

Efficient downregulation of ErbB-2 induces TACC1 upregulation in breast cancer cell lines

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Abstract. The ErbB-2 gene, whose overexpression is observed in many types of tumors including breast cancer, plays an important role in carcinoma formation. Dysregulation of the human transforming acidic coiled-coil 1 (TACC1) and ErbB-2 genes is thought to be important in the development and progression of breast cancer. However, a putative interaction between ErbB-2 and TACC1 remains undetermined in breast cancer. After infecting BT474 cells with lentiviral-mediated ErbB2-specific shRNA, we detected the expression of ErbB-2 and TACC1 by real-time PCR and western blotting. ErbB-2 mRNA expression was decreased in the Lenti-ShERBB2 infected cells, and western blotting indicated a concordant reduction in ErbB-2 protein. TACC1 expression at the mRNA and protein levels was significantly upregulated by ErbB-2 silencing in BT474 cells. CCK-8 assay indicated that the inhibition of ErbB-2 expression increased the sensitivity of BT474 cells to docetaxel treatment. These findings provide proof and the foundation for the molecular and biological relationships of ErbB-2 and TACC1 in breast cancer.

Introduction

The human epidermal growth factor receptor 2 (HER-2) gene, also called ErbB-2, is one of the most confirmed and commonly amplified genes in breast cancer. Overexpression of ErbB-2 usually results in malignant transformation of cells and accounts for approximately 25% of all breast cancer cases (1). ErbB-2 is consistently associated with more aggressive tumor phenotypes, a greater likelihood of lymph node involvement, and increased resistance to chemotherapy and endocrine therapy (2-4). The overall survival rate and time of

relapse for ErbB-2-positive breast cancer patients are significantly shorter than for patients without ErbB-2 overexpression. Therefore, ErbB-2 is a logical target for breast cancer therapy, and inhibition of ErbB-2 expression leads to the apoptosis of tumor cells (2,5-7). A monoclonal humanized antibody against ErbB-2 (trastuzumab) has been successfully applied in the treatment of ErbB-2-positive breast cancer. ErbB-2 is a cell surface receptor tyrosine kinase (RTK) and becomes internalized upon ligand binding which may trigger a multitude of signaling pathways, such as MAPKs and PI3K. Consequently, ErbB-2 has been shown to trigger signal transduction leading to cell growth and differentiation. Tumors exhibiting ErbB-2 amplification/overexpression have been shown to demonstrate increased aggressiveness and metastatic potential and are associated with decreased overall patient survival (8,9). Therefore, agents targeted to ErbB-2 can be utilized for breast cancer treatment.

Members of the transforming acidic coiled-coil (TACC) family of proteins have all been implicated in human cancer (10,11). TACC1 is located on a chromosomal region that is amplified in 10-15% of human breast cancers (12), and is a gene cloned from the breast cancer amplicon 8p11 (11). The TACC1 mRNA is ubiquitously expressed and encodes a protein with an apparent molecular mass of 125 kDa; it is cytoplasmic and mainly perinuclear (11). A full-length cDNA of 7758 bp encoding human TACC1 was found in the databases (GenBank, AF049910). Recently, research on TACC1 found that TACC1 is involved in the regulation of interaction between centrosomes and microtubules (13). Overexpression of TACC1 in mouse fibroblasts results in cellular transformation and anchorage independent growth (11). Moreover, research revealed that TACC1 mRNA and/or protein expression is downregulated in various types of tumors, such as breast and lung cancers. Thus, it was speculated that downregulation of TACC1 may alter the control of mRNA homeostasis in polarized cells and may participate in oncogenic processes (14). Ovarian tumors (78.5%) lack appreciable expression of TACC1, confirming inferences made from published SAGE analysis that TACC1 can be upregulated or lost in cancer (15,16). Another investigator described that the interaction of the TACC1 protein with several protein partners makes it a good candidate to participate in microtubule-associated processes in normal and tumoral cells (17).

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Table I. Four coding regions corresponding to targeted human ErbB-2.

Oligo name	Oligo sequence
shERBB2-1	5'-TTGTCAGTATCCAGGCTTTGTA CTCTCGAGTACAAAGCCTGGATACTGACATTTTTTC-3' 5'-TCGAGAAAAATGTCAGTATCCAGGCTTTGTA CTCTCGAGTACAAAGCCTGGATACTGACAA-3'
shERBB2-2	5'-TGATCACAGGTTACCTATACATCTCGAGATGTATAGGTAACCTGTGATCTTTTTTC-3' 5'-TCGAGAAAAAGATCACAGGTTACCTATACATCTCGAGATGTATAGGTAACCTGTGATCA-3'
shERBB2-3	5'-TGAATATGTGAACCAGCCAGATCTCGAGATCTGGCTGGTTCACATATTCTTTTTTC-3' 5'-TCGAGAAAAAGAATATGTGAACCAGCCAGATCTCGAGATCTGGCTGGTTCACATATTCA-3'
shERBB2-4	5'-TGAAGTACACGATGCGGAGACTCTCGAGAGTCTCCGCATCGTGTACTTCTTTTTTC-3' 5'-TCGAGAAAAAGAAGTACACGATGCGGAGACTCTCGAGAGTCTCCGCATCGTGTACTTCA-3'
Negative sh-control	5'-TGCGCGCTTTGTAGGATTCGCTCGAGCGAATCCTACAAAGCGCGCTTTTTTC-3' 5'-TCGAGAAAAAGCGCGCTTTGTAGGATTCGCTCGAGCGAATCCTACAAAGCGCGCA-3'

Interference sequence specific for the ErbB-2 gene, namely pLL2G-shERBB2-1, pLL2G-shERBB2-2, pLL2G-shERBB2-3, pLL2G-shERBB2-4 and negative sh-control, respectively.

An opposing view raises the possibility that amplification of TACC1 promotes malignant growth, thereby making TACC1 an attractive candidate gene for promoting tumorigenicity in human breast cancers. Still *et al* (11) found that deregulation of TACC1 gene expression, directly, through amplification of the entire gene, or through disruption of transcriptional regulatory elements, contributes to the aggressive phenotype noted in breast tumors, and the amplification of TACC1 may contribute to cancer. TACC1 was found to be significantly overexpressed in samples from ER-positive breast cancer patients who relapsed after tamoxifen treatment when compared with samples from patients who did not. TACC1 was found to be upregulated and to act as an oncogene in breast cancer, and TACC1 was correlated with significantly shorter relapse-free survival (18). Another study identified TACC1, NOV and PTTG1 as new candidate genes associated with endocrine therapy resistance in breast cancer (19). TACC1 has also been associated with other genes, such as FGFR1, BRCA1, Aurora B, BRCA2, AZU-1 and WT1 (17,20,21). Accordingly, we hypothesized that TACC1 is highly expressed in some types of tumors, but downregulated in others. Wilson *et al* used cDNA microarray technology that allows for the simultaneous evaluation of expression, at the mRNA level, of thousands of genes. A limited number of additional genes were found to be upregulated or downregulated by ErbB-2. These included TACC1, which was found to be downregulated both in ErbB2-positive breast cancer cell lines and in ErbB2-overexpressing breast cancers (22). Therefore, in the present study, we investigated the relationship between TACC1 and ErbB-2.

In the present study, we constructed a lentiviral vector mediating RNAi targeting ErbB-2 (pLL2G-shERBB2). The efficiency of pLL2G-shERBB2 plasmids in interfering with ErbB-2 expression was confirmed by western blotting. We used lentiviral infection to knock down ErbB-2 in BT474 cells resulting in reduced expression. After downregulating the expression of ErbB-2, we detected the changes in TACC1 expression at the mRNA and protein levels by real-time PCR and western blotting, respectively. Our results demonstrated

changes in the sensitivity of the cells to chemotherapeutic drugs after silencing of the ErbB-2 gene.

Materials and methods

Cell lines and cell culture. BT474 [ERBB2(+), TACC1(-)] and MCF-7 [ERBB2(-), TACC1(+)] (22) cancer cells were obtained from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. The cells were maintained in RPMI-1640 (Gibco, Carlsbad, CA, USA) supplemented with 10% FBS (fetal bovine serum) (v/v) and penicillin (100 U/ml)/streptomycin (100 mg/ml) at 37°C in 5% CO₂, 95% air.

Construction and production of lentiviral vectors. We designed and cloned an shRNA template into a lentiviral vector. A self-inactivating lentiviral vector containing a GFP reporter and a U6 promoter upstream of the cloning restriction sites (*HpaI* and *XhoI*) was used. The introduction of oligonucleotides encoding shRNAs between these restriction sites enables the production of the shRNA *in vitro*. Four coding regions corresponding to targeted human ERBB2 starting at different positions were used. We constructed four shRNA-ERBB2 lentiviral vectors, namely pLL2G-shERBB2-1, pLL2G-shERBB2-2, pLL2G-shERBB2-3 and pLL2G-shERBB2-4, respectively (Table I). Briefly, oligonucleotides were annealed, digested and inserted between the *HpaI* and *XhoI* restriction sites of the plasmid vector. Some mutations were introduced in the sense sequence of the hairpin structure to facilitate sequence and avoid destruction by bacteria during amplification in the bacterial host. Correct insertions of shRNA cassettes were confirmed by restriction mapping and direct DNA sequencing. We constructed a vector, pLL2G-shERBB2 (Fig. 1), co-infected with recombinant lentiviral vectors into 293T cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). To detect the interference effects of the target, the expression of ErbB-2 mRNA and protein was determined using real-time PCR and western blotting, respectively. Recombinant and control lentiviral vectors

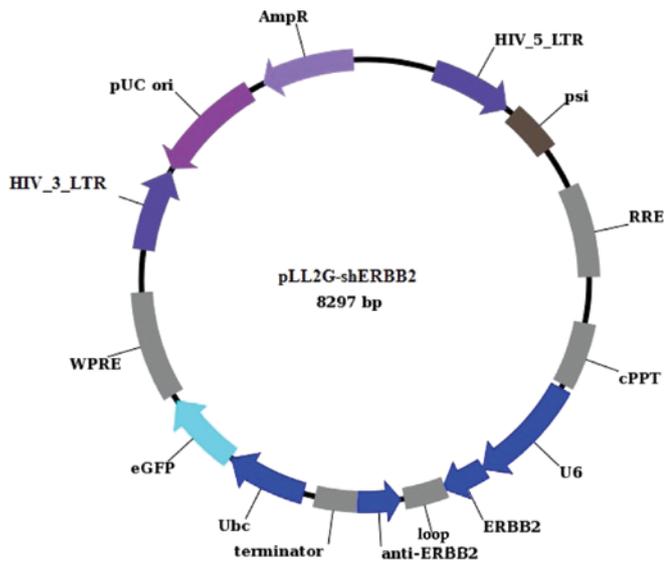


Figure 1. Construction of a lentiviral-mediated shRNA of ErbB-2. Lentiviral vector pLL2G-shERBB2 containing an eGFP reporter and a U6 promoter upstream of cloning restriction sites (*HpaI* and *XhoI*) to allow the introduction of oligonucleotides encoding shRNAs.

were produced by co-transfecting 293T cells with the lentiviral expression plasmid and packaging plasmids (pLV/helper-SL3, pLV/helper-SL4 and pLV/helper-SL5). Infectious lentiviral vectors were harvested at 48 h post-infection, centrifuged to remove cell debris, and filtered through 0.45- μ m cellulose acetate filters. The infectious titer was determined by hole-by-dilution titer assay. The virus titers produced were $\sim 4.9 \times 10^7$ TU/ml.

Lentiviral infection. Cells grown in 6-well culture plates (500,000 cells/well) were treated as required. When the cells were $\sim 80\%$ confluent in complete RPMI-1640 medium, they were infected with the lentiviral constructs. BT474 cells infected with lentiviral-mediated Lenti-shERBB2-eGFP were used as the study cells; cells infected with the negative shRNA (Lenti-sh-control) were used as the negative controls, and cells without infection were considered as the blank controls.

Quantification by real-time PCR. Total RNA was isolated using RNAiso (Takara, Japan). The concentration and purity of RNA were determined using a spectrophotometer. Reverse transcriptase was used to create cDNA for further analyses. Reverse transcriptase and quantitative real-time polymerase chain reaction (RT-PCR) assays were carried out using SYBR Premix Ex Taq II (Perfect Real-Time) (Takara) and real-time PCR amplification equipment. Reverse transcriptase and real-time PCR was carried out according to the instructions provided in the kit. The PCR primers (synthesized by Sangon Biotech, Shanghai, China) used to detect ErbB-2, TACC1 and GAPDH were as follows: ErbB-2, sense strand 5'-CATGTCA TCGTCTCCAGCAG-3', and antisense strand 5'-TTGACTC TGAATGTCGCCAA-3', with a product length of 161 bp; TACC1, sense strand 5'-TGGTCAGAAATCAGCTGGTGC-3', and antisense strand 5'-GCACAGTGGACCCGTCTTCTT-3', with a product length of 230 bp; GAPDH, sense strand 5'-CT GCACCACCAACTGCTTAG-3', and antisense strand 5'-TG

AAGTCAGAGGAGACCACC-3', with a product length of 135 bp. The real-time PCR procedure consisted of two steps: one cycle at 95°C for 10 sec, followed by 40 cycles at 95°C for 5 sec and 60°C for 20 sec. The expression of ErbB-2 was determined by normalization of the threshold cycle of these genes to that of the control housekeeping gene (GAPDH). Data were analyzed using the comparative $\Delta\Delta$ CT method.

Western blot analysis. Whole-cell proteins in various breast cancer cell lines were isolated. The lysates were centrifuged, and the supernatant was collected and stored at -80°C according to the manufacturer's instructions. Total protein (10 μ l) was loaded per well, separated by 10-15% SDS-PAGE, and transferred to polyvinylidene difluoride membranes at 60 V for 1 h at 4°C. The membranes were blocked and incubated with primary antibodies (Bioworld Co., USA; diluted 1:1,000 in TBS-A). The membranes were rinsed thrice with 0.1% Tween 20-PBS for 30 min. The secondary antibodies (Abcam Co., Cambridge, UK; diluted 1:1,200 in TBS-A) were used with Peroxidase-conjugated Affinipure goat anti-mouse IgG (1:8,000) and Peroxidase-conjugated Affinipure goat anti-rabbit IgG (1:8,000) for 1 h at room temperature. The blotted membranes were washed three times with 0.1% Tween 20-PBS for 15 min and three times with PBS for 15 min. The immunoblots were detected using an electrochemiluminescence kit and exposed to the Vilber Fusion FX5 automatic gel imaging analysis system (Vilber, Marne La Vallée, France).

Cell counting kit-8 (CCK-8) assay. The drug sensitivity assay was carried out using the mitochondrial reduction activity assay. According to the protocol of the CCK-8 assay kit (Dojindo, Kumamoto, Japan), cells grown in 96-well culture plates (8,000 cells/well) were treated as required. The experimental group consisted of cells infected with the lentivirus and the control group consisted of non-infected cells. After a 24-h culture, docetaxel (Aventis Pharma, Guildford, UK) was added at the following concentrations: 0.01, 0.05, 0.25, 0.5, 0.75, 1, 5 and 25 μ g/ml. Next, cells in each well were incubated with 10 μ l of CCK-8 at 37°C for 4 h. The optical density (OD) for each well was measured at 450 nm using a microplate reader (Bio-Rad Model 550; Bio-Rad Laboratories, Hercules, CA, USA). CCK-8 experiments were repeated three times on different days. The means and standard deviations of the optical density (OD) of the replicates were calculated for each well. The cell inhibitory rate was calculated according to the following equation: Cell inhibitory rate = $[1 - (\text{OD}_{\text{experiment}} - \text{OD}_{\text{blank}}) / (\text{OD}_{\text{control}} - \text{OD}_{\text{blank}})] \times 100\%$. The 50% inhibition concentration (IC_{50}) of the drug was determined by chartography.

Statistical analysis. For all measurements as needed, the statistical significance between groups was assessed by one-way ANOVA based on the homogeneity of variance test (SPSS 13.0, SPSS, Inc., Chicago, IL, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Selection of the most effective ErbB2-specific shRNA expression vector. Four plasmids containing shERBB2 (pLL2G-shERBB2)

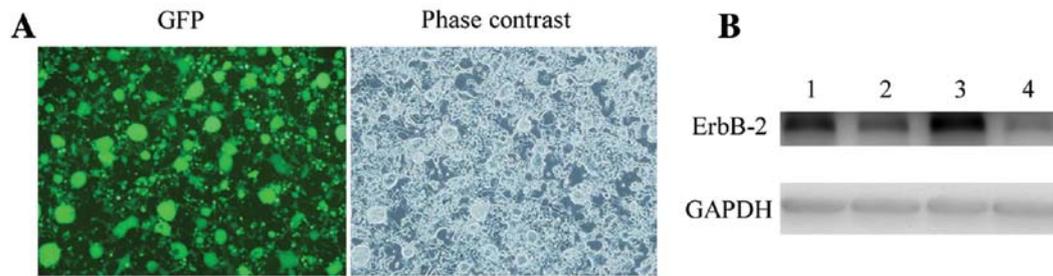


Figure 2. Selection of the most effective ErbB-2-specific shRNA expression vector in 293T cells. (A) Phase contrast and GFP expression under a fluorescence microscope was captured after 48 h in 293T cells. Infection of pLL2G-shERBB2 vector in the 293T cells (original magnification, x200). (B) Protein level of ErbB-2 in the 293T cells was detected by western blotting. Lanes 1-4, pLL2G-shERBB2-1, -2, -3 and -4, respectively. pLL2G-shERBB2-4 significantly knocked down expression of ErbB-2 at the protein level.

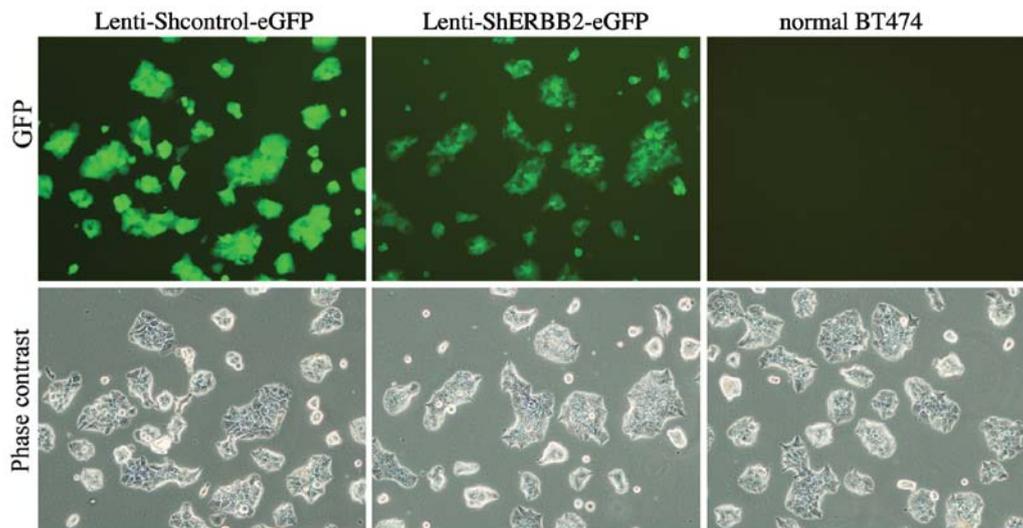


Figure 3. Phase contrast and GFP expression under a fluorescence microscope was captured after 48 h in the BT474 cells (original magnification, x200). It divided three groups of BT474 cells. Lenti-shcontrol-eGFP groups is added control lentivirus. Lenti-shERBB2-eGFP groups are infected with pLL2G-shERBB2-4. The third group of BT474 cells were without treatment.

were coinfecting into 293T cells, respectively. GFP expression in the 293T cells was observed under a fluorescence microscope 48 h after infection with pLL2G-shERBB2. According to the results of the western blotting assay, pLL2G-shERBB2-4 was the most effective lentiviral vector and, thus, was used in the subsequent research (Fig. 2).

Infection efficiency of the viral delivery in vitro. BT474 cells were infected with the lentiviral vector encoding GFP, resulting in GFP expression in the majority of cultured cells. The infection efficiency was assayed by fluorescence microscopy after cells were infected by the lentivirus for 48 h. A robust infection efficiency of 79% was observed (Fig. 3). The fluorescence gradually weakened and the infected cells began to undergo apoptosis on the fifth day resulting in the gradual decline of the infection rate. BT474 cells that were infected with Lenti-shERBB2-eGFP and non-infected cells co-cultured showed no synergistic growth inhibition effect when treated using the same protocols. We observed that the proliferation of BT474 cells was significantly inhibited after infection with Lenti-shERBB2-eGFP when compared with the Lenti-shcontrol-eGFP and normal BT474 cells ($P < 0.05$). Real-time PCR and western blot analysis showed that the mRNA

and protein expression levels of ErbB-2 were lower in the cells infected with Lenti-shERBB2-eGFP than in the cells infected with the Lenti-shcontrol and the normal BT474 cells ($P < 0.05$) (Figs. 4 and 5).

mRNA expression of ErbB-2 and TACC1. Following lentiviral infection of BT474 cells, the mRNA expression of ErbB-2 and TACC1 was examined by real-time PCR. A significant difference was noted between the silenced and control cells ($P < 0.05$), a reduction in the expression of ErbB-2 was noted. The expression of ErbB-2 mRNA in the BT474 cells was successfully knocked down. On the other hand, the expression of ErbB-2 mRNA showed no significant difference between the blank control group and the negative control-shRNA group ($P > 0.05$). As shown in Fig. 4B, the expression of TACC1 mRNA was affected following ErbB-2 knockdown, with a different expression level noted in the ErbB-2-silenced cells when compared to the control cells. The expression of TACC1 in the ErbB-2-positive cells (BT474) was expressed at a low level. However, after interference of the expression of ErbB-2, expression of TACC1 was upregulated. The result of real-time PCR showed that silencing of the ErbB-2 gene markedly increased the levels of TACC1 mRNA ($P < 0.05$) (Fig. 4).

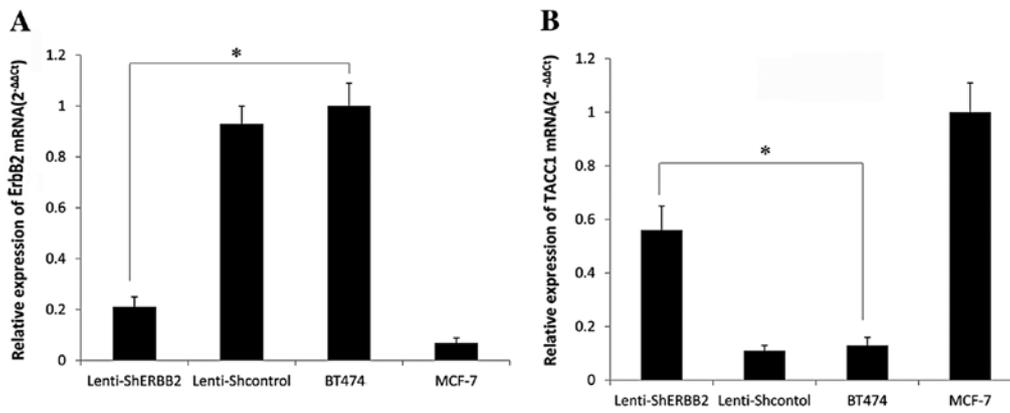


Figure 4. mRNA levels of ErbB-2 and TACC1 detected by real-time PCR after BT474 cells were treated with Lenti-shERBB2 and Lenti-shcontrol ($P < 0.05$). (A) ErbB2-shRNA-infected cells exhibited significantly lower ErbB-2 mRNA expression (0.21 ± 0.01) when compared with that of the blank control group (1 ± 0.03) and negative control-shRNA group (0.93 ± 0.02). The relative expression of ErbB-2 in MCF-7 cells was 0.069 ± 0.01 . (B) The relative expression of TACC1 in Lenti-shERBB2 BT474 cells, Lenti-shcontrol BT474 cells, BT474 cells and MCF-7 cells was 0.56 ± 0.03 , 0.1 ± 0.01 , 0.13 ± 0.015 and 1 ± 0.035 , respectively.

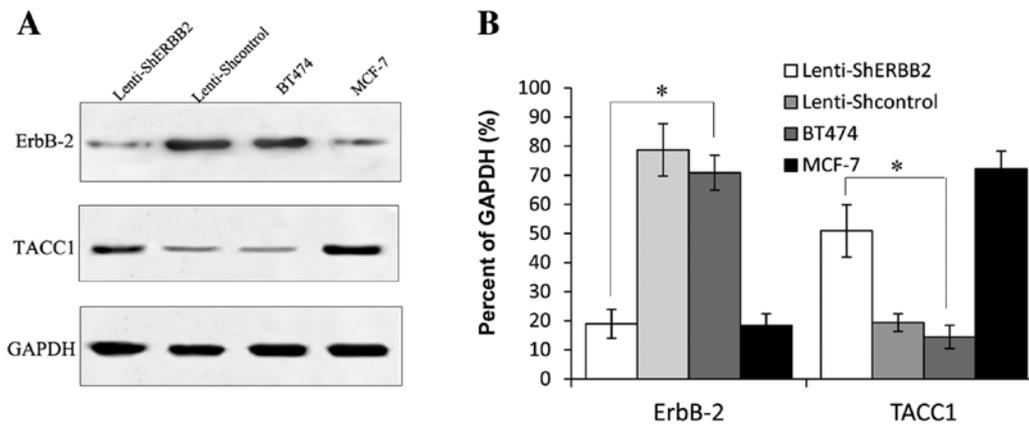


Figure 5. Protein levels of ErbB-2 and TACC1 after BT474 cells were infected with Lenti-shERBB2 and Lenti-shcontrol as detected by western blotting ($P < 0.05$). Lenti-shERBB2 significantly knocked down expression of ErbB-2 at the protein level ($P < 0.05$), compared with Lenti-shcontrol and BT474 cells. After interference of the expression of ErbB-2, TACC1 expression was significantly upregulated ($P < 0.05$).

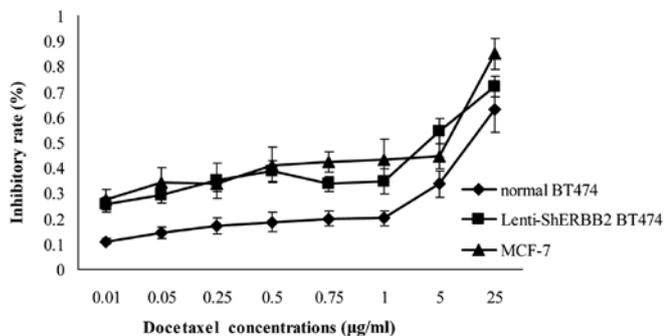


Figure 6. BT474 and MCF-7 cell lines were treated with various concentrations of docetaxel (0.01, 0.05, 0.25, 0.75, 1, 5 and 25 $\mu\text{g/ml}$) for 24 h for the CCK-8 assay. The inhibitory rates of infected BT474 cells, uninfected BT474 cells and MCF-7 cells are shown. The sensitivity to chemotherapeutic drugs (docetaxel) of BT474 cells increased after ErbB-2 interference, and the difference was statistically significant ($P < 0.05$). Chemotherapy drug sensitivity of infected BT474 and MCF-7 cells was similar, but the difference was statistically significant.

gray scale of the stained area was measured under identical conditions. The average optical density for ErbB-2 protein expression in the ErbB2-shRNA-infected cells was lower when compared with the value in the blank control and the negative control-shRNA cells, respectively. This indicated marked reduction in ErbB-2 protein following ErbB2-shRNA infection; the observed differences were significant ($P < 0.05$) (Fig. 5). Consistent with the above mRNA result, the average optical density for TACC1 protein expression in the BT474, MCF-7, Lenti-shcontrol and Lenti-shERBB-2 cells showed significant differences between the cell groups ($P < 0.05$) (Fig. 5). TACC1 was overexpressed when the ErbB-2 protein was markedly inhibited by ErbB2-shRNA in BT474 cells. The results were in accordance with the mRNA levels (Fig. 4B).

Effects of Lenti-shERBB2-eGFP on cell sensitivity to docetaxel treatment. Breast cancer cells that overexpress ErbB-2 are more resistant to chemotherapeutic agents such as paclitaxel (Taxol) and docetaxel (Taxotere) than cells that do not overexpress ErbB-2 (23). After inhibiting ErbB-2 expression using Lenti-shERBB2-eGFP infection, the IC_{50}

ErbB-2 and TACC1 protein expression. The protein expression of ErbB-2 and TACC1 was evaluated by western blotting. The

(index of chemotherapy sensitivity to docetaxel) of the Lenti-shERBB2-infected cells was $2.77 \pm 0.04 \mu\text{g/ml}$. The IC_{50} of the BT474 cells was $30.01 \pm 0.13 \mu\text{g/ml}$, and this value for the MCF-7 cells was $1.15 \pm 0.03 \mu\text{g/ml}$. That is, after ErbB-2 was inhibited, the breast cancer cell sensitivity to chemotherapy improved 10-fold. The result indicated that the suppression of ErbB-2 expression inhibits the proliferation of BT474 cells and increases the cell sensitivity to docetaxel treatment; this difference was statistically significant (Fig. 6).

Discussion

This study confirmed the knockdown of ErbB-2 in BT474 cells by lentiviral infection and that TACC1 exhibited higher expression when ErbB-2 was expressed at a low level. Lenti-shERBB2-eGFP obviously inhibited the expression of ErbB-2 mRNA and protein in BT474 breast cancer cells and also increased the chemotherapy sensitivities to docetaxel *in vitro*. We also observed that infection with the lentivirus inhibited BT474 cell growth and promoted apoptosis.

ErbB receptors, in particular ErbB-2, play an important role in carcinoma formation, and dysfunction promotes tumorigenesis. Downregulation of ErbB-2 was found to result in an increase in cells in the G1 phase and the induction of apoptosis (24). Overexpression of the ErbB-2 gene has frequently been observed in human tumors, including those of the breast, stomach, lung and oral cavity (25-27). Capable of stable and highly specific silencing of gene expression, shRNAs have been extensively applied to silence abnormal gene expression in the treatment of cancer. Given that ErbB-2 was found to be overexpressed in the BT474 cell line (28), we examined the functional consequence of ErbB-2 silencing in BT474 cells. In the present study, after Lenti-shERBB2 was infected into the BT474 breast cancer cells, ErbB-2 expression was significantly reduced at the mRNA and protein levels. Since ErbB family-mediated signaling plays a critical role in cell growth, survival, adhesion and motility (8), our data suggest that the ErbB-2 gene is a feasible RNAi target for gene silencing. The data described here provide proof that siRNA can be used therapeutically for human cancer. ErbB-2 as well as TACC1 may be referred to as novel cancer therapeutic targets.

It has been documented that TACC1 is downregulated in ErbB-2-positive breast cancer cells and tissues (22). Yet, few studies have examined the functional relationship of TACC1 and ErbB-2 in breast cancer cells. In this study, we investigated the relationship between ErbB-2 and TACC1, specifically in BT474 cells. The expression of TACC1 was examined following Lenti-shERBB2-mediated silencing of the ErbB-2 gene in BT474 cells. We found that silencing of ErbB-2 induced changes in TACC1 expression. Although TACC1 and ErbB-2 both contribute to the progression of certain types of tumors, our data suggest that TACC1 expression may be strictly associated with ErbB-2 amplification in BT474 cells.

In addition to the regulation of expression, TACC1 may be regulated at multiple levels, such as mutations, loss of heterozygosity, promoter methylation, or activation of alternative signaling pathways. Several studies have shown synergistic effects of TACC1 with other genes during tumorigenesis (17,20,21). On the other hand, there is also evidence that TACC1 is involved in drug resistance to chemotherapy

and tumor progression (29). Consistent with this hypothesis, research has found that TACC1 overexpression appears to promote paclitaxel-induced cell death (30). It is possible that TACC1 may promote chemosensitivity, resulting from the ability of TACC1 to activate Ras, PI3K and PKB (29,31), whereas the chemosensitivity of TACC1-overexpressing cells may be due to the interaction of TACC1 with microtubules and the mitotic apparatus. In these signaling pathways, TACC1 has been described as transforming (11) and, yet, is downregulated in anthracyclin-treated mammary tumors (14). TACC1 expression results in an increase in both ERK and PKB phosphorylation (32) in mammary tissues. Knuefermann *et al* (33) delineated a pathway that involves HER2/PI-3K/Akt in mediating multidrug resistance in human breast cancer cells. They found that infection of ErbB-2 in MCF7 breast cancer cells that express HER3 resulted in a phosphoinositide-3 kinase (PI-3K)-dependent activation of Akt, and was associated with an increased resistance of cells to multiple chemotherapeutic agents, including paclitaxel. Regarding PI-3K-dependent Akt activation, while the latter is associated with cell resistance, following the selective inhibition of PI-3K or Akt, cell sensitivity to chemotherapy significantly increased (29). There appears to be a certain correlation between ErbB-2 and TACC1 in PI-3K/Akt-related chemotherapy resistance. If true, overexpression of TACC1 may serve as a useful marker for chemosensitive tumors.

In summary, we demonstrated that ErbB-2 was effectively silenced in BT474 cells via lentiviral infection, providing evidence that the use of lentiviral shRNA can sensitize docetaxel-resistant ErbB-2-overexpressing breast cancer cells to the drug by repressing ErbB-2 expression. This in turn may have important implications for the development of a novel therapy that combines chemotherapy and gene therapy. In addition to new diagnostic and prognostic markers, TACC1 may be a novel target for breast cancer therapy. Future studies are required to examine the molecular and biological relationship of ErbB-2 and TACC1 in breast cancer cells in greater detail.

Acknowledgements

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