Alterations of Metastasis-Related Genes Identified Using an Oligonucleotide Microarray of Genistein-Treated HCC1395 Breast Cancer Cells

Wen-Ying Lee, Soon-Cen Huang, Ching-Cherng Tzeng, Tsui-Ling Chang, and Keng-Fu Hsu

Abstract: Genistein, one of the major isoflavones, potently inhibits the growth and metastasis of breast cancer. However, the precise molecular mechanism in metastasis inhibition is not clear. We investigated the effect of genistein in HCC1395 cells, a cell line derived from an early-stage primary breast cancer. Genistein dose dependently both decreased cell viability and inhibited the invasion potential. We used human oligonucleotide microarrays to determine the gene expression profile altered by genistein treatment. TFPI-2, ATF3, DNMT1, and MTCBP-1, which inhibit invasion and metastasis, were upregulated, and MMP-2, MMP-7, and CXCL12, which promote invasion and metastasis, were downregulated. We used quantitative real-time polymerase chain reaction to verify the microarray data at the mRNA level. We conclude that genistein-induced alternations of gene expression involving metastasis may be exploited for devising chemopreventive and therapeutic strategies, particularly for early-stage breast cancer.

Introduction

Asian women who consume a diet high in soy isoflavones have a lower incidence and mortality rate of breast cancer than women in Western countries (1,2). Plasma and urinary levels of isoflavones are inversely associated with breast cancer risk (3,4). Genistein (4,5,7-trihydroxyisoflavone), one of the major isoflavones, stimulates the growth of estrogen receptor (ER)-positive breast cancer cell lines MCF-7 and T47D in vitro (5,6) and MCF-7 cell tumors in vivo (5) at low and physiologically relevant concentrations (<1 µM), whereas a high concentration of genistein (>10 µM) potently inhibits the growth of both ER-positive and ER-negative breast cancer cells (6–8). These studies suggest that different mechanisms for genistein growth stimulation and growth inhibition: genistein at low concentrations stimulates growth via estrogen-dependent mechanisms, but genistein at higher concentrations inhibits tumor growth mediated by estrogen-independent mechanisms (6–8). The chemopreventive effects of genistein are not limited to inhibiting tumor cell proliferation but also include the ability to inhibit tumor cell invasion (9,10), which is mediated by matrix metalloproteinase (MMP) downregulation and tissue inhibitor of metalloproteinase (TIMP) upregulation. These results indicate that genistein may be of great value for preventing the metastasis of breast cancer.

Metastasis is the most significant cause of mobility and mortality in breast cancer (11). Despite the low risk of disease recurrence in node-negative breast cancer patients, up to 30% of early-stage breast cancer patients will relapse within 10 years after surgery and eventually die of distant metastasis (12). Metastatic breast cancer may respond to a variety of hormonal and chemotherapeutic interventions; however, such therapy is rarely curative (13,14). Thus, it is needed to develop mechanism-based and targeted therapeutic strategies to prevent metastatic disease. DNA microarrays allow a comprehensive analysis of gene responses under specific conditions; they reveal gene networks and provide significant clues about their mechanisms (15). Using this technology will contribute to the development of chemopreventive and therapeutic strategies. In order to better understand the precise molecular mechanisms of genistein’s inhibition of invasion and metastasis in breast cancer, we used oligonucleotide microarrays to determine the gene expression profile altered by genistein treatment in human HCC1395 breast cancer cells. The HCC1395 cell line is derived from a tumor node metastasis (TNM) stage I primary breast cancer without axillary node metastasis and represents a model of primary breast cancer (16). We hope that this study will provide new
insights for using genistein in chemopreventive therapy to inhibit metastasis in early-stage primary breast cancers.

**Materials and Methods**

**Cell Line and Culture**

An HCC1395 human breast cancer cell line, obtained from American Type Culture Collection (Manassas, VA), was cultured in RPMI 1640 (Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal bovine serum in a 5% CO2 atmosphere at 37°C. The HCC1395 cell line was initiated from a primary ductal carcinoma, which was classified as TNM stage I, grade 3, invasive ductal carcinoma with 0 out of 34 lymph node metastasis. The cells were positive for estrogen receptors, negative for progesterone receptors, negative for human epidermal growth factor receptor 2/neu, and positive for p53.

**Cell Culture and Cell Proliferation Assay**

To inhibit their growth, HCC1395 cells were treated with 1, 5, 10, 20, 50, 100, and 200 µM genistein, respectively (Sigma-Aldrich, St. Louis, MO) or 0.1% dimethyl sulfoxide (DMSO; vehicle control) for 72 h. Cell viability was determined using a modified tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTS) assay (CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit; Promega, Madison, WI) according to the manufacturer’s instructions. Briefly, 2 × 10^6 cells were seeded on Matrigel invasion chamber plates and cultured with serum free medium containing 0.1% bovine serum albumin in the presence of 30 µM or 50 µM of genistein. The control group was cultured with the same media in 0.1% DMSO. Culture media with 0.1% bovine serum albumin and fibronectin (Sigma-Aldrich, St. Louis, MO) were placed in the lower compartment as the chemotactrant. Cells were incubated for 24 h at 37°C in a humidified incubator with 5% CO2. Nonmigratory cells on the upper surface of the filter were removed by wiping them off with a cotton swab. Cells invading through the Matrigel and filter membrane were fixed with methanol and stained with hematoxylin. The cell number was counted under a microscope (magnification: ×100). Results are expressed as the average cell number per filter. Invasion indexes are expressed as a percentage of the control value.

**Oligonucleotide Microarray Analysis**

HCC1395 cells were treated with 50 µM genistein or 0.1% DMSO (vehicle control) for 72 h. Total RNA was extracted using a reagent (Trizol; Invitrogen Corp., Carlsbad, CA) and purified using a kit (RNeasy Mini Kit; Qiagen GmbH, Hilden, Germany). The purity and integrity of the RNA was evaluated by measuring the 260/280 nm optical density ratio using a bioanalyzer (Agilent 2100; Agilent Technologies, Inc., Santa Clara, CA). Total RNA (0.5 µg) was amplified using a low RNA input fluorescent linear amplification kit (Agilent) and labeled with Cy3 or Cy5 (CyDye; PerkinElmer Life and Analytical Sciences, Inc., Boston, MA). Cy-labeled cRNA (2 µg) was fragmented to an average size of about 50–100 nucleotides. Fragmented labeled cRNA was then hybridized to Human 1A (version 2) oligonucleotide microarrays (Agilent) representing 18,716 known genes. Microarrays were scanned using a microarray scanner (Agilent), and the scanned images were analyzed using commercial software (Feature Extraction version 9.1; Agilent), an image analysis and normalization software used to quantify signal and background intensity for each feature and to substantially normalize the data by rank-consistency-filtering using the Lowess method. The software’s error-model module was used to adjust P values for identifying genes whose expression differed significantly between the 50-µM genistein-treated sample and the DMSO control sample. A P value threshold of 0.05 and a twofold...
change in the expression levels between the 2 samples were considered statistically significant.

Validation: Quantitative Reverse Transcription (RT) Polymerase Chain Reaction (PCR; RT-PCR)

Total RNA (5 µg) was reverse transcribed using Moloney Murine Leukemia Virus reverse transcriptase (Promega). Each cDNA pool was stored at -20°C until further real-time PCR analysis. Specific oligonucleotide primer pairs were selected from the Roche Universal ProbeLibrary (https://www.roche-applied-science.com/sis/rtpcr/upl/index.jsp) and used for real-time PCR (Table 1). The specificity of each primer pair was tested using common reference RNA (Stratagene, La Jolla, CA) as a DNA template to perform real-time PCR reactions and followed by DNA 500 chip run on the Agilent 2100 bioanalyzer to check the size of the PCR product. Real-time PCR reactions were on a PCR system (LightCycler 1.5 Instrument; Roche Diagnostics Corporation, Roche Applied Science, Indianapolis, IN) using a kit (LightCycler FastStart DNA MasterPLUS SYBR Green I; Cat. 03 515 885) and used for real-time PCR (Table 1). Each sample was run in triplicate. During each real-time PCR run, the data were automatically analyzed by the system, and an amplification plot was generated for each cDNA sample. From each of these plots, the LightCycler3 Data analysis software automatically calculated Cp values (crossing point, the turning point corresponds to the first maximum of the second derivative curve), which imply the beginning of exponential amplification. The fold expression or repression of the target gene relative to β-actin in each sample was then calculated using the formula: \(2^{-\Delta \Delta Cp}\), where \(\Delta Cp = Cp_{target\ gene} - Cp_{\beta-actin}\) and \(\Delta \Delta Cp = \Delta Cp_{treated\ sample} - \Delta Cp_{control\ sample}\).

### Table 1. Primers Used for RT-PCR Verification

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
<th>Orientation</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2</td>
<td>ataacctggatgcgcggctg</td>
<td>Sense</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>aagcaccctggaagagttg</td>
<td>Antisense</td>
<td></td>
</tr>
<tr>
<td>MMP-7</td>
<td>tgtggcaggttgacgcgt</td>
<td>Sense</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>tctcattctcagttggtg</td>
<td>Antisense</td>
<td></td>
</tr>
<tr>
<td>CXCL12</td>
<td>ttgacccgaagtaaagttg</td>
<td>Sense</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>cccctcattcagttggtg</td>
<td>Antisense</td>
<td></td>
</tr>
<tr>
<td>TFPI-2</td>
<td>ttgcgacccacaaagaaat</td>
<td>Sense</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>ttcgctgaacatatgcaatc</td>
<td>Antisense</td>
<td></td>
</tr>
<tr>
<td>ATF3</td>
<td>tggcactcagaaacgaacg</td>
<td>Sense</td>
<td>121</td>
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<tr>
<td></td>
<td>catctctccagggctactc</td>
<td>Antisense</td>
<td></td>
</tr>
<tr>
<td>DNMT1</td>
<td>cagggaccacatctgtaaga</td>
<td>Sense</td>
<td>105</td>
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<tr>
<td></td>
<td>acctgacagttgcccagat</td>
<td>Antisense</td>
<td></td>
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<tr>
<td>MTCBP-1</td>
<td>cttgccgggtgcctccagggg</td>
<td>Sense</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>tgtccgacgtagcttttcgga</td>
<td>Antisense</td>
<td></td>
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</tbody>
</table>

*Abbreviations are as follows: RT-PCR, reverse transcription polymerase chain reaction; MMP, matrix metalloproteinase; CXCL12, chemokine (C-X-C motif) ligand 12; TFPI-2, tissue factor pathway inhibitor-2; ATF3, activating transcription factor 3; DNMT1, DNA methyltransferase 1; MTCBP-1, membrane-type 1 matrix metalloproteinase cytoplasmic tail-binding protein-1.

Statistical Analysis of Biological Assays

All experiments were repeated at least in triplicate, and all treatments were expressed relative to the control, which was set at 100%. Results are expressed as means ± standard error of mean. The significance of differences between group means was determined by Student’s t-test or 1-way analysis of variance. Statistical significance was set at \(P < 0.05\).

Results

Effect of Genistein on Cell Growth Inhibition and Cell Cycle Distribution

Genistein dose dependently decreased HCC1395 cell viability, with 50% inhibition (IC\(_{50}\)) about at 30 µM for 72 h (Fig. 1). Flow cytometry showed that genistein at IC\(_{50}\) significantly arrested tumor cells at G2/M phase (\(P < 0.001\)), decreased the percentage of cells in S phase (\(P < 0.001\)), and increased the sub-G1 apoptotic peak (\(P < 0.01\)) (Fig. 2 and Table 2).

Effect of Genistein on Invasion Assay

The effect of genistein on the invasiveness of HCC1395 cells was studied by culturing the cells without or with 30 µM and 50 µM genistein. Genistein dose dependently inhibited the invasion potential of cancer cells (\(P < 0.01\) for genistein at 30 µM vs. control; \(P < 0.01\) for genistein at 50 µM vs. control) (Fig. 3).

Effect of Genistein on Gene Expression Profiles

Of the 397 genes with a >twofold change after 72 h of treatment with 50 µM of genistein, 154 were upregulated and 243 were downregulated (\(P < 0.05\)). A closer inspection of differentially expressed genes of interest showed that genistein downregulated the MMP-2, MMP-7, and chemokine (C-X-C motif) ligand 12 (CXCL12) genes, which are linked to promoting invasion and metastasis, and upregulated the tissue factor pathway inhibitor-2 (TFPI-2), activating transcription

### Table 2. Sub-G1 Apoptotic Peak and Cell Cycle Distribution in the Control Group and in the Genistein-Treated Group at 30 µM

<table>
<thead>
<tr>
<th>Group</th>
<th>Sub-G1</th>
<th>G0/G1</th>
<th>S</th>
<th>G2/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.9 ± 0.4</td>
<td>86.3 ± 0.4</td>
<td>9.6 ± 0.3</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>Genistein 30 µM</td>
<td>6.5 ± 1.1†</td>
<td>87.5 ± 0.3</td>
<td>4.1 ± 0.06*</td>
<td>8.6 ± 0.06*</td>
</tr>
</tbody>
</table>

*The values are the mean ± standard error of the mean of 3 independent experiments.* \(P < 0.001\) vs. control; †, \(P < 0.01\) vs. control. Sub-G1, apoptotic peak; G0/G1, border quiescent to gap 1 phase; S, DNA synthesis phase; G2/M, border gap 2 to mitosis phase.
factor 3 (ATF3), DNA methyltransferase 1 (DNMT1), and membrane-type 1 matrix metalloproteinase cytoplasmic tail-binding protein-1 (MTCBP-1) genes, which are linked to inhibiting invasion and metastasis. Quantitative real-time RT-PCR verified the data of microarray at the level of mRNA (Table 3).

Discussion

At high concentrations, genistein, a natural inhibitor of protein tyrosine kinase (17), inhibits the growth of breast cancer cells in vitro (6–8). In the present study, genistein inhibited the proliferation by arresting tumor cells at G2/M phase and dose dependently inhibited tumor invasion in HCC13995 cells, a cell line derived from an early-stage and ER-positive primary breast cancer. Using oligonucleotide microarrays, we found that a high concentration of genistein altered a group of genes critically involved in the control of invasion and metastasis: TFPI-2, ATF3, DNMT1, and MTCBP-1, 4 genes that inhibit invasion and metastasis, were upregulated; and MMP-2, MMP-7, and CXCL12, 3 genes that promote invasion and metastasis, were downregulated. Quantitative real-time PCR verified the data of microarray at the level of mRNA.

The evidence that links MMP with breast cancer invasion and metastasis is now extensive (18). The primary cancer-promotion mechanism of MMP is degrading extracellular matrices (ECM), which consist of basement membranes and interstitial connective tissue. MMP, the principal matrix-degrading proteinase (19), have traditionally been divided into 4 main subgroups: collagenases (MMP-1, -8, and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -7, -10, and -11), and membrane types (MT1 to MT6-MMP) (20). To establish metastatic growth, breast cancer cells must pass through basement membranes at least 3 times. Breast cancer cells initially cross basement membranes to become an invasive lesion and then transverse these membranes during both entry into and exit from the bloodstream. MMP-2 and
MMP-9 can degrade type IV collagen, which is the main component of basement membranes. In addition, MMP-2 has been shown to be the major MMP in breast cancer (21), and MMP-2 expression is strongly associated with shortened survival independent of major prognostic indicators in patients with breast carcinoma (22). Therefore, MMP-2 may be critical in breast cancer metastasis.

MMP-7, also known as matrilysin, promotes cancer invasion not only by degrading ECM substrates but also by regulating the activities of non-ECM proteins (23). E-cadherin, a transmembrane protein, is involved in the positive regulation of cell adhesion via its interaction with catenins. MMP-7 cleaves the ectodomain of E-cadherin, disrupting the E-cadherin/catenin complex and then promoting the migration and invasion of cancer cells (23). MMP-7 is overexpressed in a variety of cancers including breast cancer (24). In one study (25), on a tissue microarray of 346 node-negative breast cancers, MMP-7 was expressed in 75% of the tumors. Furthermore, another study (26) showed that MMP-7 overexpression correlated with breast cancer invasion in vitro and that MMP-7 promoted invasion by increasing the secretion and activation of proMMP-2 and proMMP-9 (26).

The chemokine CXCL12 (stromal cell-derived factor-1) and its receptor CXC receptor 4 (CXCR4) have recently been shown to play an important role in regulating the directional migration of breast cancer cells to sites of metastasis (27). The binding of CXCL12 to CXCR4 activates phosphatidylinositol-3-kinase (PI3K)/Akt, mitogen-activated protein kinase (MAPK), and Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways, resulting in increased breast cancer migration and invasion (27). In addition, CXCL12 binding to CXCR4 induces the polymerization of actin to promote cell motility. A recent study (28) demonstrated that breast cancer cells that express CXCL12, and therefore have an active CXCL12/CXCR4 pathway, are more invasive and motile and thus have a more aggressive phenotype. The study also found that high levels of CXCL12 were significantly correlated with lymph node metastasis, local recurrence, and poor outcomes in patients with breast cancer. Furthermore, CXCL12 has been shown to stimulate the activation of MMP-2 and MMP-9, which provide a new mechanism for CXCL12-induced metastasis in breast cancer cells (29).

TFPI-2, an ECM-associated serine protease inhibitor, directly inhibits plasmin (derived from plasminogen by uPA and a broad-spectrum serine protease), thereby inhibiting ECM degradation and invasion (30). TFPI-2 also strongly inhibits the activation of proMMP-1 and proMMP-3 (31). Although reports (32,33) on TFPI-2 in breast cancer are limited, many studies have shown that TFPI-2 inhibits invasion in a variety of malignant tumor cells in vitro and in vivo.

ATF3 suppresses tumor invasion by inhibiting MMP-2 promoter (34,35). Recently, one study (36) using microarray

**Figure 3.** The effects of genistein at 30 and 50 µM on the invasion of HCC1395 breast cancer cells. Representative images of cells invading through the Matrigel and filter membrane in the control group (A) and the genistein-treated group at 50 µM (B). C: Invasion indexes expressed as the mean ± standard error of the mean of 3 independent experiments. * Statistically significant differences between the genistein-treated and control cells: \( P < 0.01 \) for genistein at 30 µM vs. control and \( P < 0.01 \) for genistein at 50 µM vs. control.
Table 3. Alterations of Invasion- and Metastasis-Related Genes Identified Using Microarray of Genistein-Treated HCC1395 Breast Cancer Cells

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>Accession no.</th>
<th>Fold change(^{\text{a}}) microarray</th>
<th>Fold change(^{\text{a}}) qRT-PCR</th>
<th>Roles in invasion and metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Downregulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-2</td>
<td>Matrix metalloproteinase 2 (gelatinase A)</td>
<td>NM_004530</td>
<td>−1.10</td>
<td>−0.86</td>
<td>Promote invasion and metastasis</td>
</tr>
<tr>
<td>MMP-7</td>
<td>Matrix metalloproteinase 7 (matrilysin)</td>
<td>NM_002423</td>
<td>−2.06</td>
<td>−2.24</td>
<td>Promote invasion and metastasis</td>
</tr>
<tr>
<td>CXCL12</td>
<td>Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)</td>
<td>NM_199168</td>
<td>−2.08</td>
<td>−1.94</td>
<td>Promote invasion and metastasis</td>
</tr>
<tr>
<td></td>
<td>Upregulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TFPI-2</td>
<td>Tissue factor pathway inhibitor 2</td>
<td>NM_006528</td>
<td>2.27</td>
<td>1.99</td>
<td>Inhibit invasion and metastasis</td>
</tr>
<tr>
<td>ATF3</td>
<td>Activating transcription factor 3</td>
<td>NM_004024</td>
<td>1.51</td>
<td>2.27</td>
<td>Inhibit invasion and metastasis</td>
</tr>
<tr>
<td>DNMT1</td>
<td>DNA (cytosine-5-)-(methyltransferase 1</td>
<td>NM_001379</td>
<td>1.32</td>
<td>1.48</td>
<td>Inhibit invasion and metastasis</td>
</tr>
<tr>
<td>MTCBP-1</td>
<td>Membrane-type I matrix metalloproteinase cytoplasmic tail-binding protein 1</td>
<td>NM_018269</td>
<td>1.67</td>
<td>2.36</td>
<td>Inhibit invasion and metastasis</td>
</tr>
</tbody>
</table>

\(^{a}\)Abbreviation is as follows: qRT-PCR, quantitative real-time reverse transcription polymerase chain reaction. *Fold changes are shown as log\(_2\) values.

Analysis revealed that ATF3 expression altered the expression of several genes related to invasion including maspin, plasminogen activator inhibitor-1 (PAI-1), and metastasis-associated protein-1 (MTA-1). Maspin, a unique serine proteinase inhibitor, inhibits tumor cell invasion and metastasis in vitro and in vivo (37). PAI-1 inhibits metastasis and invasion by blocking angiogenesis in tumor cells (38). ATF3 overexpression repressed MTA-1, which increases tumor cell migration and invasion in vitro and in vivo (39). Therefore, ATF3 is a novel mechanism through which genistein exerts its anti-invasive and antimetastatic activity. Urokinase-type plasminogen activator (uPA), a member of the serine protease family, breaks down various components of the ECM to promote invasion and metastasis in several malignancies including breast cancer (40). Upregulating DNMT1 leads to the methylation and silencing of uPA and the inhibition of the invasiveness of breast cancer cells (41).

Figure 4. Schematic representation of the potential mechanism of genistein regulating the invasion and metastasis-related genes. Genistein downregulated the expression of the matrix metalloproteinases-2 (MMP-2), MMP-7, and chemokine (C-X-C motif) ligand 12 (CXCL12) genes, which promoted tumor invasion and metastasis. Genistein upregulated the expression of the tissue factor pathway inhibitor-2 (TFPI-2), activating transcription factor 3 (ATF3), DNA methyltransferase 1 (DNMT1), and membrane-type 1 matrix metalloproteinase cytoplasmic tail-binding protein-1 (MTCBP-1) genes, which inhibited tumor invasion and metastasis. CXCR4, CXC receptor 4.
MTCBP-1, a 19-kDa protein, binds the cytoplasmic tail of membrane-type 1 matrix metalloproteinase (MT1-MMP/MMP-14) (42). Forced expression of MTCBP-1 inhibits tumor cell migration and invasion induced by MT1-MMP.

Our current findings provide potential molecular mechanisms (Fig. 4) by which genistein at high concentrations inhibits the invasion and metastasis of HCC1937 primary breast cancer cells. Other breast cancer cell lines—viz. MCF-7, T47D, and MDA-MB-231—were derived from metastatic tumors in pleural effusion (data from the American Type Culture Collection, Manassas, VA) and are suitable for investigating chemotherapeutic effects rather than the chemoprevention of metastatic disease. This is the first study to show that genistein alters genes critically involved in invasion and metastasis in a breast cancer cell line initiated from an early-stage primary tumor. Our findings may open up exciting avenues for devising therapeutic strategies and provide a basis for Preventing metastasis in early-stage breast cancer. However, our observations are based only on one cell line. More research on other breast cell lines from early-stage primary tumors is needed to verify the role of genistein as a promising agent for the chemoprevention of metastasis in early-stage breast cancer.

Also of great concern is translating the effective in vitro dosages of genistein at high concentrations of genistein to physiologically achievable serum levels in vivo. Although the plasma level of genistein is up to 1–5 μM even in humans on a soy-rich diet (43), the bioavailability of genistein in vivo may be greater than it is in vitro (44). Therefore, genistein may also inhibit the invasion and metastasis of cancer cells in vivo. However, anticancer-specific concentrations of genistein in humans are still not known. Additional investigation into the dose-response effect of genistein in humans is needed.

Acknowledgments and Notes

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