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Berberine Reverses the Chemoresistance of Breast Cancer to 5- Fluorouracil by Downregulating Metadherin

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Abstract

Background: Breast cancer is one of the most common types of malignancies among women worldwide. Chemoresistance has long been a very important obstacle in treatment of breast cancer. Metadherin (MTDH) is an oncogene that has been reported to be related to chemoresistance. Our study was to confirm the chemoresistance to 5-fluorouracil (5-Fu) for the breast cancer cells overexpressing MTDH and explore the effect of berberine on the chemoresistance of breast cancer.

Methods: We used MCF-7 cells to establish the pcDNA3.1-MTDH (3.1-MTDH) expressing cells and pcDNA3.1-vector (3.1-vector) control cells. Quantitative reverse-transcription PCR, western blotting, and MTT assay were used to determine the cell viability, expression of MTDH, and effect of berberine on chemoresistance.

Results: Overexpression of MTDH induced the cell resistance to 5-Fu (IC_{50}: 423.16 ± 39.89 ug/ml for 3.1-MTDH cells *vs*. 103.14 ± 4.93 ug/ml for 3.1-vector control cells, $P = 0.026$) and berberine could reverse this resistance by downregulating MTDH (5-Fu IC_{50}: 41.01 ± 5.31 ug/ml for 3.1-MTDH cells *vs*. 175.83 ± 41.25 ug/ml for 3.1-vector control cells, $P = 0.034$).

Conclusions: MTDH is a key oncogene, which plays an important role in chemoresistance to 5-Fu in breast cancer and berberine may reverse this resistance and improve the efficacy of 5-Fu to breast cancer.

Keywords: Breast cancer, metadherin, berberine, 5-fluorouracil, chemoresistance

Introduction

Breast cancer is one of the most common types of malignancies and the leading cancer killer among women in countries at different levels of development (1, 2). Other than surgery, chemotherapy is one of the most important therapies of breast cancer, which improves the survival of breast cancer patients. However, there are still about one-third of the patients who are diagnosed as early-stage breast cancer but eventually die of recurrent disease due to the development of drug resistance to chemotherapy (3). The underlying mechanisms of drug resistance in breast cancer are still unknown and seem to be heterogeneous and multifactorial. Certain oncogenes such as metadherin (MTDH) and their signal transduction cascades have been suggested to be related to drug resistance of breast cancer (4).

MTDH (also known as AEG-1 and LYRIC), located at 8q22, is a multifunctional oncogene which is initially cloned in 2002. It has been found that MTDH overexpresses in various cancers including esophageal squamous cell carcinoma, breast cancer, melanoma, hepatocellular carcinoma, prostate cancer, and epithelial ovarian cancers (5-9). Many studies have indicated that MTDH plays an important role in promoting tumor proliferation, migration, metastasis, and angiogenesis by regulating multiple molecular pathways (4, 5, 7, 8, 10). In breast cancer, our group has demonstrated that MTDH promotes the resistance of poorly prognostic breast cancer to chemotherapeutic agents (doxorubicin, cisplatin, and paclitaxel) (4). In human hepatocellular carcinoma, MTDH induces resistance to 5-fluorouracil (5-Fu) through upregulating the expression of transcription factor LSF, which regulates the expression of thymidylate synthase, a target of 5-Fu. In addition, MTDH also enhances the expression of dihydropyrimidine dehydrogenase, an enzyme that catalyzes the rate-limiting step in the catabolism of 5-Fu (11). However, the effect of MTDH on chemoresistance to 5-Fu has not been reported yet in breast cancer.

Berberine is a main alkaloid component in Huang Lian and many other medicinal herbs. Some studies have shown that berberine has antitumor activity in a wide variety of cancer cells (12-15). In breast cancer, studies have demonstrated that berberine can induce tumor cell apoptosis and cell cycle arrest, and inhibit growth and tumor metastasis (16-18). Berberine has also been reported to have a radiosensitizing effect (19). Besides these anticancer functions, the effect of berberine on chemosensitivity in breast cancer is still unknown.

In this study, we demonstrated that overexpression of MTDH could induce chemoresistance of breast cancer cells to 5-Fu. We also identified that berberine could reverse this resistance to 5-Fu in the breast cancer cells overexpressing MTDH, and this effect of berberine was achieved by downregulating the expression of MTDH.

Materials and Methods

Cell line and reagents

Breast cancer cell line MCF-7 was obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Gibco-BRL (Rockville, IN, USA). Fetal bovine serum (FBS) was supplied by Haoyang Biological Manufacturer Co., Ltd (Tianjin, China). Rabbit anti-MTDH antibody was from Invitrogen (Carlsbad, CA, USA). Anti-mouse IgG horseradish peroxidase (HRP) antibody was from ZhongShan Goldenbridge (Beijing,

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China). Pro-lighting HRP agent for Western blotting detection was from Tiangen Biotech Co. Ltd (Beijing, China). Cell lysis buffer for Western blotting was purchased from Beyotime Institute of Biotechnology (Jiangsu, China). Other reagents were from Sigma–Aldrich (St. Louis, MO, USA) unless described elsewhere.

Cell culture

MCF-7 cells were routinely cultured in DMEM (Gibco-BRL, 12800-017) with 10% FBS, 100 U/ml penicillin, and 100 ug/ml streptomycin at 37 ℃ with 5% CO_2.

Plasmid construction and transfection

The plasmid construction was performed as described previously (4). Briefly, the cDNA representing the complete open reading frame of MTDH was cloned into the BamHI-XhoI vector fragment derived from the pcDNA3.1 vector (Invitrogen) to generate pcDNA3.1-MTDH (3.1-MTDH) cells. The expression plasmid was verified by sequencing of both strands and was used to transfect MCF-7 cells using lipofectamine 2000 transfection reagent according to the manufacturer's protocol (Invitrogen). Cells were then selected with 600 μg/ml G418 (Invitrogen) for 2 weeks and individual colonies were isolated, expanded, and maintained with 300 μg /ml G418. The overexpression of MTDH in these clones was confirmed with Western blot analysis and RT-PCR, and these clones were then mixed for further experiments. Empty pcDNA3.1 plasmid was used similarly to establish pcDNA3.1-vector (3.1-vector) cells.

Quantitative reverse-transcription PCR

Total RNA were extracted with TRIZOL (Invitrogen) according to the manufacturer's protocol. cDNA was synthesized with a PrimerScript RT Reagent kit (TaKaRa, Japan). Real-time quantitative RT-PCR (QRT-PCR) was performed with a SYBR green PCR mix in Applied Biosystems StepOne and StepOne-Plus Real-Time PCR Systems. The gene expression ΔCt values of mRNA from each sample were calculated by normalizing with endogenous control GAPDH. All the experiments were repeated in triplicate to confirm the results.

Western blotting

Cells were washed three times with cold phosphate-buffered saline (PBS) and lysed with ice-cold RIPA buffer (Shennengbocai) [1xPBS, 1% NP40, 0.1% sodium dodecyl sulfate (SDS), 5 mM EDTA, and 0.5% sodium deoxycholate] containing protein inhibitor cocktail (SIGMA), phosphatase inhibitor sodium fluoride (NaF), and sodium vanadate (NaVO3). Protein was quantified with BCA Protein Assay Kit (Merck) and separated with 5–10% SDS-PAGE. They were electrotransferred to polyvinylidene fluoride membranes (ImmobilonP; Millipore, Bedford, MA, USA) and blocked in 5% non-fat dry milk in Tris-buffered saline, pH 7.5 (100 mM NaCl, 50 mM Tris, and 0.1% Tween-20). Membranes were immunoblotted overnight at 4 ℃ with the corresponding primary antibodies, followed by their respective horseradish peroxidase conjugated secondary antibodies. Signals were detected by enhanced chemiluminescence. Beta-actin was used as the loading control.

Cytotoxicity assay

The sensitivity of tumor cells to the drug was measured with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, M2128) assay. Cells were plated in 96-well culture plates at a density of 3.5×10^3 cells/well. On the next day, drugs were added to the 100 μl culture medium at varied concentrations. The DMEM medium alone was utilized as a blank control and the cells without drugs were used as a negative control. After incubation for 48 hours (h) at 37 ℃ with 5% CO_2, 20 μl MTT was added into each well. Cells were incubated for 4 more h. After removal of the culture medium, 100 ul DMSO was added to each well and the reduction of MTT was quantified for the absorbance at the wavelength of 490 nm with a Microplate Reader (Bio-Rad, Hercules, CA, USA).

Statistical analysis

Statistical analysis was carried out using SPSS 13.0 for Windows. Student's t-test was chosen to analyze the statistical difference. Results were presented as mean ± standard deviation (SD). $P < 0.05$ was considered statistically significant and all the experiments were repeated at least three times.

Results

Overexpression of MTDH in breast cancer cell line

We transfected the 3.1-MTDH expression plasmids to MCF-7 cell line and generated MTDH-overexpressing cells. The expression of MTDH after transfection was confirmed with RT-PCR and Western blotting. As shown in Figure 1, cells transfected with 3.1-MTDH showed significantly increased MTDH at both mRNA and protein levels compared with the control 3.1-vector MCF-7 cells.

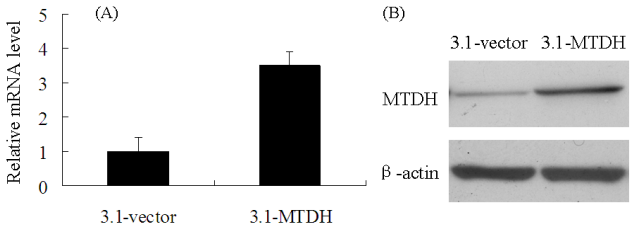

Figure 1. Forced overexpression of metadherin (MTDH) in breast cancer MCF-7 cell line. (A): mRNA levels of MTDH in MCF-7 cells transfected with 3.1 and 3.1-MTDH vector, respectively. (B): protein levels of MTDH measured by Western blotting.

Overexpression of MTDH induced chemoresistance to 5-Fu

To determine the chemosensitivity of breast cancer cells with overexpressed MTDH to 5-Fu, two selected cell clones were treated with varied concentrations of 5-Fu for 48 h, and MTT was used to quantify the changes in cell viability. As shown in Figure 2, 5-Fu reduced the cell viability in a dose-dependent manner, and the 3.1-MTDH cells were significantly more resistant to 5-Fu than the control cells (IC_{50}: 423.16 ± 39.89 ug/ml for 3.1-MTDH cells *vs*. 103.14 ± 4.93 ug/ml for 3.1-vector cells, $P = 0.026$).

Berberine reversed the chemoresistance to 5-Fu for breast cancers cells with MTDH overexpression

To explore the effect of berberine on chemoresistance of breast cells with overexpressed MTDH, on the next day after plating cells in 96-well plate, we added 25 uM berberine together with varied concentrations of 5-Fu to each well and incubated for 48 h. MTT and DMSO were then added as described in Materials and Methods. The results are shown in Figure 3. We found that berberine reversed the chemoresistance to 5-Fu for breast cancer cells with MTDH overexpression, and the 3.1-MTDH cells became more sensitive to 5-Fu than 3.1-vector control cells (IC_{50}: 41.01 ± 5.31ug/ml for 3.1-MTDH cells *vs*. 175.83 ± 41.25 ug/ml for 3.1-vector cells, $P = 0.034$).

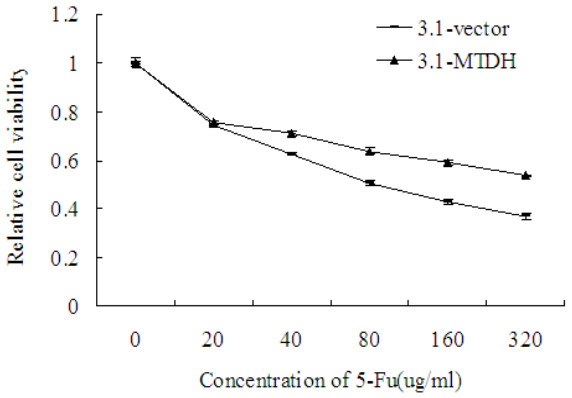

Figure 2. Overexpression of MTDH induces the chemoresistance to 5-Fu. IC_{50}: 423.16 ± 39.89 ug/ml for 3.1-MTDH cells *vs*. 103.14 ± 4.93 ug/ml for 3.1-vector control cells, $P = 0.026$.

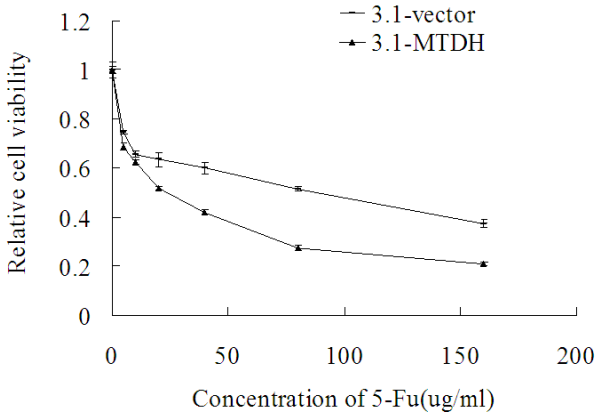

Figure 3. Berberine reverses the chemoresistance to 5-Fu for 3.1-MTDH cells. IC_{50}: 41.01 ± 5.31 ug/ml for 3.1-MTDH cells vs. 175.83 ± 41.25 ug/ml for 3.1-vector cells, $P = 0.034$.

Berberine down-regulated the expression of MTDH

In order to explore the mechanism underlying the chemoresistance reversal by berberine to 5-Fu in breast cancer cells which overexpress MTDH, we treated the cells with 25 uM berberine and extracted proteins to detect the expression of MTDH after incubation for 24, 48, and 72 h, respectively. As shown in Figure 4(A), the expression of MTDH significantly reduced in 3.1-MTDH cells after incubation with berberine, while there were almost no changes in 3.1-vector cells. Also, the expression of MTDH in 3.1-MTDH cells was lower than that in 3.1-vector cells.

In addition, we treated the parental MCF-7 cells with 25 uM and 50 uM berberine for 24, 48, and 72 h, respectively. As shown in Figure 4(B), the expression of MTDH in MCF-7 cells reduced significantly after treatment with 50 uM berberine. However there was no obvious difference among the MCF-7 cells treated with 25 uM berberine for the defined times.

Discussion

Breast cancer is one of the most common types of malignancies and the leading cancer killer among women in countries at different levels of development [1, 2]. Resistance of tumors to diverse kinds of chemotherapeutic drugs remains an important issue. The mechanisms of chemoresistance are still unknown. One potential mechanism is the abnormalities of certain oncogenes and their signal transduction cascades. If we can identify the key oncogenes that play an important role in chemoresistance of breast cancer, we will be able to develop effective targeted therapy to overcome the chemoresistance.

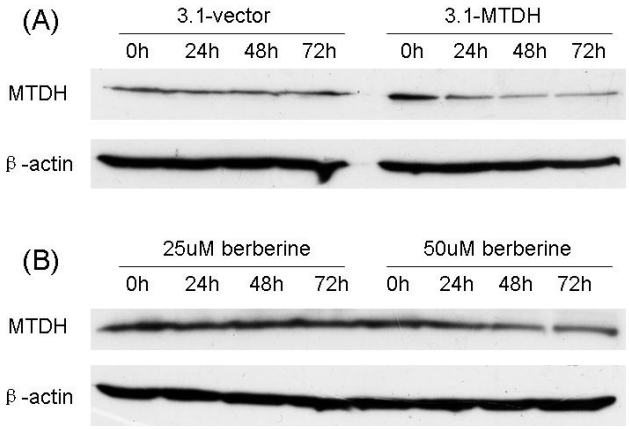

Figure 4. Berberine down-regulates the expression of MTDH. (A): The expression of MTDH significantly reduced in 3.1-MTDH cells, but almost no changes in 3.1-vector cells after incubation with berberine for 0, 24, 48, and 72 h, respectively. The expression of MTDH in 3.1-MTDH cells was lower than that in 3.1-vector cells. (B): The expression of MTDH in parental MCF-7 cells reduced significantly after incubation with 50 uM berberine for 0, 24, 48, and 72 h, respectively. However, in MCF-7 cells treated with 25 uM berberine, there was no obvious difference.

MTDH is a multifunctional oncogene, which has been found to be overexpressed in various cancers including breast cancer (5-9). Our group has completed some studies about the function of MTDH and we have demonstrated that more than 40% of tumors overexpressed MTDH, which was related to worse clinical outcomes (4, 20). MTDH could enhance the invasion of breast cancer cells by inducing epithelial to mesenchymal transition (21). MTDH also promoted chemoresistance of breast cancer with poor prognosis (doxorubicin, cisplatin, paclitaxel) (4). In this study, we further confirmed that overexpression of MTDH could induce the resistance of MCF-7 cells to 5-Fu. Thus MTDH may be a key oncogene and play an important role in chemoresistance of breast cancer. MTDH can be a new gene target for the treatment of breast cancer.

Berberine is a main alkaloid component in Huang Lian and many other medicinal herbs and it is the most commonly used medicine for gastrointestinal discomfort in China. Recently berberine has been reported to have antitumor activities in a wide variety of cancer cells (12-15). In breast cancer, studies have shown that berberine can induce the cell apoptosis and cell cycle arrest, and inhibit the tumor growth and metastasis (16-18). In addition, berberine has also been reported to have a radiosensitizing effect (19). In our study we found that in the breast cancer cells with overexpressed MTDH (3.1-MTDH cells), berberine reversed their chemoresistance to 5-Fu. Cells with a high level of MTDH and resistance to 5-Fu (IC_{50}: 423.16 ± 39.89 ug/ml for 3.1-MTDH cells *vs*. 103.14 ± 4.93 ug/ml for 3.1-vector cells, $P = 0.026$) became more sensitive than the vector control cells (IC_{50}: 41.01 ± 5.31 ug/ml for 3.1-MTDH cells *vs*. 175.83 ± 41.25 ug/ml

for 3.1-vector cells, P = 0.034). After incubation with 25 uM berberine for 24, 48, and 72 h, the expression of MTDH in 3.1-MTDH cells reduced obviously. Compared with 25 uM berberine, 50 uM berberine decreased the expression of MTDH more significantly in parental MCF-7 cells. All these results indicated that berberine could reverse the chemoresistance to 5-Fu in breast cancer by downregulating the expression of MTDH. Thus our study provided the new effect of berberine on breast cancer.

Based on our study, combination of berberine with chemotherapeutic drugs such as 5-Fu, may prevent and/or reverse the chemoresistance of breast cancer cells to therapeutic drugs and benefit the breast cancer patients. Further research is necessary to better understand the molecular mechanisms underlying the reversal of chemoresistance and clinical trials are warranted to evaluate the clinical application of berberine.

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References

1. Parkin DM, Pisani P, and Ferlay J, Estimates of the worldwide incidence of 25 major cancers in 1990. Int J Cancer 1999; 80(6): 827-41.
2. Parkin DM, Bray F, Ferlay J, and Pisani P, Global cancer statistics, 2002. CA Cancer J Clin 2005; 55(2): 74-108.
3. Frieboes HB, Edgerton ME, Fruehauf JP, Rose FR, Worrall LK, Gatenby RA, Ferrari M, and Cristini V, Prediction of drug response in breast cancer using integrative experimental/computational modeling. Cancer Res 2009; 69(10): 4484-92.
4. Hu G, Chong RA, Yang Q, Wei Y, Blanco MA, Li F, Reiss M, Au JL, Haffty BG, and Kang Y, MTDH activation by 8q22 genomic gain promotes chemoresistance and metastasis of poor-prognosis breast cancer. Cancer Cell 2009; 15(1): 9-20.
5. Yu C, Chen K, Zheng H, Guo X, Jia W, Li M, Zeng M, Li J, and Song L, Overexpression of astrocyte elevated gene-1 (AEG-1) is associated with esophageal squamous cell carcinoma (ESCC) progression and pathogenesis. Carcinogenesis 2009; 30(5): 894-901.
6. Li J, Zhang N, Song LB, Liao WT, Jiang LL, Gong LY, Wu J, Yuan J, Zhang HZ, Zeng MS, and Li M, Astrocyte elevated gene-1 is a novel prognostic marker for breast cancer progression and overall patient survival. Clin Cancer Res 2008; 14(11): 3319-26.
7. Yoo BK, Emdad L, Su ZZ, Villanueva A, Chiang DY, Mukhopadhyay ND, Mills AS, Waxman S, Fisher RA, Llovet JM, Fisher PB, and Sarkar D, Astrocyte elevated gene-1 regulates hepatocellular carcinoma development and progression. J Clin Invest 2009; 119(3): 465-77.
8. Kikuno N, Shiina H, Urakami S, Kawamoto K, Hirata H, Tanaka Y, Place RF, Pookot D, Majid S, Igawa M, and Dahiya R, Knockdown of astrocyte-elevated gene-1 inhibits prostate cancer progression through upregulation of FOXO3a activity. Oncogene 2007; 26(55): 7647-55.
9. Li C, Liu J, Lu R, Yu G, Wang X, Zhao Y, Song H, Lin P, Sun X, Yu X, Zhang Y, Ning X, and Geng J, AEG -1 overexpression: a novel indicator for peritoneal dissemination and lymph node metastasis in epithelial ovarian cancers. Int J Gynecol Cancer 2011; 21(4): 602-8.
10. Emdad L, Lee SG, Su ZZ, Jeon HY, Boukerche H, Sarkar D, and Fisher PB, Astrocyte elevated gene-1 (AEG-1) functions as an oncogene and regulates angiogenesis. Proc Natl Acad Sci U S A 2009; 106(50): 21300-5.
11. Yoo BK, Gredler R, Vozhilla N, Su ZZ, Chen D, Forcier T, Shah K, Saxena U, Hansen U, Fisher PB, and Sarkar D, Identification of genes conferring resistance to 5-fluorouracil. Proc Natl Acad Sci U S A 2009; 106(31): 12938-43.
12. Sanders MM, Liu AA, Li TK, Wu HY, Desai SD, Mao Y, Rubin EH, LaVoie EJ, Makhey D, and Liu LF, Selective cytotoxicity of topoisomerase-directed protoberberines against glioblastoma cells. Biochem Pharmacol 1998; 56(9): 1157-66.
13. Lin CC, Yang JS, Chen JT, Fan S, Yu FS, Yang JL, Lu CC, Kao MC, Huang AC, Lu HF, and Chung JG, Berberine induces apoptosis in human HSC-3 oral cancer cells via simultaneous activation of the death receptor-mediated and mitochondrial pathway. Anticancer Res 2007; 27(5A): 3371-8.
14. Lin JP, Yang JS, Lee JH, Hsieh WT, and Chung JG, Berberine induces cell cycle arrest and apoptosis in human gastric carcinoma SNU-5 cell line. World J Gastroenterol 2006; 12(1): 21-8.
15. Mantena SK, Sharma SD, and Katiyar SK, Berberine, a natural product, induces G1-phase cell cycle arrest and caspase-3-dependent apoptosis in human prostate carcinoma cells. Mol Cancer Ther 2006; 5(2): 296-308.
16. Kuo HP, Chuang TC, Tsai SC, Tseng HH, Hsu SC, Chen YC, Kuo CL, Kuo YH, Liu JY, and Kao MC, Berberine, an isoquinoline alkaloid, inhibits the metastatic potential of breast cancer cells via Akt pathway modulation. J Agric Food Chem 2012.
17. Patil JB, Kim J, and Jayaprakasha GK, Berberine induces apoptosis in breast cancer cells (MCF-7) through mitochondrial-dependent pathway. Eur J Pharmacol 2010; 645(1-3): 70-8.
18. Kim JB, Lee KM, Ko E, Han W, Lee JE, Shin I, Bae JY, Kim S, and Noh DY, Berberine inhibits growth of the breast cancer cell lines MCF-7 and MDA-MB-231. Planta Med 2008; 74(1): 39-42.
19. Wang J, Liu Q, and Yang Q, Radiosensitization effects of berberine on human breast cancer cells. Int J Mol Med 2012; 30(5): 1166-72.
20. Su P, Zhang Q, and Yang Q, Immunohistochemical analysis of Metadherin in proliferative and cancerous breast tissue. Diagn Pathol 2010; 5: 38.
21. Li X, Kong X, Huo Q, Guo H, Yan S, Yuan C, Moran MS, Shao C, and Yang Q, Metadherin enhances the invasiveness of breast cancer cells by inducing epithelial to mesenchymal transition. Cancer Sci 2011; 102(6): 1151-7.