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# Expression of the Breast Cancer Metastasis Suppressor 1 (BRMS1) maintains *in vitro* chemosensitivity of breast cancer cells

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ABSTRACT

The Breast Cancer Metastasis Suppressor 1 (BRMS1) belongs to an expanding category of proteins called *metastasis suppressors* that demonstrate *in vivo* metastasis suppression while still allowing growth of the orthotopic tumor. Since BRMS1 decreases either the expression or function of multiple mediators implicated in resistance to chemotherapy (NF-κB, AKT, EGFR), we asked whether breast carcinoma cells expressing BRMS1 could be sensitized upon exposure to commonly used therapeutic agents that inhibit some of these same cellular mediators as BRMS1. In this report, we demonstrate that chemosensitivity of breast cancer cells is preserved in the presence of BRMS1. Further, BRMS1 does not change expression of AKT isoforms or PTEN, implicated in chemoresistance to common drug agents. Overall, our data with two different metastatic breast cancer cell lines indicates that BRMS1 expression status may not interfere with the response to commonly used chemotherapeutic agents in the management of solid tumors such as breast cancer. Since tumor protein expression analysis increasingly guides therapy decisions, our data may be of clinical benefit in disease management including profiling for BRMS1 expression before start of therapy.



## 1. Introduction

The Breast Cancer Metastasis Suppressor 1 (BRMS1) belongs to an expanding class of proteins called *metastasis suppressors* that demonstrate *in vivo* metastasis suppression while allowing growth of the orthotopic tumor [1–3]. BRMS1 functions as a metastasis suppressor in animal models of breast [4], melanoma [5], and ovarian carcinomas [6]. Recent studies with clinical samples have indicated a correlation between loss of BRMS1 expression and poor prognosis in a subset of patients [7–9]. Experimentally, loss of metastasis suppressors, including BRMS1 may be reversed using therapeutic agents [10,11] suggesting use of BRMS1 and other metastasis suppressors as markers and a potential adjuvant role of such "re-expression therapy" in the management of metastasis. Experimentally, BRMS1 expression increases susceptibility to anoikis which is proposed to contribute, in part, to metastasis suppression [12,13]. BRMS1 is part of the Sin3-HDAC chromatin remodeling complexes [14,15] that regulate gene expression and which could potentially alter chemotherapeutic responses [16]. Consequently, BRMS1 regulates expression of several signaling intermediates including epidermal growth factor receptor [17],

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osteopontin [18,19], phosphatidylinositol (4,5) bisphosphate (PtdIns(4,5)$P_2$) [20], urokinase plasminogen activator [21], fascin [6], and connexins [22]. Further, BRMS1 regulates nuclear factor-kappa B (NF-κB) activity [21] and AKT phosphorylation [17] in response to exogenous stimuli implicated in chemoresistance in a number of cancer models [23–25]. Recently, Rivera and colleagues suggested that BRMS1 expression may increase chemosensitivity as a consequence of downregulation of 14-3-3-γ, sorcin, and Hsp27 [26].

Taken together, since BRMS1 decreases either the expression or activity of multiple mediators implicated in resistance to chemotherapy (e.g. NF-κB, AKT, and EGFR) and increases susceptibility to anoikis, we asked whether breast carcinoma cells expressing BRMS1 could respond differently upon exposure to commonly used therapeutic agents in the treatment of breast cancer. In this report, using multiple approaches we evaluated that chemosensitivity of breast cancer cells is preserved in the presence of BRMS1. Further, BRMS1 does not change expression of AKT isoforms or PTEN, implicated in chemoresistance to common drug agents. Information from these studies may be potentially used in the clinic in stratifying patients and designing treatment courses in the management of metastatic disease.

## 2. Materials and methods

### 2.1. Cell culture

MDA-MB-231 and MDA-MB-435 breast adenocarcinoma cells [27] that lack endogenous BRMS1 expression [13,28] were transfected with a lentiviral vector construct expressing BRMS1 under the control of a cytomegalovirus promoter [13]. MDA-MB-231/435 vector transfectants (231/435), and $231^{BRMS1}$/$435^{BRMS1}$ were cultured in a 1:1 mixture of Dulbecco's-modified essential medium (DMEM) and Ham's F-12 medium supplemented with 1% non-essential amino acids, and L-glutamine (Invitrogen, Carlsbad, CA) and containing 5% fetal bovine serum (cDMEM-F12). 231 and $231^{BRMS1}$ cells were passaged using 0.125% trypsin and 2 mM EDTA solution (Invitrogen, Carlsbad, CA) and 435 and $435^{BRMS1}$ cells were passaged using 2 mM EDTA in $Ca^{2+}$/$Mg^{2+}$-free PBS. Cell lines were confirmed to be free of Mycoplasma contamination using PCR (TaKaRa, Japan). No antibiotics or antimycotics were used.

### 2.2. Chemotherapeutic agents

Doxorubicin, vincristine were dissolved in water and 5-fluorouracil (5-FU), paclitaxel were dissolved in dimethyl sulfoxide. Stock solutions of doxorubicin (10 mM), vincristine (1 mM) were stored at 4 °C and 5-FU (500 mM), paclitaxel (1 mM) were stored at −20 °C according to manufacturer's instructions. For final drug concentrations, solutions were serially diluted in medium and added to wells. The highest doses of doxorubicin, vincristine, 5-FU, and paclitaxel used for assays were 20 μM, 1 μM, 2000 μM and 1 μM, respectively. All drugs were purchased from Sigma–Aldrich, St. Louis, MO and were used within one week of preparation.

### 2.3. Clonogenic assay

Cells (231/$231^{BRMS1}$ and 435/$435^{BRMS1}$) were passaged and allowed to proliferate to 70% confluence in 10 cm dishes for at least two passages to ensure log growth before harvesting for seeding. Cells were seeded in triplicate at a density of 1000 cells/well onto 6-well plates (Corning) in a final volume of 2 ml medium and allowed to attach overnight. The following day, drugs were added at the indicated final concentrations in a volume of 2 ml medium and incubated with cells for 4 h. Drug containing medium was aspirated gently after 4 h and replaced with 5 ml fresh pre-warmed medium in each well [29]. Cells were left undisturbed in a humidified incubator at 37 °C for 7 days and colonies formed were counted by staining with a 1:3 solution of acetic acid:methanol containing 0.01% crystal violet (Carnoy's fixative). Experiments were performed in triplicate and repeated at least twice independently.

### 2.4. MTT indirect assay for proliferation

Exponentially growing 231/$231^{BRMS1}$ and 435/$435^{BRMS1}$ cells were plated at a density of 2500 cells/well in 24-well plates in quadruplicate and allowed to attach overnight. The next day, drugs were added at the indicated concentrations in a final volume of 500 μl and cells were exposed to drugs for 4 h. After 4 h, drug containing medium was gently aspirated and replaced with 1 ml of fresh medium and cells were incubated undisturbed in a humidified incubator until the time of assay. Every second day beginning the day of drug addition, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT; Sigma–Aldrich, St. Louis, MO) was added to the medium in each well at a final concentration of 0.5 mg/ml and incubated with cells for 3 h. Following incubation with MTT, medium was aspirated gently and 500 μl of DMSO was added to each well and plates were shaken on a horizontal shaker for 30 min to dissolve the formazan crystals. Absorbance was read in a plate reader at 490 nm and experiments were repeated independently at least twice.

### 2.5. Chemosensitivity in 3D-culture

Exponentially growing 231/$231^{BRMS1}$ and 435/$435^{BRMS1}$ cells were plated at a density of 5000 cells/well on 8-well chamber slides (Nunc, Nalgene). Before plating cells, each well was coated with a Matrigel cushion (40 μl). The final concentration of Matrigel above the cushion layer was adjusted to 10%. Medium containing cells (200 μl) was mixed with a 20% Matrigel suspension in cold medium containing drug at 2× of the indicated concentrations (200 μl). The two suspensions were mixed together gently to ensure uniform distribution of cells and drugs and incubated in a humidified incubator at 37 °C. Cells were incubated for nine days and colonies formed were counted. Experiments were repeated independently at least twice.