Wilms’ Tumor 1 Suppressor Gene Mediates Antiestrogen Resistance via Down-Regulation of Estrogen Receptor-α Expression in Breast Cancer Cells

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Abstract
The antiestrogen tamoxifen has been used in the treatment of hormone-responsive breast cancer for over a decade. The loss of estrogen receptor (ER) expression is the most common mechanism for de novo antiestrogen resistance. Wilms’ tumor 1 suppressor gene (WT1) is a clinically useful marker that is associated with poor prognosis in breast cancer patients; its high level expression is frequently observed in cases of breast cancer that are estrogen and progesterone receptor negative. The lack of expression of these receptors is characteristic of tumor cells that are not responsive to hormonal manipulation. To determine whether there is a linkage between WT1 expression and antiestrogen resistance in breast cancer cells, we studied the effect of WT1 on tamoxifen responsiveness in ERα-positive MCF-7 cells. We found that overexpression of WT1 in MCF-7 markedly abrogated tamoxifen-induced cell apoptosis and 17β-estradiol (E2)–mediated cell proliferation. The expressions of ERα and its downstream target genes were significantly repressed following overexpression of WT1, whereas the down-regulation of WT1 by WT1 shRNA could enhance ERα expression and the sensitivity to tamoxifen treatment in ERα-negative MDA468 and HCC1954 cells that express high levels of WT1. Furthermore, we have confirmed that the WT1 protein can bind to endogenous WT1 consensus sites in the proximal promoter of ERα and thus inhibit the transcriptional activity of the ERα promoter in a WT1 site sequence–specific manner. Our study clearly implicates WT1 as a mediator of antiestrogen resistance in breast cancer through down-regulation of ERα expression and supports the development of WT1 inhibitors as a potential means of restoring antiestrogen responsiveness in breast cancer therapy.

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Introduction
The steroid hormone estrogen is a key regulator of growth and differentiation in normal mammary glands and plays a major role in the growth and behavior of breast cancer cells (1). The effects of 17β-estradiol (E2) are mediated through its binding to estrogen receptor (ER)-α and ERβ, which function as ligand-activated transcription factors for growth-promoting genes (2). The antiestrogen tamoxifen, a widely used drug in the treatment of receptor positive breast cancer, primarily acts by competing with estrogen for binding to ER, leading to either cell growth arrest or apoptotic cell death (3, 4). In contrast, ER-negative breast cancers are much less responsive to endocrine manipulation (5).

The Wilms’ tumor 1 suppressor gene (WT1) was initially identified as a tumor suppressor gene in a subset of pediatric Wilms’ tumors. Despite its original reputation as a tumor suppressor, a growing body of experimental evidences indicates that WT1 functions as an oncogene in leukemias and a variety of solid tumors including breast cancer (6). The expression of WT1 in breast cancer has both prognostic and biological effects. Several groups have found that high levels of WT1 expression in breast cancer are associated with a worse prognosis (7-9). This is possibly due to a proliferation and survival effect provided by WT1 because down-regulation of WT1 inhibits breast cancer growth (10).

WT1 is a modular transcription factor with an NH2-terminal glutamine and proline-rich domain involved in self-association, transcriptional repression, and transcriptional activation. The four Cys-Cys-His-His type zinc finger structure in the COOH terminus is involved in DNA and RNA binding, nuclear localization, and protein-protein interactions (11-13). Through alternative splicing, there are four predominant protein isoforms of WT1 that differ by the presence of a 17-amino-acid insert between the activation/ repression and zinc finger domains and a 3-amino-acid insert (KTS) that is found between the third and fourth zinc fingers
The different isoforms are referred to as A, B, C, and D, where A lacks both the 17-amino-acid and KTS inserts; B contains the 17-amino-acid insert but lacks KTS; C lacks the 17-amino-acid insert but contains KTS; and D contains both the 17-amino-acid and KTS inserts.

Previous publications suggest an inverse correlation between WT1 expression and ER status in breast cancer. Silberstein et al. (16) found that 22% of ER-positive tumors are WT1 positive whereas 60% of more advanced ER-negative tumors are WT1 positive. In the current publication, we have investigated whether there is any relationship between the level of ER and WT1 in breast cancer cells. Using cell lines, we have found that the overexpression of WT1 directly results in the down-regulation of ER expression and the induction of antiestrogen resistance in ER-positive cells.
Results

E₂-Induced Cell Proliferation and Tamoxifen-Mediated Apoptosis Are Abolished by Overexpression of WT1 Protein

To determine the effect of WT1 on cell viability, trypan blue exclusion was used to compare cells treated with E₂ or tamoxifen; results are presented as percent change in cells excluding trypan blue. As can be seen in Fig. 1A, the addition of E₂ to parental MCF-7 cells enhanced cell viability by ~1.75 times at 4 days, whereas viability was reduced by 50% in tamoxifen-treated cells at the same time point. In contrast, MCF-7 cells overexpressing WT1B or WT1D did not show any change in cell viability with the addition of either E₂ or tamoxifen. To confirm this, we further analyzed cell apoptosis by flow cytometry. The stably transfected MCF-7 cells were simultaneously stained with fluorescent dyes Annexin V and 7-amino-actinomycin D (7-AAD) that serve as indicators for apoptosis at early and later stages, respectively. As expected, 4 days of treatment with E₂ resulted in a decrease of the subpopulation undergoing apoptosis from 2.9% to 0.9% in control MCF-7 cells. In contrast, and in keeping with the trypan blue results, tamoxifen treatment increased the population of cells undergoing apoptosis to 15.1%. Significantly, both the E₂-enhanced growth and tamoxifen induction of apoptosis were completely abolished in WT1-overexpressing MCF-7 cells (Fig. 1B).

WT1 Down-Regulates Expressions of ERα and Its Downstream Target Genes

The above-noted change in responsiveness to E₂ and tamoxifen in an ER-positive cell line might be explained if the cells were no longer dependent on ER signaling for survival, possibly through the loss of ERα receptors. To test this, we measured the level of ERα protein in WT1-overexpressing MCF-7 cells. In keeping with our prediction, we found that concomitant with the increase in the level of WT1 protein, there was a sharp reduction in the amount of endogenous ERα protein (Fig. 2A). In agreement with this, cells overexpressing WT1B and WT1D had reduced levels of ERα RNA regardless of the growth conditions (Fig. 2B and C). We further sought confirmation of this observation by searching published microarray data of gene expression in breast cancer cell lines (17). As shown in Supplementary Fig. S1, higher level of WT1 expression was associated with lower levels of ERα expression in the majority of breast cancer cell lines.

Because ERα is a transcription factor, it is predicted that loss of ERα would result in decreased expression of its normal
targets in WT1-overexpressing cells. To test this, we assessed the levels of two ERα-regulated genes, progesterone receptor (PR) and PS2 (18-21). As can be seen in Fig. 2D and E, the basal level of PR is reduced in WT1-expressing cells. The addition of E2 resulted in increased expression of PR and PS2 in both parental and WT1-expressing MCF-7 cells. However, the degree of response was substantially reduced in WT1-overexpressing cells. This is consistent with the reduced levels of ERα in those cells.

WT1 Gene Silencing Enhances the Expression and Activity of ERα

If WT1 inhibits the expression of ERα, then it is predicted that reducing the level of WT1 should result in increased ERα expression. MDA468 and HCC1954 cells are ERα-negative breast cancer cell lines with high level of endogenous WT1 (Supplementary Fig. S1). To test if knockdown of WT1 expression would alter endogenous ERα abundance, we transfected transiently WT1 shRNAs into MDA468 and

**FIGURE 3.** Enhanced expressions of ERα and its target genes in MDA468 and HCC1954 cells transfected with WT1 shRNA. A, Western blots show the abundance of WT1 and ß-actin (middle) in the transiently transfected MDA468 and HCC1954 cells with control pSuper vector lacking insert (pS), control nonsilencing shRNA (pSCN), or WT1 shRNAs [pSWT1a (a) and pSWT1b (b)] for 1 d. B to D, MDA468 and HCC1954 cells were transfected with either control nonsilencing shRNA or WT1 shRNAs (pSWT1a and pSWT1b) for 1 d, and then treated with either ethanol or 10 nmol/L E2 for 1 d. Quantitative real-time PCRs were done with primers against hWT1 (B), ERα (C), and PS2 (D). The expressions in pSCN-transfected cells treated with ethanol are represented as 1.0. Columns, average of three experiments; bars, SD.
HCC1954 cells and examined the effect on ERα expression. As shown in Fig. 3A, both WT1 shRNAs markedly decreased the level of endogenous WT1 protein in these cell lines, whereas control nonsilencing shRNA showed no significant effect on the level of WT1 protein. The WT1 gene silencing by WT1 shRNAs was further confirmed by quantitative real-time PCR with primers specific for human WT1 (Fig. 3B). Coincident with the knockdown of WT1 RNA expression, ERα RNA expression was significantly up-regulated, a process irrespective of E2 treatment (Fig. 3C). Moreover, the expression of PS2, an ERα target gene, was obviously increased on E2 stimulation in WT1 knockdown cells (Fig. 3D). The above results further indicate that WT1 is indeed involved in down-regulating the expression and activity of ERα.

**WT1 Protein Is Specifically Bound to a WT1 Site in the Endogenous ERα Promoter**

To determine if the observed repression of ERα requires direct binding of WT1 to the ERα promoter, we conducted chromatin immunoprecipitation assays followed by PCR with DNA from MCF-7 cells or MCF-7 cells engineered to overexpress His-tagged WT1B or WT1D (Fig. 4A). The primers bracketed a potential WT1 binding site in the ERα promoter (nucleotides −254 to −116; ref. 22). MCF-7 cells have low but detectable levels of endogenous WT1 and express ERα, whereas the WT1-overexpressing cells have reduced ERα. Antibody against acetylated histone H4 was included as a positive control. In the control MCF-7 cell line, the PCR-amplifiable fragment was immunoprecipitated with antibody against WT1 but not with anti-His antibody. In cells expressing His-tagged WT1 constructs, the fragment was precipitated with both antibodies, indicating that exogenous as well as the endogenous WT1 proteins were recruited to the proximal ERα promoter. Conversely, when WT1 was knocked down in MDA468 cells, there was a marked reduction in the amount of ERα promoter that could be precipitated with the WT1 antibody (Fig. 4B). Taken together, our observations indicate that the WT1 protein specifically binds to DNA containing a WT1 site in the proximal ERα promoter, and that this is important in abrogating the transcriptional activation of the promoter in breast cancer cells.

**WT1 Regulates the Transcriptional Activity of the ERα Promoter**

In an effort to further elucidate the effect of WT1 on ERα transcriptional activity and to directly show sequence specificity requirement of the WT1 binding site, we used luciferase reporters driven by the ERα promoter that harbored a single WT1 binding site. As shown in Fig. 5, reporter expression was obviously enhanced when the WT binding site mutant was transfected into MCF-7 or MDA468 cells, as compared with the native form of the promoter. Cotransfection of WT1B or D with the wild-type promoter into MCF-7 cells further decreased wild-type reporter expression. In contrast, cotransfection of the wild-type promoter along with WT1 shRNA into MDA468 cells significantly increased the reporter expression as compared with control pSuper vector lacking insert. These effects were abolished with the reporter construct containing WT1 site mutant, thus further confirming the regulation of the ERα promoter by binding of WT1 to the ERα WT1 element in breast cancer cell lines.

**Knockdown of WT1 Increases Cell Sensitivity to Tamoxifen Treatment**

Because the above results show that down-regulation of ERα by WT1 results in antiestrogen resistance in breast cancer cells, we sought to see if sensitivity of cells to tamoxifen is restored following the down-regulation of WT1 in ERα-negative cells. To test this, we transfected MDA468 and HCC1954 cells with WT1 shRNA and treated the cells with E2 or tamoxifen for 4 days. As expected, the down-regulation of WT1 resulted in a reduction of cell viability (Fig. 6). Tamoxifen treatment further decreased viability by ~20%.
respectively, as indicated.

vector, control nonsilencing shRNA, WT1 shRNAa, or WT1 shRNAb), pcDNA3 vector, WT1B, or WT1D) as indicated. Luciferase activity was shown as fold induction as compared with luciferase vector control experiments.

In the above-mentioned study that a variant of MCF-7 cells was isolated by prolonged growth in the absence of estrogen. These MCF-7 C4 cells had markedly reduced levels of ERα protein, there was reduced expression of two bona fide ERα target genes, PR and PS2 (18-21). Conversely, the reduction of WT1 in the ERα-negative cell line MDA468 restored ERα expression. Using chromatin immunoprecipitation, we confirm the binding of WT1 to an upstream region of the ERα promoter that contains a WT1 binding site. Furthermore, we found that the effect of WT1 on ERα-promoter-luciferase constructs was dependent on an intact WT1 binding site. Together, these results are in keeping with the suggestion that the effect of WT1 is through direct binding to a canonical WT1 site in the ERα promoter DNA.

A potential interaction between WT1 and ERα has previously been reported in another setting in breast cancer cells. In those studies, it was shown that the ERα and WT1 proteins could physically interact in breast cancer cells and that this association reduced or blocked ERα-mediated induction of insulin-like growth factor I receptor (IGF-IR; ref. 36). These studies do not contradict the work presented in this article, but rather highlight the variability of WT1 function dependent on the cellular context within which it is expressed. It is interesting to note in the above-mentioned study that a variant of MCF-7 cells was isolated by prolonged growth in the absence of estrogen. These MCF-7 C4 cells had markedly reduced levels of ERα, reduced levels of IGF-IR, and increased levels of WT1. This phenotype that is ERα-negative, IGF-IR low/negative, and WT1 positive is similar to that found in some cases of metastatic breast cancer. We postulate that MCF-7 C4 cells mimic this form of metastatic breast cancer. Furthermore, we predict that the inhibition of WT1 in these cells by shRNA would lead to repression of ERα.

WT1 is a zinc finger transcription factor initially identified as the gene mutated in a minority of cases of Wilms’ tumor. Further research has shown that WT1 in its native form is highly expressed in a variety of tumors and is associated with an adverse prognosis (7, 9). In the present study, we have found that WT1 negatively regulates the expression of ERα. In the same cells, we have found that WT1 can enhance the

Clinical Association between WT1 and ERα in Primary Breast Cancer

The results presented above suggest that breast cancer samples having high level of WT1 expression should have low levels of ERα (i.e., ER-negative disease). To see if this is the case, we used Oncomine to assess the expression of WT1 in ER-positive and ER-negative cases of breast cancer. For this study, we assessed microarray data provided by Perou et al. (23) and van de Vijver et al. (24). In keeping with the prediction, there was a significant overexpression of WT1 in ERα-negative as compared with ERα-positive samples (P < 0.05).

Discussion

There are a number of key descriptors of breast cancer with biological and therapeutic relevance, one of the more important being the status of expression of ERα (25). Whereas ER-positive tumors tend to be localized and respond well to hormonal manipulation (26), ER-negative tumors are more aggressive, disseminate widely, and do not respond to hormone therapy (5). A number of mechanisms for the development of ERα negativity have been identified (27-32). This includes hypermethylation of the ERα promoter in ~ 60% of cases (33) and repression by transcription factors such as pRB2/p130 and LMO4 (34, 35).

In the present study, we provide evidence that WT1 can interfere with both the growth-enhancing effect of estrogen and the antiestrogen effect of tamoxifen in MCF-7 cells. Shown herein are the results with WT1B and WT1D isoforms; similar results were obtained with WT1A and WT1C (data not shown). This effect was found to be due to reduced levels of ERα in the WT1-overexpressing cells. In keeping with the reduction in ERα protein, there was reduced expression of two bona fide ERα target genes, PR and PS2 (18-21). Conversely, the reduction of WT1 in the ERα-negative cell line MDA468 restored ERα expression. Using chromatin immunoprecipitation, we confirm the binding of WT1 to an upstream region of the ERα promoter that contains a WT1 binding site. Furthermore, we found that the effect of WT1 on ERα-promoter-luciferase constructs was dependent on an intact WT1 binding site. Together, these results are in keeping with the suggestion that the effect of WT1 is through direct binding to a canonical WT1 site in the ERα promoter DNA.

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![Diagram](image_url)
expression of c-myc (37). It is not known what mechanisms are at play that allow the same protein to have such opposite roles in the same cell. However, the ability of WT1 to increase the expression of a gene associated with proliferation and to reduce expression of a gene associated with proliferation and to reduce expression of c-myc (37). It is not known what mechanisms are at play that allow the same protein to have such opposite roles in the same cell. However, the ability of WT1 to increase the expression of a gene associated with proliferation and to reduce expression of a gene associated with proliferation and to reduce expression of a gene whose loss is associated with resistance to hormonal manipulation helps in our understanding of how WT1 is associated with an inferior prognosis in patients with breast cancer. This observation argues for further research aimed at blocking WT1 activity in breast cancer.

Materials and Methods

Cell Culture

The human breast cancer lines MCF-7 (ATCC HTB22) and MDA 468 (ATCC HTB132) were routinely subcultured in MEM supplemented with 10% heat-inactivated fetal bovine serum and antibiotics (penicillin/streptomycin/fungizone) in a humidified atmosphere of 5% CO2 at 37°C. The third human breast cancer line, HCC1954, was cultured in RPMI 1640 containing 10% fetal bovine serum, 10 mmol/L HEPES, 1.5 g/L bicarbonate, 4.5 g/L glucose, 1 mmol/L pyruvate, and antibiotics. The breast cancer cells engineered through gene transfer to overexpress WT1 were cultured in selective medium containing 0.5 mg/mL G418. For the treatment of cells with E2 or tamoxifen, cells were seeded in triplicate onto 10-cm plates (2 × 105 per plate) and allowed to reach logarithmic growth phase in culture medium supplemented with 10% fetal bovine serum. At 30% confluence, cells were washed with PBS and cultured for 48 h in phenol-red−free medium supplemented with 10% dextran-charcoal−stripped serum (Hyclone). Cells were then treated with 10 nmol/L E2 (Sigma) or 1 μmol/L tamoxifen (Sigma) for the indicated times.

Plasmids

The expression plasmids for mouse WT1B and WT1D isoforms were constructed by cloning the corresponding coding region respectively into pcDNA3 or pcDNA3.1/His vectors (Invitrogen) under a cytomegalovirus promoter. To generate plasmid expressing WT1 shRNA, double-stranded oligonucleotides were cloned into the HindIII/BglII sites in pSuper vector (Oligoengine, Inc.). The sequences of WT1 shRNAs used are gatccccTCAGGGTTACAGCACGGTCttcaa-gagaGACCGTGCTGTAACCCCTGAtttttggaa and gatccccTGA-CATCCCAGCTTGAATGttcaagagaCATTCAAGCTGG-GATGTCAttttttgaa, respectively. The uppercase letters represent WT1 specific sequence and lowercase letters represent hairpin sequences. The control nonsilencing shRNA sequence (Qiagen) used is gatccccTTCTCCGAACGTGTCttcaagaGACCGTGCTGTAACCCCTGAtttttggaa and gatccccTGA-CATCCCAGCTTGAATGttcaagagaCATTCAAGCTGG-GATGTCAttttttgaa. The reporter ERPWT/Luc was generated by amplifying the human ERα promoter (from nucleotides −287 to +202 relative to the predominant transcription start site) with PCR amplification and cloning into the KpnI/BglII sites immediately upstream of luciferase in pGL2 enhancer vector (Promega; ref. 38). Mutagenesis in situ of the ERWT/Luc plasmid (ERPmut/ Luc) was done using Transformer Site-Directed Mutagenesis Kit (Clontech) in accordance with the manufacturer’s instructions. WT1 site CGCCCCCGC was changed to CGCAAGCTGG-GATGTCAttttttgaa. All constructs were verified with restriction enzyme digests and direct nucleotide sequencing.

Transfection and Luciferase Assays

The cells were transiently cotransfected using Lipofectamine (Invitrogen) into a 12-well plate with 0.5 μg of luciferase reporter (pGL2 vectors as control), 0.05 μg of β-galactosidase internal control vector, and 1 μg of plasmid expressing WT1 (pcDNA3 containing no insert as control) or plasmid expressing human WT1 shRNA (pSuper containing no insert as control) in each well according to the manufacturer’s protocol. After 48 h, cells were washed twice with PBS and harvested for the assay of luciferase and β-Gal activities according to standard methods (Promega). Luciferase activity was determined by normalizing each plate to β-galactosidase activity. Luciferase induction was calculated by dividing the luciferase activity from cells transfected with luciferase reporter by that from cells transfected with pGL2 control.

Quantitative Real-time PCR

The total RNA was extracted using RNeasy Mini Kits (Qiagen) and converted into cDNA with reverse transcriptase (Life Technologies, Inc.) at 42°C for 45 min. The PCR
reactions containing SYBR Green Master Mix (PE Applied Biosystems), 100 nmol/L primer, and template cDNA in a 25-μL final volume were done in an ABI PRISM 7799 sequence detector. The SYBR Green PCR program consisted of an initial denaturation at 95°C for 10 min, followed by 40 PCR cycles: 95°C for 15 s, 60°C for 60 s with the continual measurement of fluorescence. The primer sequences for gene amplification were as follows: hWT1, 5′-GAGAGCCAGGCGCTACAT-3′ (forward) and 5′-CATGGGATCCATCTAGTCTG-3′ (reverse); mWT1, 5′-TCAAGGACTGCGAGAGG-3′ (forward) and 5′-TGTGGTGTTGCTTTCAAGATGG-3′ (reverse); ERα, 5′-GAC-AGGGAGCTGTTCACATG-3′ (forward) and 5′-ACGAGACCAATCTCAGGATCTC-3′ (reverse); PR, 5′-GAGCCCACA-ATACAGCCTTG-3′ (forward) and 5′-TCCAAGTGCCTTCA-CAACTGAC-3′ (reverse); PS2, 5′-CATGGGAGAACAGGTGATCG-3′ (forward) and 5′-CGAACCGTGTGTCG-GAAAC-3′ (reverse); and GAPDH, 5′-GAACTGGAAGGAGAGAAGG-3′ (forward) and 5′-TCAAGGACTGCGAGAGG-3′ (reverse). The DNA specificity of the primers was shown by a lack of amplification in the samples of no-template control and no-amplification control. Quantitation of gene expression was done using the comparative Ct (threshold cycle) method (PE Applied Biosystems) and normalized to human GAPDH level. The expression levels were shown as fold change relative to untreated control in empty vector–transfected cells.

Chromatin Immunoprecipitation

Chromatin immunoprecipitations were done using the Chromatin Immunoprecipitation Assay Kit (Upstate Biotech) with a slight modification. Approximately 1 × 10⁵ cells were cross-linked with 1% formaldehyde at 37°C for 15 min. The cells were collected, resuspended in 200-μL SDS lysis buffer (1% SDS, 10 mmol/L EDTA, 50 mmol/L Tris-HCl, pH 8.1), and incubated on ice for 15 min. Chromatin was then sonicated on ice to an average length of ~200 bp using a Fisher Scientific Dismembranator 100 with 7- to 15-s pulses at a 4-W power output setting. The sheared chromatin was preclarified with salmon sperm DNA/protein A agarose for 30 min at 4°C and subjected to immunoprecipitation with 2 μg of preimmune serum, anti-WT1 (C-19; Santa Cruz Biotech), anti-His serum, anti-WT1 (C-19; Santa Cruz Biotech), or anti–acetyl-histone H4 antibody (Upstate Biotech) overnight at 4°C. Following this, the mixture was incubated with secondary antibody for an additional 1 h at 4°C and washed with elution buffer (50 mmol/L NaHCO₃ and 1% SDS). One immunoprecipitated chromatin complex was then washed with salmon sperm DNA/protein A agarose for 30 min at 4°C. Following reversal of cross-links by incubation with 0.3 mol/L NaCl for 4 h at 65°C and subsequent proteinase K digestion for 2 h at 45°C, the DNA was extracted with phenol/chloroform, precipitated with ethanol, and resuspended in 30 μL of water. PCR reactions containing 3 μL of the above DNA preparation, primers, and Platinum Taq (Invitrogen) in a 50-μL final volume were done with an initial denaturation at 95°C for 4 min, followed by 35 PCR cycles (95°C for 45 s, 55°C for 30 s, and 72°C for 1 min) and a final extension at 72°C for 10 min. The sequences of primers against endogenous human ERα promoter were 5′-TGGGCCACCTTTAGCA-GATC-3′ (forward) and 5′-CTCCAGGCACAACTGATTG-3′ (reverse). The DNA specificity of the primers was shown by the nucleotide sequence of single correctly sized band visualized by agarose gel electrophoresis.

Apoptotic Assay

The cells were stained with Annexin V-phycocerythrin and 7-AAD (BD PharMingen) according to the manufacturer’s recommendation. Appropriate gates based on individual controls, defining the Annexin V-phycocerythrin and 7-AAD stained cells, were used to determine the percentages of cell population at earlier stage (Annexin V positive, 7-AAD negative) and later stage (Annexin V positive, 7-AAD positive) of apoptosis.

Data Mining

The Oncomine database was queried for the expression of WT1 in breast cancer specimens using the differential expression tool (39).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

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