Loss of B-Cell Translocation Gene-2 in Estrogen Receptor–Positive Breast Carcinoma Is Associated with Tumor Grade and Overexpression of Cyclin D1 Protein

Hirofumi Kawakubo,1 Elena Brachtel,2 Tetsu Hayashida,2 Giminna Yeo,2 Joshua Kish,1 Alona Muzikansky,2 Paul D. Walden,3 and Shyamala Maheswaran2
1Department of Pathology and 2Surgical Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts and 3Department of Urology, NYU Medical Center, New York, New York

Abstract
The B-cell translocation gene-2 (BTG2) is present in the nuclei of epithelial cells in many tissues, including the mammary gland where its expression is regulated during glandular proliferation and differentiation in pregnancy. In immortalized mammary epithelial cells and breast cancer cells, BTG2 protein localized predominantly to the nucleus and cytoplasm, respectively. The highly conserved domains (BTG boxes A, B, and C) were required for regulating localization, suppression of cyclin D1 and growth inhibitory function of BTG2. Expression analysis of BTG2 protein in human breast carcinomas showed a significant correlation with cyclin D1 protein overexpression, suggesting that loss of BTG2 may be a factor involved in deregulating cyclin D1 expression in human breast cancer. (Cancer Res 2006; 66(14): 7075-82)

Introduction
The B-cell translocation gene-2 (BTG2) belongs to a class of proteins known as the Tob and BTG antiproliferative protein family, which is defined by the presence of two highly conserved domains known as BTG boxes A and B separated by a spacer sequence of 20 to 25 nonconserved amino acids. A short stretch of amino acids designated box C is present only in BTG1 and BTG2 and is located between positions 118 and 128 (1–5). BTG2 can be induced by p53 and is a key effector of p53-dependent growth arrest of mouse and human fibroblasts transduced with oncogenic ras (6, 7). BTG2 expression is also regulated by activators of nuclear factor-κB (NFκB)–positive growth factor–α (EGF)–positive breast tumors correlated significantly with increased histologic grade and tumor size. Consistent with its ability to suppress cyclin D1 transcription, loss of nuclear BTG2 expression in ER-positive breast carcinomas showed a significant correlation with cyclin D1 protein overexpression, suggesting that loss of BTG2 may be a factor involved in deregulating cyclin D1 expression in human breast cancer.

Note: H. Kawakubo and E. Brachtel contributed equally to this work.
Requests for reprints: Shyamala Maheswaran, Surgical Oncology, Massachusetts General Hospital, Jackson 904, 55 Fruit Street, Boston, MA 02114. Phone: 617-724-6552; Fax: 617-726-9623; E-mail: maheswaran@helix.mgh.harvard.edu.

DOI: 10.1158/0008-5472.CAN-06-0379
grade, tumor size, and overexpression of cyclin D1, suggesting that loss of BTG2 may be a factor involved in deregulating cyclin D1 expression in human breast cancer.

Materials and Methods

Cell lines. MCF7 cells were grown in DMEM supplemented with 10% female fetal bovine serum, glutamine, and penicillin/streptomycin. MCF10A (American Type Culture Collection, Rockville, MD) were grown in mammary epithelial growth medium (Clonetics, San Diego, CA) supplemented with 100 ng/mL cholera toxin (Calbiochem, San Diego, CA).

Generation of BTG2 mutants. BTG2 constructs lacking boxes A, B, and C were generated by PCR amplification of fragments flanking the NH2 terminus and COOH terminus of the deletions such that ligation of the two fragments would fuse in order to generate proteins lacking the intended amino acid sequences with no other change in sequence.

The primers used to generate the NH2-terminal fragment flanking box A were forward, CCCCA(GAATTC)CGCGACATGAGCCACGGGAAG and reverse, CCCCA(AGTACT)GAAGACCTTAAGCCTCTGCTC. The sequences within parentheses in the forward and reverse primers represent EcoRI and ScaI restriction sites, respectively. The primers used to generate the COOH-terminal fragment flanking box A were forward, CCCCA(AATATT)CGCATACCAAGAAGGGAC and reverse, CCCCA(AGTACT)GAAGACCTTAAGCCTCTGCTC. The sequences within parentheses in the forward and reverse primers represent SfI and SalI restriction sites, respectively. The other primers used to generate BTG2 boxes B and C are described below.

The reverse primer used to generate the NH2-terminal fragment flanking box B was CCCCA(AGTACT)GGGCCAGCATGGTTGCAGCTG. The sequence within parenthesis represents a ScaI restriction site. The forward primer used to generate the COOH-terminal fragment flanking box B was CCCCA(AATATT)GGGCCAGCATGGTTGCAGCTG. The sequence within parenthesis represents a SfI restriction site.

The reverse primer used to generate the NH2-terminal fragment flanking box C was CCCCA(GAATTC)CTCCCAATGGGCGTAGGAC. The sequence within parenthesis represents an EcoRV restriction site. The forward primer used to generate the COOH-terminal fragment flanking box C was CCCCA(GAATTC)CTCCCAATGGGCGTAGGAC. The sequence within parenthesis represents a SfI restriction site.

The amplified fragments were gel purified and ligated in frame into a cytomegalovirus (CMV) expression vector containing a hemagglutinin (HA) tag. The constructs were sequenced to ensure that they did not contain additional deletions or mutations.

Antibodies and Western blot analysis. MCF7 cells were transfected with wild-type or mutant BTG2 using Fugene-6. Proteins were extracted with radioimmunoprecipitation assay buffer and analyzed by Western blot as described (20). The E-cadherin, mouse monoclonal anti-HA, and anti-human cyclin D1 antibodies were from Zymed (South San Francisco, CA), Covance (Berkeley, CA), and Novocastra (Newcastle, United Kingdom), respectively. The mouse anti-c-myc and the rabbit anti-tubulin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The mouse anti-c-myc and the rabbit anti-tubulin antibodies were from Covance (Berkeley, CA), and Novocastra (Newcastle, United Kingdom), respectively. The mouse anti-c-myc and the rabbit anti-tubulin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The mouse anti-c-myc and the rabbit anti-tubulin antibodies were purchased from Covance (Berkeley, CA), and Novocastra (Newcastle, United Kingdom), respectively. The mouse anti-c-myc and the rabbit anti-tubulin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The mouse anti-c-myc and the rabbit anti-tubulin antibodies were purchased from Covance (Berkeley, CA), and Novocastra (Newcastle, United Kingdom), respectively. The mouse anti-c-myc and the rabbit anti-tubulin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The mouse anti-c-myc and the rabbit anti-tubulin antibodies were purchased from Covance (Berkeley, CA), and Novocastra (Newcastle, United Kingdom), respectively. The mouse anti-c-myc and the rabbit anti-tubulin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Antibodies and Western blot analysis. MCF7 cells were transfected with wild-type or mutant BTG2 using Fugene-6. Proteins were extracted with radioimmunoprecipitation assay buffer and analyzed by Western blot as described (20). The E-cadherin, mouse monoclonal anti-HA, and anti-human cyclin D1 antibodies were from Zymed (South San Francisco, CA), Covance (Berkeley, CA), and Novocastra (Newcastle, United Kingdom), respectively. The mouse anti-c-myc and the rabbit anti-tubulin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The mouse anti-c-myc and the rabbit anti-tubulin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The mouse anti-c-myc and the rabbit anti-tubulin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The mouse anti-c-myc and the rabbit anti-tubulin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

To analyze nuclear localization, wild-type and mutant BTG2 expression constructs were transfected into MCF7 cells. After 48 hours, cells were plated on coverslips for 24 hours before transfection, and the pcMV vector and wild-type or mutant BTG2 expression constructs were transfected using Fugene-6 or Cellfectin. After 48 hours, cells were fixed with 4% paraformaldehyde in PBS and permeabilized with 0.1% Triton X-100 in PBS for 3 minutes. The permeabilized cells were treated with 3% bovine serum albumin (fraction V from Fisher Scientific, Fairlawn, NJ) in PBS for 30 minutes and incubated with mouse anti-HA antibodies at a dilution of 1:1,000 for 1 hour at room temperature. After extensively washing with PBS, cells were incubated with FITC-labeled goat anti-mouse IgG (Jackson Immunoresearch Lab, Inc., West Grove, PA) followed by staining with 4,6-diamidino-2-phenyindole (Molecular Probes, Eugene, OR).

Growth inhibition assays. To determine the ability of wild-type and mutant BTG2 to inhibit cellular proliferation, MCF7 cells were transfected with empty pcDNA vector and wild-type or mutant BTG2 expression constructs along with a hygromycin resistance plasmid. Cells were grown in hygromycin-containing medium for 2 to 3 weeks and stained with crystal violet, and drug-resistant colonies were counted.

Patients and tissue samples. One hundred forty-eight cases of human breast carcinoma were selected from the files of the Massachusetts General Hospital (MGH) Pathology Department according to protocols approved by the Human Research Committee at MGH. The samples consisted of formalin-fixed, paraffin-embedded tissue from 135 invasive ductal carcinomas and 13 invasive lobular or mixed ductal-lobular carcinomas from 110 excisions and 38 mastectomies. Tissues were obtained after all diagnostic tests were completed and included tumor and uninvolved breast parenchyma. Histologic diagnosis and grading of tumors were made in accordance with the WHO classification criteria (21). Tumor size, lymph node involvement, and distant metastases were recorded following the American Joint Committee on Cancer staging system (22).

Case-matched H&E stains were reviewed for each patient. Clinical history and follow-up relevant to mammary pathology were obtained by review of the clinical notes. Patient age ranged from 31 to 94 years with a median of 57 years. The status of hormone receptor proteins (ERα and progesterone receptor) was determined by immunohistochemistry. Tumor size ranged from 0.5 to 10 cm with a median of 2.3 cm. There were 79 patients who had no lymph node metastasis (N0); 37 showed metastatic carcinoma in one to three ipsilateral lymph nodes (N1); 10 patients showed metastatic carcinoma in four to nine lymph nodes (N2); 6 patients showed metastatic carcinoma in ≥10 lymph nodes (N3); and nodal status was unknown (N0) in 16 patients. There was no metastatic disease at presentation in 118 patients (M0); 11 patients showed metastatic disease, and metastatic status was unknown for 19 patients (M3). Follow-up was available for up to 93 months with a median of 53 months. On follow-up, five patients had died (three of metastatic disease and two of other causes); 24 were alive with metastatic disease; 2 were alive with metastatic other tumors; and 111 were alive with no evident disease.

Immunohistochemical analyses. Immunohistochemistry was done on formalin-fixed, paraffin-embedded 5-μm tissue sections according to standard procedures as described (8). Briefly, deparaffinized tissue sections were treated with 3% hydrogen peroxide to inhibit endogenous peroxidase. After antigen retrieval, sections were incubated with 2.5% normal goat serum to block nonspecific protein binding. For BTG2 detection, sections were incubated with rabbit antibodies against human BTG2 at a final antibody concentration of 1 μg/mL diluted in blocking solution serum. For cyclin D1 staining, the antibody was used at a dilution of 5 μL/mL. After incubation with biotinylated link antibody and peroxidase-labeled streptavidin, staining was developed by reaction with diamobenzidine or Nova Red. Detection chemistries were purchased from Vector Laboratories (Burlingame, CA) and used according to manufacturer’s instructions. The sections were lightly counterstained with hematoxylin and mounted.

The stains for ERα protein had been done during the initial pathologic evaluation for diagnostic purposes. For quantification of immunohistochemistry, slides were evaluated semiquantitatively for staining intensity and extent as described in ref. (23), and the predominant staining intensity of BTG2 and cyclin D1 was compared with adjacent uninvolved breast tissue on the same slide. Membranous Her2/c-erbB protein expression was assessed during the initial pathologic evaluation for diagnostic purposes as previously described (24).

Statistical analysis. χ2 test was done to assess the significance of the association between BTG2 expression and other tumor characteristics.
(grade, tumor size of <2 or >2 cm, Her2 and cyclin D1 expression, nodal status, and metastasis). The analysis was repeated in the subgroups classified by ERα status. \( P_s < 0.05 \) were considered statistically significant.

**Results**

Conserved domains of BTG2 regulate nuclear localization, repression of cyclin D1, and growth inhibitory function. We had previously shown that BTG2 nuclear localization is impaired in breast carcinoma (8). BTG2 mutants lacking the conserved boxes A, B, and C were generated to characterize their role in directing nuclear localization and function of the protein. A schematic diagram representing the three mutants, which lack amino acids 41 to 70 (BTG2 box A), amino acids 100 to 115 (BTG2 box B), and 118 to 129 (BTG2 Box C), is shown (Fig. 1A). The constructs were transfected into MCF7 cells, and protein expression was analyzed by Western blot using an anti-HA antibody (Fig. 1B). The synthesis of two proteins occurs due to the presence of an internal translation initiation site located nine codons downstream of the initiator codon. Both ATG codons are flanked by an identical 5' sequence (CGACATG). The expression of wild-type BTG2, BTG2

---

**Figure 1.** Conserved domains of BTG2 regulate nuclear localization. A, schematic representation of BTG2 deletion mutants. B, the constructs shown above were transfected into MCF7 cells. Protein expression was analyzed by Western blot using the anti-HA antibody. The blot was probed with an anti-myc antibody to control for loading. C, top, MCF10A cells were transfected with 2 \( \mu \)g of wild-type BTG2, BTG2 box A, BTG2 box B, and BTG2 box C expression constructs. BTG2 protein expression in cells was analyzed by indirect immunofluorescence using an anti-HA antibody. Three fields, each containing a single cell expressing the wild-type or mutant BTG2 protein (top three). D, 4,6-Diamidino-2-phenylindole (DAPI) staining of nuclei and super imposition of the images in a single field (bottom). Bottom, MCF7 cells were transfected with 2 \( \mu \)g of wild-type BTG2, BTG2 box A, BTG2 box B, and BTG2 box C expression plasmids. BTG2 protein expression in cells was analyzed by indirect immunofluorescence using an anti-HA antibody. Higher magnification of insets, propidium iodide (PI) staining of nuclei, and super imposition of the two images (bottom three). D, MCF7 cells were transfected with wild-type and mutant BTG2 expression constructs. After 48 hours, nuclear and cytoplasmic protein fractions were analyzed by Western blot using the anti-HA antibody. The purity of the fractions was examined by probing the blots with anti-c-myc and anti-tubulin antibodies. C, cytoplasmic fraction; N, nuclear fraction.
Loss of nuclear BTG2 expression correlates with increasing tumor grade. Characterization of BTG2 expression in breast carcinoma samples showed that BTG2 protein was present in the nuclei of uninvolved glands and absent in the tumor in 15 of 23 cases examined (8). To characterize whether aberrant BTG2 expression in breast tumors correlated with tumor characteristics, we examined 148 breast carcinomas for which clinicopathologic data and follow-up were available (Table 1).

In agreement with previously reported results (8), BTG2 protein localized to the nuclei of epithelial cells lining the normal ducts and lobules but was absent in 46% of the tumors. BTG2 immunostaining of tumor and adjacent normal tissue and the corresponding H&E stains (Fig. 3A) of a representative case of grade 3 breast carcinoma are shown. Although 44% of ERα-positive breast tumors and 50% of ERα-negative breast tumors showed loss of nuclear BTG2 expression, loss of BTG2 did not correlate significantly with ERα status of the tumor. The percentage of tumors showing loss of BTG2 protein significantly increased with the tumor grade: 19% of grade 1, 45% of grade 2, and 54% of grade 3 tumors showed the loss of nuclear BTG2 protein (P = 0.033). In ERα-positive patients, the correlation between loss of nuclear BTG2 expression and the histologic grade of the tumor was more pronounced; loss of expression was observed in 19% of grade 1, 39% of grade 2, and 69% of grade 3 tumors (P = 0.0033). Moreover, decreased BTG2 expression in tumors associated with increasing tumor size (P < 0.020). No correlation existed between the absence of nuclear BTG2 expression and tumor grade in ERα-negative tumors.

Loss of nuclear BTG2 expression in breast carcinoma did not show a statistically significant correlation with nodal status, metastasis to other organs, or survival (P > 0.05). However, the association between loss of BTG2 and overexpression of Her2 in ERα-positive tumors almost approached statistical significance (P = 0.051). The loss of nuclear BTG2 expression in a tumor overexpressing Her2, nuclear BTG2 expression in adjacent normal glands, and H&E stains of the tumor and uninvolved tissue are shown (Fig. 3B).

Loss of BTG2 expression in ER-positive breast tumors correlates with increase in cyclin D1 protein. Because BTG2 suppresses cyclin D1 expression (12), and because its growth inhibitory function seems to be associated with its ability to suppress cyclin D1 (Fig. 2), we analyzed whether the loss of nuclear BTG2 expression in breast tumors would be related to overexpression of cyclin D1. Cyclin D1 immunostaining in breast tumors was nuclear with faint cytoplasmic staining. Staining

Figure 2. Conserved domains of BTG2 are required for suppression of cyclin D1 and growth. A, MCF7 cells grown in six-well plates were transfected with 2 μg of wild-type or mutant BTG2 expression constructs along with 0.2 μg of hygromycin resistance plasmid. Cells were grown in medium containing 100 μg/mL of hygromycin, and colonies were stained with crystal violet. Number of colonies on each plate was counted (n = 3). Representative culture plate for each sample (bottom). B, wild-type and mutant BTG2 constructs shown were transfected into MCF7 cells. Protein expression was analyzed by Western blot using anti-HA and cyclin D1 antibodies. The blot was probed with an E-cadherin antibody to control for loading.
usually was homogenous, and specimens were considered as overexpressing cyclin D1 if they showed moderate to strong nuclear staining compared with that observed in the adjacent matched normal glands. Cyclin D1 protein was overexpressed in 46% of breast tumors compared with that seen in uninvolved matched normal breast tissue. A representative case in which cyclin D1 is overexpressed in the tumor compared with adjacent normal glands along with H&E stains (Fig. 4A) is shown. As reported previously (25–29), overexpression of cyclin D1 was associated with hormone receptor positivity; 48% of ER-negative tumors and 26% of ER-positive tumors overexpressed of cyclin D1.

Characterization of BTG2 and cyclin D1 protein expression in breast carcinoma showed an inverse correlation (Fig. 4A and B). In a tumor overexpressing cyclin D1, nuclear expression of BTG2 was not detected, whereas in the matched normal glands, BTG2 expression was nuclear, and cyclin D1 protein expression was low (Fig. 4A). In another tumor where BTG2 expression was nuclear, cyclin D1 expression was almost undetectable, whereas the normal glands showed nuclear BTG2 expression (Fig. 4B).

Cyclin D1 was overexpressed in 39% of tumors in which BTG2 expression was detected and in 53% of tumors in which nuclear BTG2 expression was absent (P = 0.097). However, in ER-positive tumors, a statistically significant inverse correlation was observed by between overexpression of cyclin D1 and loss of nuclear BTG2 expression; 68% of samples, which had no nuclear BTG2 protein, overexpressed cyclin D1, whereas only 43% of those that retained BTG2 expression overexpressed cyclin D1 (P = 0.018). No significant correlation between loss of BTG2 and increased cyclin D1 expression was observed in ER-negative breast cancer.

### Discussion

The expression patterns of BTG2 in the normal breast and breast cancer suggest that BTG2 may be a regulator of growth and differentiation in the mammary gland. Immunolocalization studies revealed that although BTG2 protein partitions between the nucleus and cytoplasm in cells, nuclear expression predominates in immortalized human mammary epithelial cells, whereas cytoplasmic expression is more prominent in breast cancer cells, suggesting that breast cancers may be expressing components that facilitate cytoplasmic retention and/or inhibit nuclear localization of BTG2 protein. Deletion of the conserved domains (boxes A, B, and C) of BTG2, which mediate the interaction of BTG2 with other cellular proteins (3, 5, 30), decreased nuclear localization and increased cytoplasmic retention.

The eukaryotic linear motif resource for predicting functional sites in proteins and “prediction and analysis of nuclear localization signals” did not identify any sequence within BTG2 that shared homology with previously described nuclear localization signals. However, Rodier et al., in a study to identify domains involved in cellular trafficking of BTG1, a BTG family member highly homologous to BTG2, reported several BTG1 domains to be involved in nuclear localization. The highly conserved B box of BTG1 induced significant nuclear localization of β-galactosidase; it was enhanced by the presence of the COOH-terminal moiety. Moreover, the NH2-terminal LxxLL motif of BTG1, which mediates interaction with members of the nuclear receptor superfamily, also favored nuclear localization (31). The domains of BTG2 required for directing nuclear localization are consistent with these observations; the conserved boxes B and C of BTG1 and

### Table 1. BTG2 and cyclin D1 expression in relation to clinicopathologic variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total cases, no. cases (%)</th>
<th>BTG2 T &lt; U, no. cases (%)</th>
<th>BTG2 T = U, no. cases (%)</th>
<th>Cyclin D1 T &gt; U, no. cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age, y (n = 148)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥50</td>
<td>96 (65)</td>
<td>40 (63)</td>
<td>44 (59)</td>
<td>37 (62)</td>
</tr>
<tr>
<td>&lt;50</td>
<td>52 (35)</td>
<td>24 (37)</td>
<td>31 (41)</td>
<td>23 (38)</td>
</tr>
<tr>
<td>ER (n = 146)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER positive</td>
<td>99 (68)</td>
<td>40 (63)</td>
<td>51 (69)</td>
<td>48 (80)</td>
</tr>
<tr>
<td>ER negative</td>
<td>47 (32)</td>
<td>23 (37)</td>
<td>23 (31)</td>
<td>12 (20)</td>
</tr>
<tr>
<td>Her2 staining (n = 77)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strong overexpression</td>
<td>9 (12)</td>
<td>6 (17)</td>
<td>3 (8)</td>
<td>5 (16)</td>
</tr>
<tr>
<td>Histologic grade (n = 148)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>18 (12)</td>
<td>3 (5)</td>
<td>13 (18)</td>
<td>4 (7)</td>
</tr>
<tr>
<td>G2</td>
<td>59 (40)</td>
<td>25 (39)</td>
<td>31 (41)</td>
<td>27 (45)</td>
</tr>
<tr>
<td>G3</td>
<td>71 (48)</td>
<td>36 (56)</td>
<td>31 (41)</td>
<td>29 (48)</td>
</tr>
<tr>
<td>Tumor size, cm (n = 145)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2 (T1)</td>
<td>84 (58)</td>
<td>34 (53)</td>
<td>47 (64)</td>
<td>37 (62)</td>
</tr>
<tr>
<td>&gt;2 (T2, T3, T4)</td>
<td>61 (42)</td>
<td>30 (47)</td>
<td>26 (36)</td>
<td>23 (38)</td>
</tr>
<tr>
<td>Lymph nodes (n = 132)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0/N1</td>
<td>116 (88)</td>
<td>46 (82)</td>
<td>64 (93)</td>
<td>46 (88)</td>
</tr>
<tr>
<td>N2/N3</td>
<td>16 (12)</td>
<td>10 (18)</td>
<td>5 (7)</td>
<td>6 (12)</td>
</tr>
<tr>
<td>Follow-up findings (n = 142)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No disease</td>
<td>111 (78)</td>
<td>48 (79)</td>
<td>58 (79)</td>
<td>28 (76)</td>
</tr>
<tr>
<td>Alive with disease</td>
<td>24 (17)</td>
<td>12 (20)</td>
<td>10 (14)</td>
<td>8 (22)</td>
</tr>
<tr>
<td>Deceased of disease</td>
<td>3 (2)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Other conditions</td>
<td>4 (3)</td>
<td>0 (0)</td>
<td>4 (6)</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

Abbreviations: T, tumor; U, uninvolved glandular tissue.
BTG2 share 100% and 80% homology, respectively. Moreover, BTG2 also contains an L(SS)LL motif between positions 19 and 23. Although nuclear localization of BTG2 is impaired in 46% of breast tumors, and although BTG2 mRNA is very low or undetectable in almost all breast cancer cell lines tested (8), analysis of 12 breast cancer cell lines and 18 primary breast tumors revealed the lack of mutations or polymorphisms in BTG2 (7). Whether loss of important protein(s) that regulate BTG2 nuclear localization and altered posttranslational modification of BTG2 are involved in aberrant localization of BTG2 in breast cancer cells needs to be determined.

Figure 3. Nuclear expression of BTG2 is absent in breast carcinoma (images in original magnification, ×400). A, tumor cells lack nuclear BTG2 protein, whereas the adjacent normal glands strongly express BTG2 in the nucleus. B, tumor cells lack nuclear BTG2 and show strong membranous Her2/C-erbB staining. BTG2 expression is nuclear in matched normal glands negative for Her2/C-erbB staining.
Moreover, epigenetic mechanisms may also play a critical role in regulating the levels and localization of BTG2 in breast cancer cells. BTG2 is a potential target of cellular microRNAs (miRNAs), a class of 17- to 24-base single-stranded RNA molecules, which associate with a cellular complex that participates in RNA interference to regulate the stability and translational efficiency of the target mRNA (32–34). Of the several members of the miRNA family that are likely regulators of BTG2 expression in cells (32–34), miR-21 is suppressed in normal mammary epithelial cells but overexpressed in breast cancer cells (35). Whether overexpression of miRNAs that target posttranscriptional processing of BTG2 and/or protein-protein interaction with cellular partners leads to aberrant regulation of BTG2 expression in breast carcinoma remains to be elucidated.

Consistent with the reports that BTG2 suppresses cyclin D1 (12), and down-regulation of BTG2 in human cells being linked to up-regulation of cyclin D1 (6), loss of BTG2 expression in ERα-positive breast tumors correlated with overexpression of cyclin D1 protein. Estrogen- and progesterone-mediated suppression of BTG2 in breast cancer cells correlated with increased cyclin D1 levels (8) and could provide a possible explanation for the statistically significant association between loss of BTG2 and overexpression of cyclin D1 in ERα-positive breast tumors. Moreover, the spatial pattern of BTG2 and cyclin D1 expression (BTG2 is predominantly expressed in the mammary epithelium, and overexpression of cyclin D1 is observed in a majority of breast tumors of luminal epithelial origin) also suggests that the loss of BTG2 and gain of

Figure 4. BTG2 and cyclin D1 protein expression shows an inverse correlation in breast carcinoma (images in original magnification, ×400). A, a representative case of breast carcinoma shows loss of nuclear BTG2 expression, whereas strong nuclear BTG2 expression is seen in the adjacent normal glands. The tumor overexpresses the cyclin D1 protein with adjacent normal glands showing weak cyclin D1 staining. B, another representative case of breast carcinoma in which BTG2 expression is nuclear in the tumor cells, which are negative for cyclin D1. Nuclear BTG2 expression and scattered nuclear staining with the cyclin D1 antibody is observed in the adjacent, uninvolved glands.
cancer. In breast cancer cells, estrogen suppresses BTG2 proteins and cyclin D1. BTG2 is a positive regulator of cell cycle progression, whereas BTG2 induces a G1 arrest in cells. In breast cancer cells, estrogen suppresses BTG2 mRNA, and BTG2 in turn mitigates estrogen-induced transactivation of a reporter construct driven by an estrogen response element (8, 30). Cyclin D1 expression is induced by estrogen and cooperates in estrogen-mediated transcription (39). Whether coordinated inhibition of BTG2 and enhancement of cyclin D1 could constitute a mechanism to increase estrogen responsiveness and proliferation in breast cancer cells and thus serve as a molecular signature to identify patients who would develop resistance to endocrine therapy is an important question that needs to be addressed in the future.

Acknowledgments

Received 1/31/2006; revised 5/9/2006; accepted 5/17/2006.

Grant support: NIH grant CA84441 (P.D. Walden), Department of Defense grant PC003031 (P.D. Walden), and NIH/National Cancer Institute grant CA89138 (S. Maheshwaran).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore hereby be marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Drs. Emmett Schmidt, Toshi Shioda, and Dennis Sgroi for critically reading this article.

References


Received on May 29, 2017. © 2006 American Association for Cancer Research.
Loss of B-Cell Translocation Gene-2 in Estrogen Receptor–Positive Breast Carcinoma Is Associated with Tumor Grade and Overexpression of Cyclin D1 Protein

Hirofumi Kawakubo, Elena Brachtel, Tetsu Hayashida, et al.


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/66/14/7075

Cited articles  This article cites 40 articles, 17 of which you can access for free at:
http://cancerres.aacrjournals.org/content/66/14/7075.full.html#ref-list-1

Citing articles  This article has been cited by 8 HighWire-hosted articles. Access the articles at:
/content/66/14/7075.full.html#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.