranscriptional regulation of MUC1 by miR-145.

Clinically, MUC1 overexpression occurs frequently in many types of cancer, including breast, ovarian, lung, colon, and pancreatic carcinomas (22, 23); its expression tends to increase as tumors progress (37, 38). Consistent with these reports, we also observed an increased level of MUC1 in more metastatic breast tumors compared with less metastatic breast tumors. However, the precise mechanism of MUC1-mediated metastasis remains to be elucidated. Mucin plays an important role in protecting normal cells from pathogens but tumor cells may use this protective shield to evade from immune system (39). In addition, MUC1 may enhance metastasis in part through interaction with adhesion molecules such as the endothelial protein ICAM-1 (40) or by serving as a ligand for galecin-3 (41).

Our study suggests that MUC1-mediated invasion may involve the oncopgenic cadherin 11 because miR-145 also causes downregulation of cadherin 11 in MDA-MB-231 cells. It has been previously shown that MDA-MB-231 has lost the epithelial marker type 1 cadherins but it expresses an oncopgenic cadherin 11 instead (27). Further evidence suggests that cadherin 11 is expressed in several types of cancers, including breast cancer; moreover, cadherin 11 expression promotes the metastasis of prostate cancer cells to bone (42). Although the underlying mechanism remains to be determined, this downregulation of cadherin 11 by miR-145 is possibly through MUC1 and β-catenin. Given that MUC1 interacts with β-catenin and enhances the β-catenin level (29), it would be reasonable to speculate that this MUC1-mediated β-catenin expression also affects cadherin 11; thus, a likely consequence of ectopic expression of miR-145 is a reduction of cadherin 11 through suppression of MUC1. In support of this, we found that miR-145 suppresses MUC1, β-catenin, and cadherin 11 (Fig. 4A and B). Moreover, suppression of MUC1 by RNAi also downregulates β-catenin and cadherin 11 (Fig. 5A). Therefore, further characterization of this pathway will provide a new insight into miR-145-mediated suppression of invasion and metastasis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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