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Original article

Exceptionally high UBE2C expression is a unique phenomenon in basal-like type breast cancer and is regulated by BRCA1

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ABSTRACT

Ubiquitin-conjugating enzyme 2C (UBE2C) is overexpressed in various types of cancer, leading to poor outcomes and drug resistance. UBE2C may also have a critical role in phenotypes associated with poor prognosis in breast cancer; however, the relationship between UBE2C expression and clinical outcome in breast cancer subtypes has not previously been investigated. We firstly analyzed breast cancer patient data and immunohistochemistry of breast cancer patient samples. We demonstrated that UBE2C was associated with poor prognosis in breast cancer, particularly basal-like breast cancer, a subtype with aggressive clinical features. Interestingly, we found that there was a close relationship between the expression of BRCA1 and UBE2C in the MCF-7 and MDA-MB-231 breast cancer cell lines. Upregulation of BRCA1 could inhibit the expression of UBE2C. In cells with BRCA1 silenced down, expression of UBE2C was obviously increased, with a concurrent decrease in cellular sensitivity to doxorubicin. Suppression of UBE2C expression by RNA interference led to decrease the mRNA expressions of BCRP, MRP1 and P-gp in doxorubicin-treated MDA-MB-231 cells. Moreover, treatment with 1 μg/ml doxorubicin led to increased expression of UBE2C. The results show high expression of UBE2C is a potential prognostic factor of poor outcome in basal-like breast cancer. Moreover, loss of BRCA1 function results in an increase in UBE2C expression and chemical resistance to doxorubicin in breast cancer cells.

1. Introduction

Breast cancer is a heterogeneous disease, with more than 1,300,000 cases and 450,000 deaths annually worldwide. Clinically, breast cancer is classified into five subtypes: normal breast-like, luminal A, luminal B, HER2/Neu-enriched, and basal-like breast cancer (BBC) [1]. BBC is characterized by high proliferation and aggression, with consequent poor patient prognosis [2].

Ubiquitin-conjugating enzyme 2C (UBE2C) is involved in cell cycle progression, mitosis regulation, and targeted degradation of short-lived proteins [3,4]. The expression level of UBE2C is overexpressed in cancers compared with normal tissue [5], and is positively correlation with poor clinical outcome in cancers of the esophagus, lung and liver [6–8]. Thus, UBE2C may also play a crucial role in acquiring phenotypes associated with poor prognosis in breast cancer; however, a relationship between expression of UBE2C and clinical outcome in breast cancer subtypes has not been demonstrated to date. The expression of UBE2C also plays an important role in resistance to cytotoxic drugs. Suppression of UBE2C sensitizes breast cancer cells to doxorubicin [9], which is a DNA helix intercalator and topoisomerase II inhibitor and regarded as the single most active agent for the therapy of breast cancer. Variation in UBE2C expression levels may provide an accessible biomarker target for patients with doxorubicin resistance.

Mutation of BRCA1, a member of the breast cancer susceptibility gene family, often leads to tumors with a basal-like phenotype [10,11]; moreover, BRCA1-associated tumors are more likely to express markers

Abbreviations: UBE2C, ubiquitin-conjugating enzyme 2C; BBC, basal-like breast cancer; HR, hazard ratio; P-gp, P-glycoprotein; MRP1, multidrug resistance protein 1; BCRP, breast cancer resistance protein; SD, standard deviation

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consistent with this phenotype [11]. A recent report has implicated the CAMK1 transport network in modulating the relationship between BRCA1 and UBE2C in other cancers [12]; however, the relationship between expression of UBE2C and BRCA1 remains uncertain in breast cancer. Defining parameters critical for prognosis and treatment is a key in anti-tumor therapy, and patients may have important concerns about how a BRCA1 mutation will affect clinical outcome and therapy design. A recent study confirmed that BRCA1-defective breast cancer cells are significantly less sensitive to doxorubicin [13]; however, the multiple pathways associated with the loss of BRCA1 in tumor drug resistance are not fully understood.

Here, we report analysis of the TCGA breast cancer data set [14] and immunohistochemistry of breast cancer patient samples demonstrating increased expression levels of UBE2C in BBC, compared with both normal breast tissue and three other subtypes of breast cancer. To further investigate the function of BRCA1, we inhibited BRCA1 synthesis by RNA interference, which resulted in the increased expression of UBE2C, further supporting a critical role for UBE2C overexpression in the progression of BBC. In addition, we examined the expression of UBE2C-related chemoresistance genes. We suggest UBE2C as a potential target of a drug resistance mechanism, where loss of BRCA1 expression leads to an increase in UBE2C expression, which is responsible for a reduction in sensitivity to doxorubicin.

2. Materials and methods

2.1. Cell culture and transfection

The MCF-7 and MDA-MB-231 breast cancer cell lines were obtained from the laboratory of pathology of Dalian Medical University. The cells were cultured in DMEM F12 and DMEM media, respectively, supplemented with 10% fetal bovine serum at 37 °C in a 5% CO_2 atmosphere. For small interfering RNA (siRNA) transfection experiments, cells were transfected with siRNA targeting UBE2C and BRCA1 or a negative control (NC) siRNA (Transgen Biotech, China). Total proteins were extracted 48 h after siRNA treatment and the levels of specific proteins evaluated by western blotting. To overexpress BRCA1 in MDA-MB-231 cells, we used flag-BRCA1 and the flag-vector alone as a control, which were purchased from Transgen Biotech (China). Varying amounts of flag-BRCA1 and flag-vector plasmids were transfected into MDA-MB-231 cells for 48 h before downstream analyses.

2.2. Human tissue samples

All breast cancer tissue samples used in this study (n = 90) were obtained during breast surgery and immediately frozen in liquid nitrogen. Breast cancer samples were collected at the Department of Medical Oncology, Second Affiliated Hospital of Dalian Medical University, Dalian medical University, Dalian, China. All the patients signed the informed consent at the beginning of our research. The study was approved by the Second Affiliated Hospital of Dalian Medical University.

2.3. Western blot analysis

Cells were washed with cold PBS and harvested, followed by the mixture of lysis and 1× RIPA buffer (Sigma). Protein concentrations were estimated by using the Easy II Protein Quantitative Kit (Transgen). Protein (50 μg) was subjected to 10% SDS-PAGE. The samples were in the segment of electrophoresis of approximately 0.5–1 hours and transferred to PVDF membranes (Immobilon). After blocking with 5% non-fat milk dissolved in TBST (TBS and 0.01% Tween-20) for 2 hours at room temperature, the PVDF membranes were incubated with the selected antibodies solution overnight at 4 °C. Selected antibodies (San Ying Biotechnology, China) were diluted as follows: BRCA1, 1:500; UBE2C, 1:500; and GAPDH (loading control), 1:1000. Then transfer membranes were incubated with anti-IgG secondary antibodies (1:16,000 in TBST) for 1 h at 37 °C [15]. The image of protein bands was performed using a machine of ODYSSEY infrared imaging system.

2.4. RNA extraction and real-time quantitative PCR

The mRNA expression levels of the drug transporters P-glycoprotein (official symbol ABCB5), multidrug resistance protein 1 (MRP1; official symbol ABCC1), and breast cancer resistance protein (BCRP; official symbol ABCG2) were quantified by real-time PCR. Cultured cells were using Trizol reagent (Transgen, China) to extract total RNA. In total, 1 μg total RNA was reverse-transcribed with All-in-one First-Strand cDNA Synthesis SuperMiX (Transgen, China). RT-qPCR was analyzed using the iCycler™ Real Time System and a SYBR Premix EX Tag Master mixture kit (Transgen, China) according to the manufacturer's protocols. Primers for the amplification of transporter genes have been described previously [16]: P-gp F, 5′-CCC ATC ATT GCA ATA GCA GG-3′; P-gp R, 5′-GTT CAA ACT TCT GCT GGT CA-3′; BCRP F, 5′-TTC GGC TTG CAA CAA CTA TG-3′; BCRP R, 5′-TCC AGA CAC ACC ACG GAT AA-3′; MRP1 F, 5′-ATG TCA CGT GGA ATA CCA GC-3′; MRP1 R, 5′-GAA GAC TGA ACT CCC TTC CT-3′. GAPDH was used as an internal standard, with the primers GAPDH F, 5′-GGC ATC GTG ATG GAC TCC G-3′; GAPDH R, 5′-GCT GGA AGG TGG ACA GCG A-3′.

2.5. Cytotoxicity assay

To detect whether BRCA1 influenced the drug sensitivity of breast cancer cells, cells were seeded in 96-well plates at a density of 1×10^5 cells/ml. Then cells were incubated at 37 °C under 5% CO_2 for 48 hours then exposed to different doses of doxorubicin. The CCK-8 assay was measured by using a cell counting kit-8 (Keygen Biotech, China) according to the manufacturer's instructions with slight modifications. Specifically, cells were treated with 0, 0.5, 1, 2 μg/ml for 48 h and washed twice with PBS. Moreover, 10 μl of CCK-8 and 100 μl medium were placed in each well for 2 hours at 37 °C. Then the absorbance at 450 nm was detected using spectrophotometer (Thermofisher Scientific). All experiments were accomplished in triplicate.

2.6. Immunohistochemical staining analysis

The distribution of UBE2C proteins in tissues was evaluated by immunohistochemical analysis. Patients should be allowed to utilize frozen slices of the patient's operation, and the patient's postoperative tissue was placed in the citric acid buffer and processing microwave heat for 20 minutes. UBE2C main monoclonal antibody (San Ying Biotechnology, China) was incubated with the sections overnight at 4 °C. After PBS washing, part of the organization and the secondary antibody was hatching 37 °C for 10 minutes, then incubated with peroxidase conjugated-biotin streptavidin complex for 10 minutes, finally examined with 3,3′-diaminobenzidine and hematoxylin. The count of positive cells was as follows: positive cell number ≤ 10%, 0 points; 11–50%, 1 point; 51–75%, 2 points; ≥76%, 3 points. The intensity of the staining was as follows: 0 = negative, 1–2 points = weakly positive, 3–4 = positive, > 5 points = strongly positive.

2.7. Analysis of gene expression

Gene data from breast invasive tumor samples were downloaded from the TCGA data portal [14]. Analyses were performed and figures generated using GraphPad Prism. The classification of breast cancers into subtypes, including basal-like, luminal A, luminal B, and Her-2 enriched was based on the PAM50 gene expression signature [14].

2.8. Survival analysis

UBE2C expression and associated survival data were downloaded

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