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Breast cancer metastasis suppressor 1 coordinately regulates metastasis-associated microRNA expression

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Abstract

Breast cancer metastasis suppressor 1 (BRMS1) suppresses metastasis of multiple tumor types without blocking tumorigenesis. BRMS1 forms complexes with SIN3, histone deacetylases and selected transcription factors that modify metastasis-associated gene expression (*e.g.*, EGFR, OPN, PI4P5K1A, PLAU). microRNA (miRNA) are a recently discovered class of regulatory, noncoding RNA, some of which are involved in neoplastic progression. Based on these data, we hypothesized that BRMS1 may also exert some of its antimetastatic effects by regulating miRNA expression. Micro-RNA arrays were done comparing small RNAs that were purified from metastatic MDA-MB-231 and MDA-MB-435 and their non-metastatic BRMS1-transfected counterparts. miRNA expression changed by BRMS1 were validated using SYBR Green RT-PCR. BRMS1 decreased metastasis-promoting (miR-10b, -373 and -520c) miRNA, with corresponding reduction of their downstream targets (*e.g.*, RhoC which is downstream of miR-10b). Concurrently, BRMS1 increased expression of metastasis suppressing miRNA (miR-146a, -146b and -335). Collectively, these data show that BRMS1 coordinately regulates expression of multiple metastasis-associated miRNA and suggests that recruitment of BRMS1-containing SIN3:HDAC complexes to, as yet undefined, miRNA promoters might be involved in the regulation of cancer metastasis.

Keywords

metastasis suppression; microRNA; BRMS1

Gene regulation by microRNA (miRNA) is a conserved mechanism in animals and plants.¹ Endogenous, miRNA range from 15 to 28 nucleotides (nt) in *Homo sapiens* and, most commonly, negatively regulate gene expression, although some gene expression is positively regulated by miRNA. miRNA acts as templates for RNA-induced silencing complexes (RISC) to target mRNA. Animal miRNA differs functionally from plant miRNA in that they have imperfect base pairing with their target mRNA and more commonly inhibit protein translation than degrade mRNA.² Imperfect or promiscuous base pairing allows animal miRNA to target

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multiple mRNA or even entire cellular pathways.^{3,4} To date, miRNA has been shown to regulate multiple cellular processes or pathways critical for neoplastic transformation and progression,⁵ including apoptosis,^{6,7} cell cycle regulation,⁸ differentiation,^{4,9,10} immune function¹¹ and metabolism.^{12,13} Up- and down-regulation of miRNA expression are correlated with development of multiple cancers,^{5,14} including breast carcinoma,^{15–17} and a growing number of miRNA also contribute to promotion and suppression of cancer invasion and metastasis.^{16,18–27}

Metastasis suppressors, defined by their ability to suppress metastasis without blocking orthotopic tumor growth, are an expanding family of >25 molecules.^{28,29} Breast cancer metastasis suppressor 1 (BRMS1) inhibits breast, melanoma, nonsmall cell lung and ovarian cancer metastasis in xenograft and syngeneic models.^{30–36} Expression of BRMS1 protein,^{30, 37} but not necessarily mRNA,^{38,39} expression generally correlates inversely with survival and development of metastasis. BRMS1 associates with SIN3:histone deacetylase complexes^{36, 40,41} which are involved in chromatin structure and selective regulation of gene expression. Collectively, these factors lead to the hypothesis that BRMS1 suppresses metastasis by altering expression of metastasis-associated genes. Previous studies have identified selective regulation of the epidermal growth factor receptor,⁴² osteopontin,^{35,36,43,44} connexins⁴⁵ and urokinase plasminogen activator.⁴⁶ We hypothesized that BRMS1 might also regulate recently discovered, metastasis-associated miRNA.

To determine whether BRMS1 regulates miRNA expression, we compared miRNA expression patterns in nonexpressing and BRMS1 re-expressing breast carcinoma cells. miRNA expression was compared using microarrays imprinted with 328 known human miR probes and a selected common subset was further validated using quantitative real-time PCR (RTQ). In this article, we report that BRMS1 alters miRNA expression in metastatic breast carcinoma cells and notably down-regulated 3 of the 4 published metastasis-promoting miRNA^{22,23,47} and up-regulated all 3 of the known metastasis-suppressing miRNA.^{16,24}

Material and methods

Cell lines and cell culture

MDA-MB-231 (231) and MDA-MB-435 (435) are human estrogen receptor- and progesterone receptor-negative cell lines derived from pleural effusions of metastatic infiltrating ductal breast carcinomas.^{48,49} Both cell lines form progressively growing tumors when injected into the mammary fat pads of immunocompromised mice. MDA-MB-435 cells develop macroscopic metastases in lungs and regional lymph nodes by 10–12 weeks postinoculation, but infrequently metastasize after direct injection into the lateral tail vein or following subcutaneous injection. In contrast, MDA-MB-231 cells form macroscopic metastases when injected intravenously, but less commonly following injection into an orthotopic site. Both lines form osteolytic metastases following injection into the left ventricle of the heart.^{31,50–52} The origin of 435 has been questioned,⁵³ however, this does not affect the interpretation of the results since BRMS1 suppresses metastasis of tumor cell lines from multiple tissue origins.^{33,34}

Parental cell lines were cultured in a mixture (1:1 v:v) of Dulbecco's modified Eagle's medium (DMEM) and Ham's-F12 medium (DMEM/F12; Invitrogen, Carlsbad, CA) supplemented with 2 mM L-glutamine, 0.02 mM nonessential amino acids and 5% fetal bovine serum (Invitrogen). All cultures were maintained without antibiotics or antimycotics on 100-mm tissue culture dishes (Corning, Corning, NY) at 37°C with 5% CO₂ in a humidified atmosphere. When cultures reached 80–90% confluence they were passaged using a solution of 2 mM EDTA in Ca²⁺/Mg²⁺-free Dulbecco's phosphate buffered saline (CMF-DPBS; Invitrogen).

All cultures were regularly tested and confirmed negative for *Mycoplasma* spp. infection using a PCR-based test (TaKaRa, Shiga, Japan).

Constructs and transductions

Cells (231 and 435) were transduced with GFP to facilitate tracking of cells *in vivo* (231^{GFP}/435^{GFP}) as previously described.⁵⁰ Full-length BRMS1 cDNA was cloned into lentiviral constructs and transduced into 231^{GFP} and 435^{GFP} cells. Single cell clones (231^{BRMS1} and 435^{BRMS1}) were isolated and BRMS1 mRNA and protein expression were verified.

Transduced cells were initially selected with puromycin (500 µg/ml) and maintained in puromycin (100 µg/ml) to ensure stable transduction. For routine culture, no antibiotic selection was used and expression has been verified to be stable for more than 2 years.

MicroRNA arrays

Cells were grown to 90% confluence, media aspirated, washed in ice-cold PBS and lysed in acid phenol:chloroform (Ambion, Austin, TX). Small RNA species (≤200 nt) were isolated using *mirVana* PARIS kit (Ambion) according to manufacturer's instructions and immediately stored at −80°C. RNA quantification was performed with use of a DU 800 spectrophotometer (Beckman Coulter, Fullerton, CA). Three independently isolated samples were collected for every cell line.

miRNA array profiling was performed at the Vanderbilt Microarray Shared Resource according to the core's standard operating procedures (<http://array.mc.vanderbilt.edu/microarray/expr/protocol.vmsr>) as summarized later. RNA species (10–40 nt) were enriched using the flashPAGE fractionator (Ambion). The 3' ends were polyadenylated with modified amines; RNA from 231^{GFP} and 435^{GFP} were labeled with Cy5 whereas RNA from 231^{BRMS1} and 435^{BRMS1} cells were labeled with Cy3. The yields on the coupling reactions were typical of miRNA labeling reactions with no detectable CyDye, but a large increase in measurable RNA indicated that poly A polymerase was effective in adding the modified dNTPs to the miRNA (data not shown). RNA samples were suspended in 3X HybTM buffer (Ambion), heated for 95°C for 2 min, allowed to cool at room temperature for 1 min and then loaded onto *mirVana* miRNA bioarrays v2 (Ambion) with Maui DC cover slips (BioMicro Systems, Salt Lake City, UT). Hybridizations were performed using the Maui hybridization station at 42°C for 16 hr with Mix D settings in Maui A1-A3. Arrays were washed once in Ambion SlideHybTM low-stringency buffer for 30 sec with mixing, followed by 2 Ambion SlideHybTM high-stringency buffer washes for 30 sec with mixing. Arrays were spun dry and scanned on the AXON 4000B scanner (Molecular Devices, Sunnyvale, CA).

Raw GeneChip files from GeneChip Operating Software (GCOS, Affymetrix, CA) were uploaded and background was subtracted. Expression changes were normalized to the respective control cells (231^{GFP} or 435^{GFP}) to calculate the intensity ratio/fold changes of the BRMS1-expressing counterparts (Cy5/Cy3). Data were sorted from greatest to least intensity. miR spots with foreground intensities less than 150 were disregarded as signals were too near background median. miR with values of −50 and −100 were also excluded as spot irregularity-affected fluorescence.

Raw data from Axon were background-subtracted, normalized to the respective control cells (231^{GFP} or 435^{GFP}) to calculate the intensity ratio/fold changes of the BRMS1-expressing counterparts (Cy5/Cy3). miR spots with foreground intensities less than 150 were disregarded as signals were too near background median. miR with values of −50 and −100 were also excluded as spot irregularity-affected fluorescence.

Antibodies and immunoblotting

BRMS1 monoclonal antibody clone 1a5.7 was used at 1:2,500 and was previously described.^{36,54} Other antibodies used in this study were purchased and used at the titer indicated: anti-Twist1 (1:1,000), anti- α tubulin (1:1,000) and anti-EGFR (1:1,000; Cell Signaling Technology, Danvers, MA), anti-HoxD10 E20 (1:200; Santa Cruz Biotechnology, Santa Cruz, CA), anti-RhoC (1:1,000; Abcam, Cambridge, MA). Western blotting was performed as previously described.^{36,54}

Validation using real-time quantitative PCR

miRNA expression was determined by collecting total RNA from 70 to 90% confluent cell cultures using Qiazol (Qiagen Inc, Valencia, CA). RNA was purified using miRNeasy (Qiagen Inc). MiScript primers (Qiagen Inc), designed to amplify specifically mature miRNA (*i.e.*, not pre-miR or pri-miR), were as follows: hsa-miR-10b_2, miR-96_1, hsa-miR-30e3p_1, hsa-miR-30e5p, hsa-miR-151, hsa-miR-320, hsa-miR-335_1, hsa-miR-373_1 and hsa-miR-520c. All samples were normalized to small nuclear RNA U6 and fold changes were calculated as previously described.⁵⁵

Results and discussion

Small RNAs (≤ 40 nt) were enriched from metastatic 231^{GFP}, 435^{GFP} and metastasis-suppressed BRMS1-transduced isogenic counterparts. BRMS1-associated mature miRNA expression changes were determined using miR-microarrays in 3 independent experiments. The top 25 consistently increased and decreased mature miRNA as a result of BRMS1 re-expression from both cell line comparisons are listed in Table I. The arrays revealed changes in mature miRNA expression that could contribute to BRMS1 metastasis suppression. For example, the oncogenic miR-155 decreased in 435 cells and tumor suppressing let-7a increased in 231 cells; however, as the arrays were used as a screen for miRNA changes observed in both cell lines, many miRNA were excluded from further study in this report. In general, the direction of miRNA expression change was consistent during the validations, but the magnitude of the changes was often under-appreciated on the arrays.

The criteria for prioritizing candidates were as follows: (i) technical replicates on the arrays were consistent; (ii) the direction of miRNA expression change was consistent in both 231/231^{BRMS1} and 435/435^{BRMS1} cell pairs; (iii) miRNA that target metastasis-associated mRNA (or proteins); and (iv) miRNA previously demonstrated to alter phenotypes associated with invasion and/or metastasis. Based on these criteria 6 miRNA were initially selected for further follow-up: miR-10b, -30e-3p, -30e-5p, -96, -151, -339 (Fig. 1).

Our original goals were to determine whether BRMS1 regulates miRNA and, if so, whether BRMS1-regulated miRNAs are downstream mediators of BRMS1 metastasis suppression. However, as these results were being collected, other laboratories reported on miRNA regulation of invasion and/or metastasis,^{16,22,23} compelling more detailed analysis of our data with regard to BRMS1 regulation of those miRNA (Table II).

The first miRNA validated to promote metastasis was miR-10b.²³ Knockdown of miR-10b in 231 cells decreased *in vitro* migration and invasion. Additionally, ectopic expression of miR-10b in HMEC and SUM149 cells promoted *in vitro* migration and invasion. In addition, there was increased dissemination to the lung and formation of macroscopic foci in the peritoneum by SUM159 cells.²³ Correspondingly, miR-10b expression was suppressed in 231^{BRMS1} and 435^{BRMS1} cells by greater than 50%, which is consistent with prior data showing that BRMS1 suppresses invasion and metastasis. Furthermore, when miR-10b expression was

examined in a panel of cells denoting breast cancer progression, BRMS1 reduced miR-10b levels toward those found in nonmetastatic cells (Fig. 2).

Ma *et al.*²³ further showed that miR-10b down-regulated HoxD10 protein which led to an increased expression of *RhoC*, which is a positive regulator of metastasis.^{56–58} Although *RhoC* mRNA expression decreased in both 231^{BRMS1} and 435^{BRMS1} cells (Fig. 3), HoxD10 mRNA increased in 435^{BRMS1} cells but decreased in 231^{BRMS1} cells. The differences observed when comparing cell lines suggest a nonlinear pathway and/or other (*i.e.*, non-HoxD10) mediators of miR-10b. Because we were unable to detect HoxD10 with the commercially available antibodies, we measured mRNA. The inconsistency of mRNA levels between cell lines is in agreement with the findings reported by Ma *et al.*²³

Relatedly, Huang *et al.*²² utilized a library of miR-transduced into human breast cancer cells to identify which miRNA promoted invasion *in vitro*. miR-373 and miR-520c stimulated migration and invasion *in vitro* and metastasis *in vivo*. Expression of miR-373 and -520c was decreased in 231^{BRMS1} and 435^{BRMS1} cells (Fig. 4 and Table II).

Tavazoie *et al.*¹⁶ compared miRNA expression patterns in paired sets of 231 variants which showed preferential organ-selective metastasis patterns. miR-335, when over-expressed by 250-fold, significantly suppressed lung and bone metastasis. Re-expression of BRMS1 suppressed metastasis to lungs and bone^{31,34,36} and increased expression of the metastasis-suppressing miR-335 by 6-fold (Fig. 4 and Table II). In the same report, miR-126 was described as metastasis suppressing¹⁶; however, it also suppressed orthotopic tumor growth, thereby excluding it as a metastasis suppressor.

Classically metastasis suppressor genes have encoded proteins; however, the demonstration that miR-335 and miR-146a/b suppress metastasis without blocking primary tumor growth shows that the types of molecules considered to be regulating metastasis needs to be more inclusive. Thus far, few pathways have been linked between known metastasis suppressors. In this article, we show for the first time that BRMS1 coordinately regulates expression of multiple miRNA and their corresponding downstream targets [*e.g.*, *RhoC* (this report) and epidermal growth factor receptor⁴²]. The findings are consistent with the emerging awareness that metastasis involves simultaneous control of multiple genes. Our observations beg the question as to whether other metastasis suppressors also mediate some or all of their effects through miRNA regulation.

Prior studies relating miRNA and metastasis have focused attention primarily toward identifying miRNA targets. Comparatively little is known about miRNA regulation.^{59,60} Ma *et al.*²³ identified TWIST1, a major regulator of epithelial-mesenchymal transition,⁶¹ as a promoter of miR-10b expression.²³ We discovered that BRMS1 decreases TWIST expression in whole cell lysates as well as nuclear fractions (Fig. 3). Moreover, BRMS1 mutants that do not suppress metastasis fail to down-regulate TWIST1 in 231 cells. Whether BRMS1 directly binds to the miR-10b promoter is still not known. But it is conceivable that BRMS1 might be part of a corepressor complex(es) recruited to miR-10b because BRMS1 is a component of several SIN3:histone deacetylase complexes.^{36,41} Similarly, the mechanism(s) by which regulation of other miRNA occurs will be the object of intense future investigation. Given the decrease in such a prominent epithelial-to-mesenchymal transition (EMT) regulator (Twist1), one could speculate that BRMS1 suppresses metastasis by invoking the reverse process (mesenchymal-to-epithelial transition, MET) in metastatic cells; however, some published and unpublished data argue against this possibility. Several recent publications indicate that miR-200 family are associated with increased expression of E-cadherin,^{20,21,26,62,63} which is typically lost during EMT.⁶⁴ Data presented in Table I show decreases in miR-200 family members when BRMS1 is expressed. This is the opposite of what would be predicted.

Moreover, changes in miR-200c are not consistent between 231 and 435 cells when BRMS1 is re-expressed. Also, we have previously reported that BRMS1 re-expression has no effect on E-cadherin mRNA or protein expression.⁶⁵ Finally, BRMS1 consistently increases the vimentin expression, which is opposite of what one would expect if BRMS1 were regulating metastasis solely by regulating EMT.

Some miRNA expression changes observed in BRMS1-expressing 231 and 435 cells are large (miR-10b; 0.35/0.36, respectively), whereas others are more subtle (miR-373; 0.69/0.53 and miR-520c; 0.65/0.59, respectively). Nonetheless, it is interesting that nearly all (6 of 7; 86%) of the known metastasis-associated miRNA are regulated in the direction predicted for a metastasis suppressor. The exception was miR-21 which promoted metastasis in breast⁴⁷ and colorectal cancer⁶⁶ cells. BRMS1 increased miR-21 expression in 231 by ~ 2-fold, but did not change in 435. It is quite possible that in 231 cells, the combined effects changing expression of the other metastasis-associated miRNA may overwhelm the miR-21 up-regulation or the miR-21 expression change may be an attempt by cells to compensate for other changes.

Among the many miRNA changes observed in BRMS1-expressing cells, miR-146a and -146b expression were increased. Coupled with our prior reports that BRMS1 inhibits NFκB activity⁴⁶ and EGFR expression,⁴² NFκB regulation by miR-146a/b^{25,67} and demonstration that EGFR is a target sequence for miR-146a/b, we tested the hypothesis that miR-146 was a downstream mediator of BRMS1 metastasis suppression. Transfection of miR-146a or miR-146b into 231 cells resulted in a significant suppression of lung metastases in experimental xenograft models.²⁴ We emphasize that, unlike prior studies testing the role of miRNA in the processes of invasion and metastasis, the data reported here are based upon near physiologic expression levels of BRMS1 and the corresponding downstream miRNA. This point is key because off-target effects are more likely if experiments involve gross over-expression.

In summary, we demonstrate that BRMS1 regulates virtually all of the known miRNA regulating invasion and/or metastasis. Given that a single metastasis suppressor regulates several key metastasis-associated miRNA, future studies should focus on which step(s) of the metastatic cascade are dysregulated in human disease and the specific miRNA expression regulatory elements that are regulated by BRMS1 directly.

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Abbreviations

BRMS1

breast cancer metastasis suppressor 1

CMF-DPBS

	calcium- and magnesium-free Dulbecco's phosphate buffered saline
Cy	cyanine
DMEM	Dulbecco's modified Eagle's medium
EGFR	epidermal growth factor receptor
EMT	epithelial-to-mesenchymal transition
hsa	<i>Homo sapiens</i>
MET	mesenchymal-to-epithelial transition (reverse of EMT)
miR	microRNA
miRNA	microRNA
nt	nucleotide
RTQ	real-time quantitative PCR
TTBS	Tween-20 containing Tris buffered saline

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Figure 1.

BRMS1 re-expression changes miR levels in metastasis suppressed breast cancer cells. Total RNA was extracted from 231/231^{BRMS1} and 435/435^{BRMS1} cell lines, reverse transcribed and miR assessed using SYBR-green real-time RT-PCR.

Figure 2.

BRMS1 restores miR-10b levels to those found in non-metastatic cells. A panel of breast cancer cell lines was evaluated for miR-10b expression. Total RNA was extracted, reverse transcribed and miR assessed using SYBR-green real-time RT-PCR.

Figure 3.

BRMS1 decreases a prometastatic pathway. (a) Whole cell and nuclear fractions were obtained and probed for TWIST1. α -tubulin and lamin A/C were used as loading controls for the whole cell and nuclear fractions, respectively. (b) Total RNA was collected from 231/231^{BRMS1} and 435/435^{BRMS1} cell lines. RNA (3 μ l) was reverse transcribed and messages detected using Taqman primer probes for RhoC and HoxD10. Results are given as fold change relative to non-BRMS1 expressing 231 and 435 cells $\pm$ S.E.M. Ribosomal S9 was used as a normalization control for equal loading.

Figure 4.

BRMS1 decreases prometastatic and increases metastasis-suppressing miR in breast cancer cells. RNA was collected using Qiazol and the miRNeasy kits. Purified RNA (6 μ l) was reverse transcribed and then amplified using miR specific primer probes. Relative amounts were calculated using the comparative cycle threshold (C_T) method. The miR amounts were normalized to small nuclear RNA-U6. Results are given as fold change relative to non-BRMS1 expressing 231 and 435 cells $\pm$ S.E.M.

TABLE I
TOP 25 MOST COMMONLY REGULATED MIR FOLLOWING RE-EXPRESSION OF BRMS1

Cell line	Direction change	microRNA	Cys5:Cy3 ratio	Locus	Accession no. Sanger database
MDA-MB-231	DOWN	hsa_miR_200a_AS	62.190	1p36	MIMAT0001620
		hsa_miR_200b	16.213	1p36	MIMAT0000318
		hsa_miR_380_3p	12.441	14q32	MIMAT0000735
		hsa_miR_326	8.200	11q13	MIMAT0000756
		hsa_miR_483	6.864	11p15	MIMAT0002173
		hsa_miR_210	3.964	11p15	MIMAT0000267
		hsa_miR_99b	3.580	19q13	MIMAT0000689
		hsa_miR_330	2.945	19q13	MIMAT0000751
		hsa_miR_324_5p	2.860	17p13	MIMAT0000761
		hsa_miR_324_3p	2.769	17p13	MIMAT0000762
		hsa_miR_320	2.684	8p21	MIMAT0000510
		hsa_miR_520h	2.621	19q13	MIMAT0002867
		hsa_miR_149	2.614	2q37	MIMAT0000450
		hsa_miR_423	2.553	17q11	MIMAT0001340
		hsa_miR_425	2.552	3p21	MIMAT0001343
		hsa_miR_501	2.478	Xp11	MIMAT0002872
		hsa_miR_452	2.430	Xq28	MIMAT0001635
		hsa_miR_511	2.414	10p12	MIMAT0002808
		hsa_miR_339	2.336	7p22	MIMAT0000764
		hsa_miR_151	2.299	8q24	MIMAT0000757
		hsa_miR_491	2.126	9p21	MIMAT0002807
		hsa_miR_362	2.064	Xp11	MIMAT0000705
		hsa_miR_17_3p	2.031	13q31	MIMAT0000071
		hsa_miR_18a_AS	1.969	13q31	MIMAT0002891
MDA-MB-231	UP	hsa_let_7a	0.295	22q13	MIMAT0000062
		hsa_let_7f	0.328	9q22	MIMAT0000067
		hsa_miR_128b	0.358	3p22	MIMAT0000676
		hsa_miR_30c	0.370	6q13	MIMAT0000244
		hsa_let_7g	0.384	3p21	MIMAT0000414
		hsa_miR_30a_3p	0.385	6q13	MIMAT0000088

Cell line	Direction change	microRNA	Cy5:Cy3 ratio	Locus	Accession no. Sanger database
MDA-MB-435	DOWN	hsa_miR_21	0.386	17q22	MIMAT0000076
		hsa_miR_10a	0.396	17q21	MIMAT0000253
		hsa_miR_128a	0.408	2q21	MIMAT0000424
		hsa_miR_146b	0.429	10q21	MIMAT0002809
		hsa_miR_181a	0.434	1q31	MIMAT0000256
		hsa_miR_182	0.434	7q32	MIMAT0000259
		hsa_miR_96	0.435	7q32	MIMAT0000095
		hsa_miR_20a	0.436	13q31	MIMAT0000075
		hsa_miR_200c	0.436	12p13	MIMAT0000617
		hsa_miR_26a	0.463	3p22	MIMAT0000082
		hsa_let_7i	0.470	12q14	MIMAT0000415
		hsa_miR_15b	0.504	3q25	MIMAT0000417
		hsa_miR_148a	0.510	7p15	MIMAT0000243
		hsa_miR_30a_5p	0.515	6q13	MIMAT0000087
		hsa_miR_15a	0.518	13q14	MIMAT0000068
		hsa_miR_27a	0.520	19p13	MIMAT0000084
		hsa_miR_30e_5p	0.527	1p34	MIMAT0000692
		hsa_miR_16	0.540	13q14	MIMAT0000069
		hsa_miR_142_5p	0.541	17q22	MIMAT0000433
		hsa_miR_34a	102.208	1p36	MIMAT0000255
		hsa_miR_126	99.692	9q34	MIMAT0000445
		hsa_miR_224	53.970	Xq28	MIMAT0000281
		hsa_let_7b	51.088	22q13	MIMAT0000063
		hsa_miR_155	50.785	21q21	MIMAT0000646
		hsa_miR_139	31.486	11q13	MIMAT0000250
		hsa_miR_199a	28.600	19p13	MIMAT0000231
		hsa_miR_452	25.500	Xq28	MIMAT0001635
		hsa_miR_148a	24.778	7p15	MIMAT0000243
		hsa_miR_200b	20.446	1p36	MIMAT0000318
		hsa_miR_222	16.220	Xp11	MIMAT0000279
		hsa_miR_200c	12.161	12p13	MIMAT0000617
		hsa_miR_380_3p	11.759	14q32	MIMAT0000735

Cell line	Direction change	microRNA	Cy5: Cy3 ratio	Locus	Accession no. Sanger database
MDA-MB-435	UP	hsa_miR_503	7.627	Xq26	MIMAT0002874
		hsa_miR_138	7.141	3p21	MIMAT0000430
		hsa_miR_485_5p	6.773	14q32	MIMAT0002175
		hsa_let_7f	6.644	9q22	MIMAT0000067
		hsa_miR_21	6.037	17q22	MIMAT0000076
		hsa_miR_152	5.913	17q21	MIMAT0000438
		hsa_miR_125b	5.000	11q24	MIMAT0000423
		hsa_miR_100	4.076	11q24	MIMAT0000098
		hsa_miR_210	4.040	11p15	MIMAT0000267
		hsa_miR_520h	4.031	19q13	MIMAT0002867
		hsa_miR_221	3.980	Xp11	MIMAT0000278
		hsa_miR_146a	0.001	5q33	MIMAT0000449
		hsa_miR_363	0.001	Xq26	MIMAT0000707
		hsa_miR_506	0.002	Xq27	MIMAT0002878
		hsa_miR_508	0.003	Xq27	MIMAT0002880
		hsa_miR_509	0.003	Xq27	MIMAT0002881
		hsa_miR_513	0.003	Xq27	MIMAT0002877
		hsa_miR_31	0.004	9p21	MIMAT0000089
		hsa_miR_105	0.006	Xq25	MIMAT0000102
		hsa_miR_211	0.009	15q13	MIMAT0000268
		hsa_miR_510	0.013	Xq27	MIMAT0002882
		hsa_miR_20b	0.016	Xq26	MIMAT0001413
		hsa_miR_146b	0.025	10q24	MIMAT0002809
		hsa_miR_9_AS	0.028	1q22	MIMAT0000442
		hsa_miR_204	0.032	9q21	MIMAT0000265
		hsa_miR_501	0.034	Xp11	MIMAT0002872
		hsa_miR_500	0.038	Xp11	MIMAT0002871
		hsa_miR_502	0.042	Xp11	MIMAT0002873
		hsa_miR_362	0.045	Xp11	MIMAT0000705
		hsa_miR_188	0.061	Xp11	MIMAT0000457
		hsa_miR_185	0.070	22q11	MIMAT0000455
		hsa_miR_497	0.079	17p13	MIMAT0002820

Cell line	Direction change	microRNA	Cy5:Cy3 ratio	Locus	Accession no. Sanger database
		hsa_miR_196a	0.087	17q21	MIMAT0000226
		hsa_miR_195	0.089	17p13	MIMAT0000461
		hsa_miR_96	0.182	7q32	MIMAT0000095

Small RNAs were isolated from cell lines not expressing (MDA-MB-231 or MDA-MB-435) or expressing BRMS1, labeled with Cy5 or Cy3 and hybridized to arrays containing 328 human microRNA. Arrays were performed 3 times in independent experiments. Only consistent changes are shown in this table. Sanger database v12: <http://microrna.sanger.ac.uk/sequences/>. Subsequent to the evaluation of miR expression on the microarrays, homologs of some miR have been submitted to the Sanger databases. The chromosomal locations provided in this table correspond to the first miR deposited into the database.

TABLE II
SUMMARY OF METASTASIS-ASSOCIATED MIR CHANGES FOLLOWING BRMS1 RE-EXPRESSION

miR	Accession no.	Change		Reported role(s) of miR in tumor progression	References
		231 ^{BRMS1}	435 ^{BRMS1} ,435		
Decrease					
10b	MI00000267	0.36	0.35	Increased migration, invasion, metastasis	23
373	MI00000781	0.53	0.69	Increased migration, invasion, metastasis	22
520c	MI00007801	0.59	0.65	Increased migration, invasion, metastasis	22
Increase					
146a	MI00000477	6	64	Decreased invasion, metastasis	24,25
146b	MI00003129	0.6	42	Decreased invasion, metastasis	24,25
335	MI00000816	6	11	Decreased migration, metastasis	16
21	MI00000077	2.1	1.1	Decreased migration, metastasis	46,59