Mitogen-inducible Gene-2 (MIG2) and Migfilin Expression Is Reduced in Samples of Human Breast Cancer

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Abstract. *Background: Cell adhesion proteins that connect each cell to neighboring cells and the extracellular matrix play a fundamental role in metastasis. Mitogen-inducible gene-2 (MIG2), is a cell-matrix adhesion protein, which through migfilin, interacts with filamin-A, being linked to actin cytoskeleton. Aim: Recent studies have implicated both MIG2 and migfilin in cancer, but little is known regarding their expression in breast cancer. In this study, we investigated this topic. Materials and Methods: mRNA and protein expression was examined in 30 breast cancer samples and compared to that of normal adjacent tissue using real time-polymerase chain reaction (PCR) and western blotting. Results: Our results showed that expression of MIG2 and migfilin was significantly reduced in the majority of the breast cancer tissues compared to normal tissues regardless of metastatic status and disease stage. Conclusion: Both MIG2 and migfilin are down-regulated in breast cancer.*

Breast cancer (BC) is the most common malignancy in women. However, 90% of patients with BC die from metastatic disease (1). Metastasis is a complex process ultimately leading to cancer cell spread, in which de-regulation of cell adhesion promotes dissociation of cells from the original tumor and invasion of surrounding tissues (2). Thus, extracellular matrix (ECM)-related adhesion proteins play important roles.

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Mitogen-inducible gene-2 (*MIG2*, also known as kindlin-2) (3), is localized at cell ECM adhesion sites and through migfilin [another cell ECM and cell-cell adhesion protein (4), also known as filamin-binding (LIM) protein 1] interacts with filamin A, thus being linked to actin cytoskeleton (5).

Recent studies have implicated both *MIG2* and migfilin in a variety of human cancer types. *MIG2* expression was increased in leiomyomas compared with normal myometrium, while it was decreased in leiomyosarcomas (6). MIG2 was also up-regulated in gastric cancer and, in fact, its expression had a significant positive correlation with metastasis and poor survival (7). *MIG2* was also shown to be highly expressed in 90% of malignant mesothelioma tumors (8), as well as in almost 100% of human bladder carcinomas (9), and in the majority of chondrosarcomas (10). Interestingly, it has been postulated that *MIG2* could function as a promising marker of tumor progression (7, 10). Little is known, however, regarding *MIG2* expression in BC. To date, there has been only one study in human BC tissues showing both up-regulation and down-regulation of *MIG2* expression in BC, suggesting that alterations in *MIG2* expression could potentially contribute to malignancy (11).

Migfilin has also been associated with various types of cancer although its role has not been extensively studied. Cytoplasmic migfilin expression has been strongly associated with higher tumor grade in leiomyosarcomas (12) and inversely-correlated with clinical metastasis of esophageal cancer cells (13). Finally, high migfilin expression was significantly correlated with tumor grade in glioma and poor prognosis (14). However, no data exist, to date, regarding migfilin expression in BC, to our knowledge.

In the present study, we investigated the expression of MIG2 and migfilin both at the mRNA and protein level, in a total of 30 samples obtained from patients with BC and results were compared to the relative expression in normal adjacent

tissue isolated from the same patient. Results were also analyzed in relation to disease stage, and metastatic status.

Materials and Methods

BC tissue samples. BC tissue samples were obtained from patients with BC during surgery at the University Hospital of Larissa (UHL), Greece. Respective normal adjacent tissue (designated as normal hereafter) was also obtained from each patient (n=30). Details concerning diagnosis, disease stage, and metastatic status were also obtained (see Table I). All samples were obtained following verbal informed consent according to a protocol approved by the Institutional Review Board of the UHL (#12549) and in accordance with ethical guidelines of the 1995 Declaration of Helsinki as reflected in *a priori* approval by the Local Ethical Committee of the UHL (#12549). Tissue samples from BC patients undergoing chemotherapy at the time of surgery were excluded from the study.

RNA isolation and real time Polymerase Chain Reaction (PCR). Total RNA was extracted from BC and normal tissue using Trizol (Life Technologies, Carlsbad, CA, USA) and purified using RNeasy mini kit (Qiagen, Valencia, CA, USA). Transcription to cDNA was performed using Superscript (Life Technologies). Quantification of *MIG2*, and Migfilin was performed by real-time PCR (BioRad, Philadelphia, PA, USA). Porphobilinogen deaminase (*PBGD*) was used as housekeeping gene. The primer sequence for *MIG2* was: forward 5'AGC TTT ATG AGC AGG CCA AA3' and reverse 5'GAA AGG GCA GCA TCA ACT TC3'; and for migfilin was: forward 5'CGA ATG CAT GGG AAG AAA CT3' and reverse 5'GCA GGT TAG GAA GGG AAA CC3'. Quantification was performed with the ΔΔCt method using Microsoft Excel software. Reactions were carried out in duplicates and at least two independent experiments were performed.

Protein extraction and western blot analysis. Western blot was performed using standard protocols, as described elsewhere (5). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as loading control.

Antibodies. Antibodies against *MIG2* (5), and Migfilin (5) were described previously. Antibody a-GAPDH antibody was purchased from Santa Cruz (Santa Cruz, CA, USA). Secondary antibodies were purchased from Life Technologies.

Statistical analysis. Comparison of means using Statgraphics software (Warrenton, VA, USA) was used for the statistical analysis. A p-value <0.05 was considered statistically significant. All graphs were generated using the Graphpad Prism software (La Jolla, CA, USA).

Results

MIG2 mRNA and protein expression is significantly reduced in samples from BC tissues compared to normal tissues. *MIG2* expression was found to be significantly reduced both at the protein (Figure 1A) and mRNA level (Figure 1B and C) in BC compared to normal tissue. In fact, *MIG2* mRNA expression was reduced in 83% of the samples (20 out of 24), while only 12.5% of samples showed elevated expression (3 out of 24 samples). However, reduction in *MIG2* mRNA expression was not statistically correlated with metastatic status (Figure 1D) or disease stage (Figure 1E).

Table I. *Clinical characteristics of the breast cancer samples used in the study.*

No	Pathology diagnosis	Stage	Metastases at the time of surgery
1	IDC	III	Yes
2	IDC	II	Yes
3	IDC	III	No
4	IDC	III	No
5	IDC	III	No
6	IDC	I	No
7	IDC	II	Yes
8	DCIS	I	No
9	IDC	III	Yes
10	IDC	III	Yes
11	IDC	II	Yes
12	IDC	II	Yes
13	IDC	III	Yes
14	IDC	I	Yes
15	IDC	II	No
16	IDC	III	Yes
17	IDC	II	Yes
18	IDC	III	Yes
19	IDC	II	No
20	IDC	II	Yes
21	IDC	III	Yes
22	IDC	II	No
23	IDC	II	No
24	IDC	III	No
25	IDC	II	Yes
26	IDC	II	Yes
27	IDC	III	Yes
28	IDC	II	No
29	IDC	II	No
30	ILC	II	No

IDC: Invasive ductal carcinoma; DCIS: ductal carcinoma *in situ*; ILC: invasive lobular carcinoma.

Migfilin mRNA expression is reduced in BC samples. Migfilin expression was also assessed in BC tissues compared to normal tissues. Migfilin protein expression was lower in BC in some of the samples tested (Figure 2A, compare lanes 3-4 and 5-6), while others appeared not to be affected. Moreover, *migfilin* mRNA expression was also significantly reduced (Figure 2B-E). In fact, reduction was observed in 70% (14 out of 20) and increase in 30% (6 out of 20) of the BC samples tested compared to normal tissue. We found no statistically significant correlation between migfilin expression and metastatic status, or disease stage (Figure 2D-E) in BC.

Discussion

As ECM-related adhesion proteins play pivotal roles in the dissociation of cancer cells from the original tumor

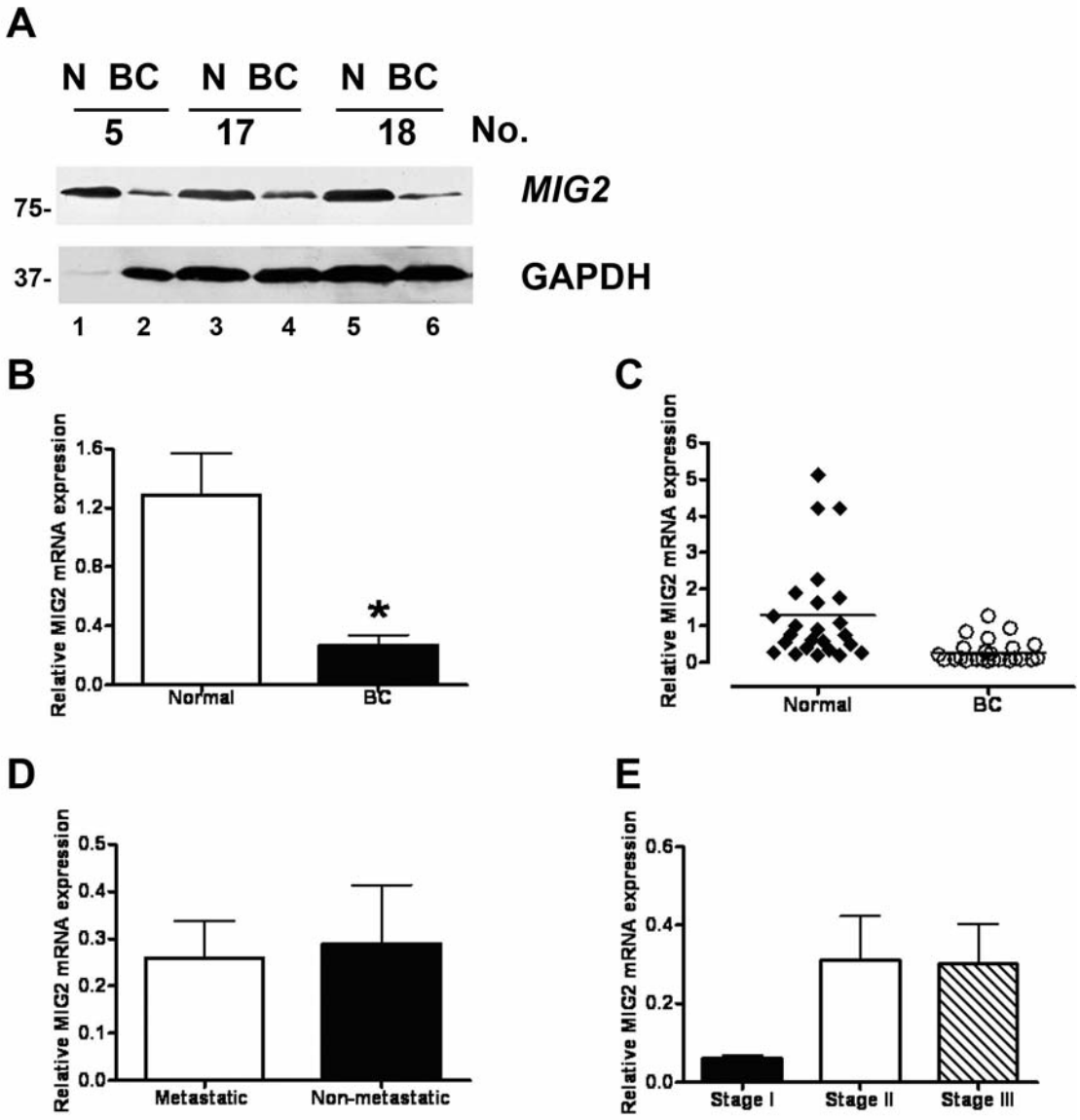

Figure 1. *A: Representative western blot showing Mitogen-inducible gene-2 (MIG2) expression in breast cancer (BC) and normal adjacent tissue (N). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as loading control. B-E: Real-time polymerase chain reaction (PCR) showing mean MIG2 mRNA expression (B) in 24 BC samples compared to normal adjacent tissues (p=0.001), sample distribution (C), and expression according to metastatic status (D), and disease stage (E). *Statistically significant difference of p<0.05. Lines in (C) indicate mean mRNA expression of MIG2. All error bars represent standard errors.*

ultimately leading to metastasis, the aim of the present study was to investigate the expression of the cell adhesion proteins MIG2 and migfilin in samples obtained from patients with BC and compare results with their expression in normal adjacent tissue isolated from the same patient.

Although both *MIG2* and migfilin have been implicated in cancer, little is known regarding their expression in BC. A study by Gozgit *et al*. showed that whereas all 21 normal human breast tissues expressed *MIG2*, half of the 34 BC cases, analyzed by immunohistochemistry, lost MIG2 expression. Interestingly, 10 of these 17 positive cases were considered to overexpress MIG2 (11). Our findings are in accordance with the study by Gozgit *et al*. (11), as we also show that normal regulation of *MIG2* is disrupted in BC. We show that MIG2 expression is significantly reduced in 83% of the BC tissues tested compared to normal adjacent tissues both at the protein and mRNA level (Figure 1). However, while Gozgit *et al*. used normal tissues from reduction-

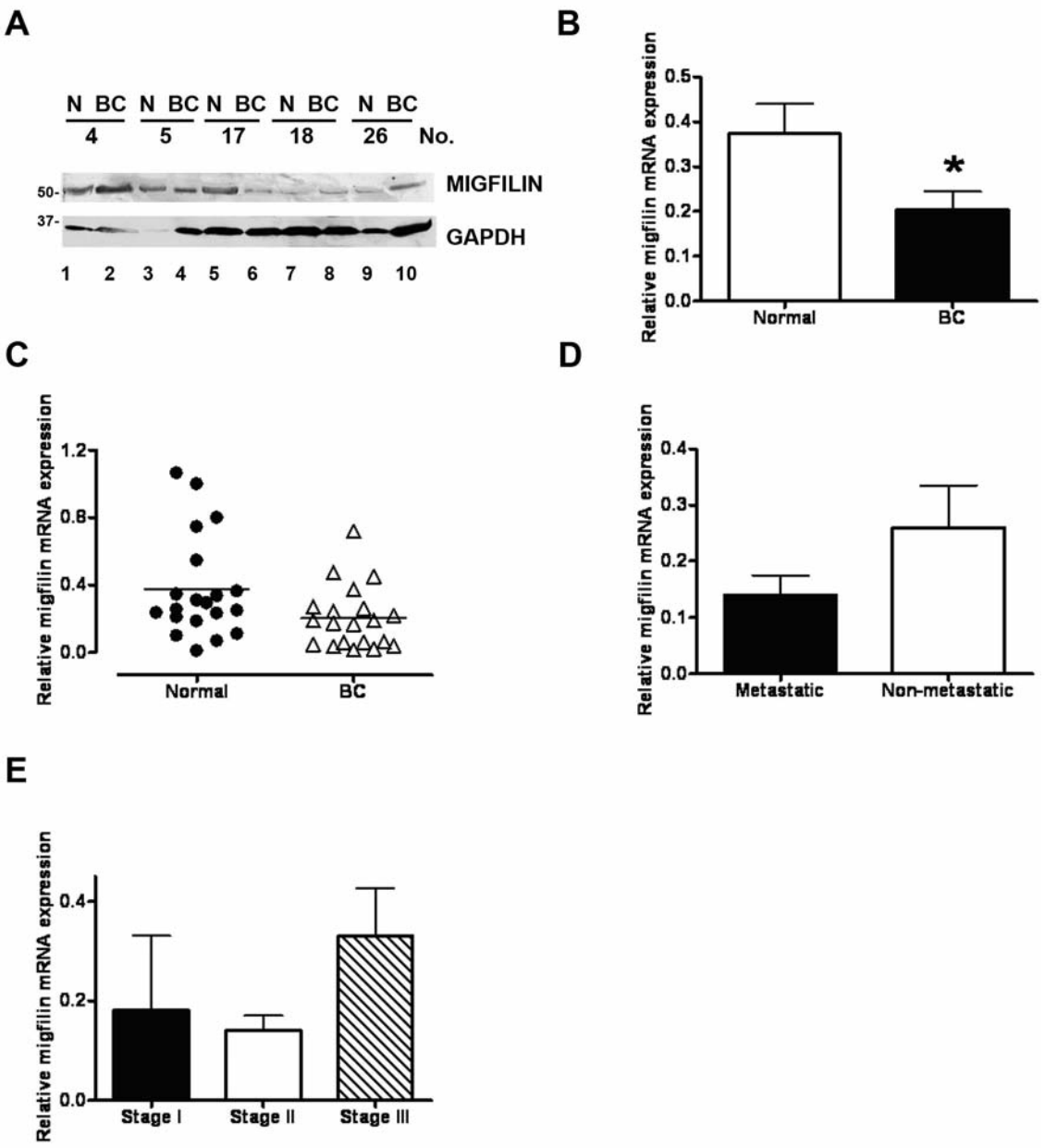

Figure 2. *A: Representative western blot showing migfilin expression in breast cancer (BC) and normal tissue (N) with Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a loading control. B-E: Real-time polymerase chain reaction (PCR) showing mean migfilin mRNA expression in 20 BC samples compared to normal adjacent tissue (p=0.039), sample distribution (C), and expression according to metastatic status (D), and disease stage (E). *Statistically significant difference of p<0.05. Lines in (C) indicate mean mRNA expression of migfilin. All error bars represent standard errors.*

mammoplasty surgeries as controls (11), we utilized normal adjacent tissues. Comparing expression in the BC tissues to those of the normal adjacent tissues offers the advantage that both tissues originate from the same patient, and they have been exposed to the same environmental factors, as they are harvested, and stored at the same time and under the same conditions. Our findings indicate that *MIG2* expression is lost in BC, and since *MIG2* has been implicated in inhibition of cell invasion (15), it is likely that reduction of *MIG2* expression enhances the malignant phenotype of cancer cells by increasing aggressive behavior and invasiveness. However, when expression data where analyzed based on the metastatic status or the stage of the disease of the BC samples tested, no statistically significant correlation was revealed.

Regarding migfilin, to date and to the best of our knowledge, this is the first time that migfilin has been investigated in BC tissue. Interestingly, our results show that migfilin expression was also significantly reduced in BC following a similar trend to that of its binding partner *MIG2* (Figure 2). However, although, recent studies have shown that expression of migfilin is negatively-correlated with clinical metastasis in esophageal cancer (13), we found no statistical correlation between its expression and metastatic status or disease stage in BC.

In summary, the present work shows that both *MIG2* and migfilin are significantly down-regulated in BC. Of course, more studies are needed in order to identify their exact mode of action in BC and tumor biology in general.

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