miR-141-Mediated Regulation of Brain Metastasis From Breast Cancer


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Abstract

Background: Brain metastasis poses a major treatment challenge and remains an unmet clinical need. Finding novel therapies to prevent and treat brain metastases requires an understanding of the biology and molecular basis of the process, which currently is constrained by a dearth of experimental models and specific therapeutic targets.

Methods: Green Fluorescent Protein (GFP)-labeled breast cancer cells were injected via tail vein into SCID/Beige mice (n = 10-15 per group), and metastatic colonization to the brain and lung was evaluated eight weeks later. Knockdown and overexpression of miR-141 were achieved with lentiviral vectors. Serum levels of miR-141 were measured from breast cancer patients (n = 105), and the association with clinical outcome was determined by Kaplan-Meier method. All statistical tests were two-sided.

Results: Novel brain metastasis mouse models were developed via tail vein injection of parental triple-negative and human epidermal growth factor receptor 2 (HER2)-overexpressing inflammatory breast cancer lines. Knockdown of miR-141 inhibited metastatic colonization to brain (miR-141 knockdown vs control: SUM149, 0/8 mice vs 6/9 mice, P = .009; MDA-IBC3, 2/14 mice vs 10/15 mice, P = .007). Ectopic expression of miR-141 in nonexpressing MDA-MB-231 enhanced brain metastatic colonization (5/9 mice vs 0/10 mice, P = .02). Furthermore, high miR-141 serum levels were associated with shorter brain metastasis–free survival (P = .04) and were an independent predictor of progression-free survival (hazard ratio [HR] = 4.77, 95% confidence interval [CI] = 2.61 to 8.71, P < .001) and overall survival (HR = 7.22, 95% CI = 3.46 to 15.06, P < .001).

Conclusions: Our study suggests miR-141 is a regulator of brain metastasis from breast cancer and should be examined as a biomarker and potential target to prevent and treat brain metastases.
with median survival times ranging from five weeks without treatment to six months with multimodality treatment (4,5). Also, the incidence of brain metastases is increasing with the advent of effective targeted systemic therapies for breast cancer such as trastuzumab (3,6,7). Despite the substantial clinical need, the molecular basis for brain metastasis is still poorly understood.

One constraint to understanding the biology of brain metastasis and developing effective treatments is the dearth of preclinical models with which to gain mechanistic insights and identify therapeutic targets. Currently available experimental models have been widely used to elucidate mechanisms by which breast cancer metastasizes to brain (8–13) and to develop potential therapies (12,14–16). However, most of these models involve technically challenging intracardiac or intracarotid-artery injections and have employed only a few human breast cancer cell lines that have been generated by consecutive rounds of in vivo selection. Beyond the limited variability of the models, direct arterial injection of cells bypasses the relevant biology involved in traversing the lungs and surviving in the circulation. Herein, we developed and characterized novel brain metastasis mouse models and investigated the potential role of miR-141 in brain metastatic colonization of breast cancer cells.

Methods

Mouse Studies

Animal protocols were pre-approved by the University of Texas MD Anderson Cancer Center under an Institutional Animal Care and Use Committee (IACUC)—approved protocol (ACUF 07-08-07213). SCID/Beige mice were purchased from Harlan Laboratories (Indianapolis, IN). For tail vein metastasis study, GFP-labeled breast cancer cells were injected into the tail veins of four- to six-week-old female SCID/Beige mice (n = 10–15). For spontaneous metastasis study, cells were transplanted into the cleared mammary fat pads of three- to five-week-old SCID/Beige mice (n = 8). Details on tumor measurement, resection and imaging are described in the Supplementary Methods (available online).

Patient Samples

The patients (n = 105) who provided serum samples are described elsewhere (17). The study was approved by the institutional review board at MD Anderson Cancer Center, and informed consent was obtained from each patient. Details on the blood collection and serum microRNA quantification are described in the Supplementary Methods (available online).

Statistical Analysis

The Fisher’s exact test was used to compare the rate of metastatic colonization to the brain and lung between treatment groups or between cell lines. The nonparametric Mann Whitney-U test was used to compare the size of brain metastasis in SUM149 and MDA-IBC3 control cells to evaluate miR-141 serum levels in metastatic and nonmetastatic patients. The Kaplan-Meier method was used to evaluate the overall survival (OS) and progression-free survival (PFS) of patients according to serum miR-141 levels, and a log-rank test was used to analyze the differences between groups. Univariate and multivariable Cox proportional hazards regression analyses were performed for PFS and OS. The proportional hazards assumption was tested using the Schoenfeld residuals after fitting the model. All variables with P values of less than .25 from the univariate analysis were included in the multivariable analyses. Data are represented in graphs as means ± SD. A P value of less than .05 in a two-sided test was considered statistically significant.

Additional methods are available in the Supplementary Methods (available online).

Results

Association of the Epithelial Phenotype and E-cadherin With Brain Metastasis

The cell line SUM149 was derived from invasive ductal carcinoma of a patient with triple-negative (ER-/PR-/HER2-) inflammatory breast cancer (IBC), an aggressive variant of breast cancer characterized by rapid progression and metastasis that has mixed populations of basal and luminal cell types (18,19). We injected GFP-labeled SUM149 cells (Figure 1A) via tail vein into immunocompromised SCID/Beige mice and using fluorescent stereomicroscopy evaluated the metastatic propensity of these cells to specific organs eight weeks after injection. We found metastatic colonization in the lung and brain (Figure 1B) but not in other organs. Cultures of these lung and brain metastases generated sublines that were morphologically distinct (Figure 1B): the lung sublines (designated LuMS for lung metastatic sublines) were mesenchymal-like, and the brain sublines (designated BrMS for brain metastatic sublines) were epithelial-like. These sublines were also molecularly distinct: the LuMS demonstrated mesenchymal molecular characteristics, with no expression of the epithelial marker E-cadherin and high expression of mesenchymal markers vimentin, N-cadherin, and Fibronectin; the BrMS expressed markers consistent with an epithelial phenotype (Figure 1C). When we cultured the widely used metastasis models MDA-231-BR (brain-seeking) (8) and MDA-231-LM2 (lung-seeking) (20), we observed an epithelial-like morphology and higher expression of the epithelial marker EpCAM/CD326 in the brain-seeking vs lung-seeking MDA-231 sublines, consistent with our findings (Supplementary Figure 1, A and B, available online). Microarray analysis of LuMS and BrMS populations revealed distinct gene expression profiles, with 3369 differentially expressed genes between the two groups (P < .001, False discovery rate [FDR] = 0.001) (Supplementary Figure 1C, available online). Immunohistochemical staining from our SUM149 xenograft models showed that E-cadherin expression was stronger and more uniform in brain metastases than in lung metastases; conversely, vimentin expression was weaker in the brain metastases (Figures 1D and 4C). These observations were supported by publicly available gene expression data. Unsupervised clustering revealed segregation of BrMS with the E-cadherin-expressing HMLE cells and LuMS with E-cadherin knockdown HMLE cells (Figure 1E) (21). Hierarchical clustering of the top 50 upregulated and downregulated genes from a recent study in SUM149 cells (22) also revealed that the BrMS clustered with E-cadherin-expressing, Zeb1-low cells (Supplementary Figure 1D, available online). Additionally, the gene expression of the CD49f+/CD326lo (mesenchymal-like) and CD49f+/-CD326hi (epithelial-like) population of SUM149 parental cells from a study by Perou’s group (23) closely resembled expression profiles from LuMS and BrMS, respectively (data not shown). Finally, unbiased transcriptome profiling along with gene set enrichment analysis (GSEA) identified enrichment of E-cadherin stabilization and cell junction
organization pathways in BrMS while proteins relevant to the extracellular matrix organization and collagen formation were overrepresented in LuMS (Figure 1F), further supporting the potential role of E-cadherin in brain metastasis colonization. Collectively, these findings suggest that E-cadherin expression and the epithelial phenotype can be pro–brain metastasis. We further determined whether the brain and lung metastases variants represent two stable, independent populations vs a single population that readily transitions between epithelial-like and mesenchymal-like states by sorting the parental SUM149 cells into CD49f⁺/CD326lo and CD49f⁺/CD326hi subpopulations (23) and then culturing cells for five days in vitro. We found that each cell gave rise to the other, suggesting both populations of cells are capable of undergoing EMT–MET cycles (Supplementary Figure 1E, available online).

Given this evidence that developmental programs rather than single regulators may be associated with specific sites of metastases and the broad range of molecular signals ascribed to both MET and EMT, we considered that broad regulation by individual microRNAs might be a programmatic regulator of brain metastases. To identify microRNAs that could mediate lung- or brain-specific metastasis, we performed microRNA microarray analysis of the LuMS and BrMS sublines. Unsupervised hierarchical cluster analysis showed that LuMS clustered distinctly from that of BrMS and the miR-200s, including miR-141, miR-200a, miR-200b, and miR-200c, were overexpressed in all of the BrMS sublines (P < .001, false discovery rate = 0.05) (red = upregulated genes, green = downregulated genes). BrMS = brain metastasis sublines, LuMS = lung metastasis sublines.

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**Figure 1.** Association of the epithelial phenotype and E-cadherin expression with brain metastasis. A) GFP-labeled SUM149 cells injected via tail vein into immunocompromised mice. Scale bar = 100 μm. B) Lung metastasis sublines (LuMS) and brain metastasis sublines (BrMS) were derived by digesting tissues from a lung and a brain metastasis from a xenograft mouse model and culturing them in an adherent culture. The BrMS were morphologically epithelial-like whereas the LuMS were mesenchymal-like. Scale bars: left = 2000 μm; right = 100 μm. C) Western blot showing that the BrMS expressed markers consistent with an epithelial phenotype whereas the LuMS showed mesenchymal molecular characteristics. D) Immunohistochemical staining shows stronger expression of E-cadherin in xenograft tissues obtained from brain metastases vs lung metastases (scale bar = 200 μm; inset = 25 μm). E) Unsupervised hierarchical clustering analysis of the 50 most differentially expressed genes revealed a segregation of the BrMS with the E-cadherin-expressing HMLE cells, and the LuMS with the metastatic profile generated from E-cadherin loss (red = upregulated genes; blue = downregulated genes) (21). F) Gene set enrichment analysis (GSEA) identified E-cadherin stabilization and cell junction organization pathways over-represented in BrMS, and extracellular matrix organization and collagen formation pathways in LuMS. G) miRNA microarray showed that the LuMS clustered distinctly from the BrMS and miR-200s (miR-141, miR-200a, miR-200b, and miR-200c) were overexpressed in all of the BrMS sublines (P < .001, false discovery rate = 0.05) (red = upregulated genes, green = downregulated genes). BrMS = brain metastasis sublines, LuMS = lung metastasis sublines.
and MDA-231 (ER/HER2). We confirmed that the expression of miR-200s and E-cadherin was statistically significantly lower whereas vimentin, Zeb1, and Zeb2 were statistically significantly higher in the claudin-low breast cancer cell lines (SUM159 and MDA-231) than in the other cell lines (Supplementary Figure 2, available online). Expression data obtained from a publically available data set of 70 normal and matched primary breast tumors revealed that the miR-200s were overexpressed in tumors (Supplementary Figure 3, available online). We confirmed these findings in samples from patients with IBC and with locally advanced non-IBC breast cancer (Supplementary Figure 4, available online).

**Generation of Novel Brain Metastasis Xenograft Mouse Models**

We assessed the metastatic colonization after tail vein injection of parental GFP-labeled SUM149 and MDA-IBC3 cell lines into immunocompromised SCID/Beige mice. The percentage of brain metastasis eight weeks after injection was 66.7% (6/9 mice) (Figure 2A) for control-transduced SUM149 cells and 66.7% (10/15 mice) (Figure 2A) for MDA-IBC3 cells. In subsequent experiments, the percentage of brain metastases from SUM149 and MDA-IBC3 ranged from 90% to 100% (data not shown). Of note, the MDA-IBC3 cell line was generated from the malignant effusion fluid of a patient with ER/PR/HER2+ IBC. As is characteristic of IBC, this tumor was HER2- and E-cadherin-positive (Supplementary Figure 5, A and B, available online). In vitro, this cell line displayed an epithelial-like morphology and expression pattern, CD44lo, CD24hi, and CD326hi (Supplementary Figure 5, C–F, available online). Consistent with the association between the epithelial phenotype and brain metastases discussed above, we observed that MDA-IBC3 had a greater propensity to colonize the brain than the lung (brain vs lung metastasis: 10/15 mice vs 3/15 mice, P = .02) (Supplementary Figure 5G, available online). Furthermore, the brain metastatic lesions from the more epithelial MDA-IBC3 cell line were larger than those of SUM149 cell line, which contains mixed mesenchymal- and epithelial-like cell populations (SUM149 median size = 258 μM, range = 89–1336 μM; vs MDA-IBC3 median size = 513 μM, range = 173–4590 μM, P < .001) (Supplementary Figure 5H, available online).

**Impact of miR-141 Knockdown on Brain Metastatic Colonization in Preclinical Mouse Models**

To investigate the role of miR-200s in brain metastasis, we selected two of the miR-200s (miR-141 and miR-200a) that were most strongly expressed in our miRNA microarray (Figure 1G). SUM149 and MDA-IBC3 cell lines were stably transduced with GFP-expressing miR-141-knockdown (miR-141 KD), miR-200a knockdown (miR-200 KD), or control lentiviral vectors. A 50% to 70% knockdown of miR-141 and miR-200a was observed in both cell lines (Supplementary Figure 6A, available online). No differences were observed in cell proliferation in knockdown vs control vector—transduced SUM149 and MDA-IBC3 cells (Supplementary Figure 6, B and C, available online). In orthotopic tumor growth delay experiments comparing miR-141 KD, miR-200a KD, or control-transduced SUM149 cell lines, no difference in tumor progression or histology was observed (Supplementary Figure 6, D and E, available online).

We assessed the metastatic colonization after tail vein injection of miR-141 KD, miR-200a KD, and control-transduced GFP-labeled SUM149 and MDA-IBC3 cell lines into SCID/Beige mice. miR-141 knockdown in both cell lines specifically inhibited brain metastases compared with control cells (SUM149 control vs miR-141 KD: 6/9 vs 0/8, P = .009; MDA-IBC3 control vs miR-141: 10/15 vs 2/14, P = .007) (Figure 2A) without affecting lung metastases (SUM149 control vs miR-141 KD: 7/9 vs 5/8; MDA-IBC3 control vs miR-141 KD: 3/15 vs 4/14, P > .05) (Figure 2A). A statistically nonsignificant reduction in metastatic colonization to the brain was observed from miR-200a KD cells in both cell lines. Representative images of lung and brain metastases from control and miR-141 KD cell lines are shown in Figure 2, B–E. Overt brain metastasis lesions generated in mouse xenografts recapitulated the receptor status of each cell line, triple-receptor-negative SUM149 and HER2-overexpressing MDA-IBC3 (Figure 3A). Also, a high level of miR-141 expression was demonstrated by in situ hybridization in the metastatic brain lesions from SUM149- and MDA-IBC3-injected mice (Figure 3B).

Immunostaining of histologic sections from the lung and brain metastases showed highly proliferative Ki-67-positive cells in both SUM149 and MDA-IBC3 xenografts; SUM149 metastases were strongly cytokeratin (CK) 5/6-positive while MDA-IBC3 were CK5/6-negative (Figure 4, A and B). E-cadherin was expressed statistically significantly more highly in tissue sections from brain metastases compared with lung metastasis sections in both cell lines (P < .03) (Figure 4C). To confirm that the observed pro–brain metastasis role in xenografts is unique to miR-141, we conducted studies after knockdown of an invariant (not differentially expressed) microRNA, miR-19a, in SUM149 cells (Supplementary Figure 7A, available online). miR-19a KD resulted in upregulation of miR-19a known target genes Tsp1 and SOCS1 (Supplementary Figure 7, B and C, available online). The incidence of brain metastasis was not statistically different between miR-19a KD and control SUM149 cells (control: 5/8 mice; miR-19a KD: 3/10 mice, P = .34). Similarly, the incidence of lung metastasis in the two groups was not different (control: 7/8 mice; miR-19a KD: 7/10 mice, P = .59).

To evaluate if miR-141 plays a similar pro–brain metastasis role in an orthotopic model, we developed a spontaneous brain metastasis model from breast cancer using the MDA-IBC-3 cell line after orthotopic transplantation into the cleared mammary fat pad followed by resection of primary tumor to allow the colonization of cells to distant organs. Two months after resection, four of seven mice developed brain metastasis from parental MDA-IBC3 while none of the mice injected with miR-141 KD MDA-IBC3 cells developed brain metastasis (P = .03) (Figure 5, A and B). Staining of the brain metastasis tissues showed a strong and uniform E-cadherin expression, similar to the tail vein mouse models (Figure 5C).

**Ectopic Expression of miR-141 in Low-Expressing Breast Cancer Cells and its Effect on Brain Metastasis**

We next sought to determine if the increased brain metastasis observed with these two parental, miR-141-expressing IBC cell lines specifically reflects the propensity of IBC cells to colonize the brain or whether miR-141 expression is sufficient to drive brain metastases in non-IBC cell lines that express low endogenous miR-141. To address this question, we selected MDA-231, a non-IBC, claudin-low breast cancer cell line that has low endogenous miR-141 expression and has not been shown previously to metastasize to the brain after tail vein injection (20,26). This cell line was transduced with GFP-labeled, miR-141-overexpressing (miR-141 OE) or control lentiviral vectors. Overexpression of miR-141 in MDA-231 cells was confirmed by real-time polymerase...
Figure 2. miR-141 knockdown and metastatic colonization in xenograft mouse models. A) Mice were injected via tail vein with $1 \times 10^6$ GFP-labeled SUM149 cells (n = 10/group) or $5 \times 10^5$ GFP-labeled MDA-IBC3 cells (n = 15/group), and brain and lung metastatic colonization were analyzed eight weeks after injection by fluorescent stereomicroscopy. Mice injected with cancer cells that either died immediately or days after injection or were found dead before the eight-week endpoint were excluded from the final analysis. Both cell lines were transduced with miR-141 knockdown (KD), miR-200a KD, or control vector. Transduction with miR-141 KD completely and specifically blocked metastasis to the brain in SUM149 cells ($P = .009$, Fisher’s exact test, two-sided) and statistically significantly reduced brain metastasis in MDA-IBC3 cells ($P = .007$, Fisher’s exact test, two-sided). B and C) Representative bright field (BF)–fluorescence images (upper panel) and hematoxylin and eosin (H&E)–stained images (lower panel) of lung metastases from mice injected with SUM149 control and miR-141 KD cells (B) (scale bars: upper = 2000 μm; lower = 1000 μm) and MDA-IBC3 control and miR-141 KD cells (C) (scale bars: upper = 2000 μm; lower = 100 μm). D and E) Representative BF–fluorescence images (upper panel) and H&E images (lower panel) of brain metastases from mice injected with control and miR-141 KD SUM149 cells (D) (scale bars: upper = 2000 μm; lower = 1000 μm) and MDA-IBC3 cells (E) (scale bars: upper = 2000 μm; lower = 100 μm). H&E = hematoxylin and eosin.

Figure 3. Receptor status of brain metastasis lesions generated from each parental cell line. A) Representative images from sections of brain metastasis lesions obtained from experimental models SUM149 and MDA-IBC3 (via tail vein injection) stained for estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (scale bar = 100 μm). B) In situ hybridization showing high levels of miR-141 in brain metastasis xenografts from SUM149 and MDA-IBC3 cell lines (scale bar = 100 μm). ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2; PR = progesterone receptor.
chain reaction (RT-PCR) (Figure 6A). We injected miR-141 OE or control-transduced MDA-231 cells into the tail veins of SCID/Beige mice and evaluated the development of metastasis eight weeks later. MiR-141 OE cells colonized the brain, but none of the control-vector-transduced MDA-231 cells metastasized to the brain (miR-141 OE: 5/9 mice; control: 0/10 mice, \( P = .02 \)) (Figure 6, B and C). One hundred percent of the control and miR-141 OE cells colonized the lung (Figure 6, B and D). We also overexpressed miR-141 in SUM159 cells, a non-IBC, metaplastic breast cancer cell line that has low endogenous miR-141 expression. Overexpression of miR-141 in SUM159 cells was confirmed by RT-PCR (Figure 6A). No statistically significant difference was observed in the percentage of brain or lung metastasis in miR-141 OE vs control SUM159 cells after tail vein injection into SCID/Beige mice; only 10% of mice had brain metastasis, but 60% of the miR-141 OE-injected mice and 56% of the control SUM159-injected mice developed lung metastasis (Figure 6, B and E).

Staining of lung and brain metastasis tissue sections showed highly proliferative cells with EMT characteristics (E-cadherin-negative and vimentin-positive) in the brain parenchyma; however, focally E-cadherin-positive cells were observed at the meninges (Figure 7A). Staining of histologic sections recapitulated the in vitro characteristics of the SUM159 cell line (Figure 7B). In situ hybridization of the metastatic brain tissues from MDA-IBC3 miR-141 OE cells exhibited high expression of miR-141 (Figure 7C). In addition to observing focally E-cadherin-positive cells in the meninges of brain lesions from miR-
miR-141 can regulate metastatic colonization to brain.

Discussion

We demonstrated that E-cadherin and epithelial expression patterns are associated with brain metastases in our xenograft mouse models and that miR-141, a known inducer of the epithelial phenotype and a key regulator of E-cadherin, is necessary for metastatic colonization of breast cancer cells to the brain. We characterized brain metastases from heterogeneous cell lines that have not previously been associated with brain metastases, and we provided clinical evidence supporting the prognostic relevance of miR-141.

EMT is widely thought to be central to cancer metastasis (27,28), and miR-200s have been shown to inhibit EMT and promote MET (24,25), suggesting that miR-200s likely suppress metastasis. However, functional studies in different models of metastasis have generated contradictory results, with some studies showing that miR-200s promote metastasis and others that miR-200s suppress metastasis (29–31). This discrepancy may be attributed to the role miR-200 plays as a potential regulator of reversible EMT-MET transition, as demonstrated by Korpal et al. (32). None of these functional studies examined the role of miR-200s on metastatic colonization specifically to the brain. Our findings demonstrated that metastasis to the brain can occur as a function of MET, rather than EMT, with expression of several 'epithelial' microRNAs, including miR-200s and E-cadherin, elevated in brain metastatic samples. In addition, knockdown of miR-141, one of the 'epithelial' microRNAs, blocked metastasis to the lung. In contrast, metastases in the lung showed EMT features and miR-141 knockdown did not prevent metastasis to the lung. Our findings suggest that EMT may be central to cancer metastasis to organs such as the lung but

![Figure 6. Ectopic expression of miR-141 in low-expressing breast cancer cells and its effect on brain metastasis. A) Overexpression of miR-141 in MDA-231 and SUM159 parental cell lines as validated by real-time polymerase chain reaction. Error bars represent standard deviation. B) Mice were injected via tail vein with $5 \times 10^5$ GFP-labeled MDA-231 cells (n=11/group) and with $5 \times 10^5$ GFP-labeled SUM159 cells (n=12/group). Mice injected with cancer cells that either died immediately or days after injection or were found dead before the eight-week endpoint were excluded from the final analysis. None of the control-vector-transduced MDA-231 cells metastasized to the brain while miR-141 OE MDA-231 cells did (P=0.02, Fisher’s exact test, two-sided). C and D) Representative bright field (BF)-fluorescence images (upper panel, scale bar=1000 µm) and hematoxylin and eosin (H&E) images (lower panel, scale bar=100 µm) of brain metastases (C) and lung metastases (D) collected from mice injected with control and miR-141 OE-transduced MDA-231 cells. E) Representative BF-fluorescence images (upper panel, scale bar=2000 µm) and H&E images (lower panel, scale bar=1000 µm) of lung metastases from mice injected with control and miR-141 OE-transduced SUM159 cells. *p=0.02, Fisher test.](https://academic.oup.com/jnci/article-abstract/108/8/djw026/2457483)
Indeed, we found that the epithelial MDA-IBC3 cell line has a more limited propensity to colonize the lung than SUM149, a cell line with mixed epithelial and mesenchymal features, and SUM159 and MDA-231, cell lines with mesenchymal features. These findings may provide insights into how epithelial and mesenchymal gene programs could determine organ-specific metastasis.

It is to be noted that neither knockdown of the miR-141 or miR-200a in both SUM149 and MDA-IBC3 cell lines affected the percentage of mice developing lung metastasis. Similarly, overexpression of miR-141 in MDA-231 only affected brain metastatic colonization without affecting lung metastatic colonization. Following tail vein injection of cancer cells, most of the cells are likely initially arrested in and subsequently colonize the lung tissue because the lung capillary bed is the first to be encountered. MiR-141 (and epithelial phenotype) may be critical to escape from the lung in order to colonize the brain. To this end, miR-141-overexpressing MDA-231 cells, generate brain metastasis upon tail vein injection. Of note, although EMT-like parental MDA-231 cells are unable to generate brain metastasis upon tail vein injection, they colonize the brain upon intracardiac and intracarotid artery injections, which bypass the lung capillary beds. Further investigation is warranted on the interactions between breast cancer cells and the lung and its microenvironment in order to understand the metastatic escape or reseeding process. Furthermore, it is not clear how epithelial differentiation promotes brain metastases. Zhang and colleagues recently demonstrated that the osteogenic niche boosts tumor cell proliferation through heterotypic adherens junctions between breast cancer cell E-cadherin and osteogenic N-cadherin (33). The role of heterotypic cadherin junctions in brain metastasis initiation and colonization needs to be explored.

Our study is not without limitations. First, we note that our study with miR-141 serum samples from metastatic breast cancer patients was limited because of the low number of cases...
with brain metastasis and the variable time of blood draw during treatment course in the samples available for this study. Second, miR-141 promoted brain metastasis in aggressive cell lines with some epithelial phenotype but not in a metaplastic cell line that is less plastic and has greater commitment to the mesenchymal phenotype, limiting generalizability of our findings.

In conclusion, we describe herein a key regulator of brain metastasis and have increased preclinical mouse models for studying brain metastasis. This study may provide insights into the regulation of brain metastasis from breast cancer and has a potential target for the prevention and treatment of brain metastases are warranted.

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Notes

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BGD and WAW conceived and designed the project. BGD, LL, SA, PD, RL, AW, WX, DS, and LL performed experiments. BGD, LL, SA, KC, AB, CW, WW, CI, PKA, XHZ, GC, SK, NTU, TAB, JMR, and WAW analyzed data; LH and JMR provided patient materials. BGD and WAW wrote the manuscript with input from all other authors.

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