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Inhibition of ubiquitin conjugating enzyme UBE2C reduces proliferation and sensitizes breast cancer cells to radiation, doxorubicin, tamoxifen and letrozole

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Abstract

Purpose The objective of this study was to determine radiation, doxorubicin, tamoxifen and letrozole sensitivity of breast cancer cells in response to functional inhibition of the ubiquitin conjugating enzyme UBE2C.

Methods Taqman Real time PCR was performed to measure UBE2C levels in breast cancer cell lines and control HBL100 and HEK293T cells. A dominant negative form of UBE2C (DN-UBE2C) was used to functionally inhibit wild type UBE2C. Cell proliferation and anchorage independent growth were measured by colorimetric and soft agar assays, respectively. Radiation, doxorubicin, tamoxifen and letrozole responses of the cell lines were assessed by colorimetric and clonogenic assays.

Results Overexpression of UBE2C was observed in all breast cancer cell lines tested using quantitative real time PCR. UBE2C expression was found to be highest in MDAMB231 and relatively lowest in MCF7 cells, compared to control cells. Both the growth rate and the anchorage independent growth of MCF7 and MDAMB231 cells transfected with DN-UBE2C were significantly reduced compared to cells transfected with vector alone. MCF7 and MDAMB231 cells expressing DN-UBE2C were significantly more sensitive to different doses of radiation and doxorubicin compared to both wild type and vector alone transfected cells. In addition, DN-UBE2C transfected MCF7 cells were more sensitive to inhibition by tamoxifen and letrozole compared to wild type and vector alone transfected cells.

Conclusions Our results show that inhibition of UBE2C sensitizes breast cancer cells to radiation, doxorubicin and hormone blocking agents. UBE2C may, therefore, serve as a potential therapeutic target aimed at inducing radiation and chemo sensitization.

Keywords UBE2C · Breast cancer · Radiation sensitivity · Doxorubicin sensitivity · Tamoxifen sensitivity · Letrozole sensitivity · Therapeutic target

1 Introduction

Breast cancer is now the most common cancer among women living in urban India. According to the Madras Metropolitan Tumor Registry (MMTR) breast cancer has overtaken cervix carcinoma, with a crude incidence rate of 30/100000. The global burden of breast cancer measured by incidence, mortality, and economic costs is substantial and on the increase [1].

Our recent gene expression study in breast cancer (data not shown) revealed that the ubiquitin-conjugating enzyme 2C (UBE2C) is up-regulated in breast tumors compared to normal breast tissues. UBE2C is a member of the ubiquitin proteosome pathway, which participates in cell cycle progression and checkpoint control through targeted degradation of short-lived proteins. The ubiquitin/proteasome system involves the activation of three distinct enzymes, i.e., ubiquitin-activating enzyme E1, ubiquitin-carrier or conjugating enzyme E2s, and ubiquitin-protein ligase E3. The process of proteolysis occurs through sequential activation of these enzymes, thereby linking chains of the polypeptide co-factor, ubiquitin, onto proteins that are to be degraded by the proteasome [2]. A major problem in the treatment of cancer is the time-dependent development of resistance to therapy (chemoresistance and radioresistance). There is, therefore, an increased interest in finding new agents for targeted therapies [3]. Targeting the

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proteosome pathway, especially UBE2C, in combination with available therapeutic modalities such as radiation or chemotherapeutic agents may result in a more favorable therapeutic outcome. In the present study, we show that functional inhibition of UBE2C enhances the sensitivity of breast cancer cells to radiation, a chemotherapy drug (doxorubicin) and hormone blocking agents.

2 Materials and methods

2.1 Cell culture

Four well-characterized breast cancer cell lines (MCF7, T47D, BT549 and MDAMB231) were selected on the basis of their ER/PR/Her2 status (ER^+/PR^+/$Her2^-$ - Luminal A - MCF7, T47D) (ER^-/PR^-/$Her2^-$ - Basal - BT549, MDAMB231) and their metastatic potential (highly metastatic - MDAMB231, BT549) (non metastatic - MCF7, T47D). The immortalized normal breast epithelial cell line HBL100 and the transformed human embryonic kidney cell line HEK293T were used as controls. The cell lines were grown in DMEM or RPMI medium (Himedia Laboratories, Mumbai, India) containing 10 % fetal calf serum (Gibco Laboratories, Carlsbad, CA) and 100 units/ml Penicillin/streptomycin (Himedia Laboratories). The cells were incubated in a humidified atmosphere of 95 % air and 5 or 10 % CO_2 at 37 °C.

2.2 UBE2C expression analysis in breast cancer cell lines

RNA was isolated from the breast cancer cell lines using a Qiagen RNeasy Kit, (Qiagen, Gmbh, Hilden) followed by cDNA synthesis using a High Capacity Reverse Transcription Kit (Applied Biosystems, Foster city, CA; Cat no: 4368814). Taqman® Real time PCR was performed to assess UBE2C expression levels using a primer probe mix (Assay ID: Hs00738962_m1). GAPDH expression was used to normalize the data.

2.3 Radiation and drug treatment

Radiation sensitivities of cell lines and clonogenic assays were performed as described before [4]. Briefly the cells were seeded into 25 cm^2 flasks and irradiated using a Cobalt-60 irradiator (THERATRON phoenix, Best® Theratronics Ltd, Canada). A single dose of 2, 4, 8 and 12 Gy was delivered at a dose rate of 130 r/min. After irradiation, cell counts were performed and 1×10^3 cells were seeded in quadruplicates into 96-well tissue culture plates (Corning Incorporated, NY, USA). Cells were allowed to grow for 7 days and cell viability was then assessed using the MTS assay (Promega Pte Ltd. Singapore). The cells were similarly treated for the clonogenic assay and the plates were incubated for 2 weeks in a

humidified atmosphere of 95 % air and 5 % CO_2 at 37 °C. The colonies were stained using a clonogenic assay reagent containing 50 % ethanol and 0.25 % 1, 9-dimethyl-methylene blue, and colony counting was performed using a hemocytometer (Tiefe Depth. Profondeur, Marienfeld, Germany).

Doxorubicin sensitivity of cell lines was determined as described before [5]. Briefly 2×10^3 cells were seeded in quadruplicates in a 96-well plate and exposed to 0.1, 0.25, 0.5, 1, 2 and 5 µg/ml of doxorubicin (Oncodria, Sun Pharmaceuticals Industries Ltd, India) for 3 h. The cells were allowed to grow for 3 days and the cell viability was then assessed using the MTS assay. For determining tamoxifen and letrozole sensitivities, 2×10^3 cells were seeded in quadruplicates in a 96-well plate and exposed to 1, 5 and 10 µg/ml of tamoxifen and letrozole (Sun Pharmaceuticals Industries Ltd, India) for 24 h. After this, the cells were washed with PBS and allowed to grow for 6 days in fresh medium supplemented with 10 % FBS and the cell viability was then assessed using the MTS assay.

2.4 Subcloning and transfection of dominant-negative UBE2C (DN-UBE2C)

Addgene plasmid 8505 - UbcH10 DN [6] was obtained from Addgene, Inc. and subcloned into a pcDNA / FLAG 3.1 mammalian expression vector using standard technique. The accuracy of subcloned DN-UBE2C, including a mutation in its active site, was confirmed by DNA sequencing using a Big dye teriminator V3.1 cycle sequencing kit (Applied Biosystems, CA, USA) and analyzed using an ABI Prism 310 Genetic Analyzer (Applied Biosystems). MCF7 and MDAMB231 cells were transfected with DN-UBE2C plasmids using a Fugene HD Transfection Reagent (Roche Diagnostics, GmbH) according to the manufacturer's instructions and transfectants were confirmed by Western blotting.

2.5 Western blotting

Cell lysates were prepared and analyzed as described before [7]. DN-UBE2C transfectants were confirmed using an anti-FLAG® (Sigma, Missouri, USA) antibody. UBE2C and cyclin B1 levels were measured using an anti-UBE2C antibody (Millipore, USA) and anti-Cyclin B1 antibody (Santa Cruz Biotechnology, Inc. USA), respectively.

2.6 Cell proliferation assay

Cell proliferation was assessed as described before [8]. The cells were seeded at a density of 1×10^3 cells/well in a 96-well plate and their proliferation was then assessed at different time points (0, 24, 48, 72 and 96 h) using a CellTiter 96 AQ$_{ueous}$ One Solution Cell Proliferation Assay (Promega), according to the manufacturer's instructions.
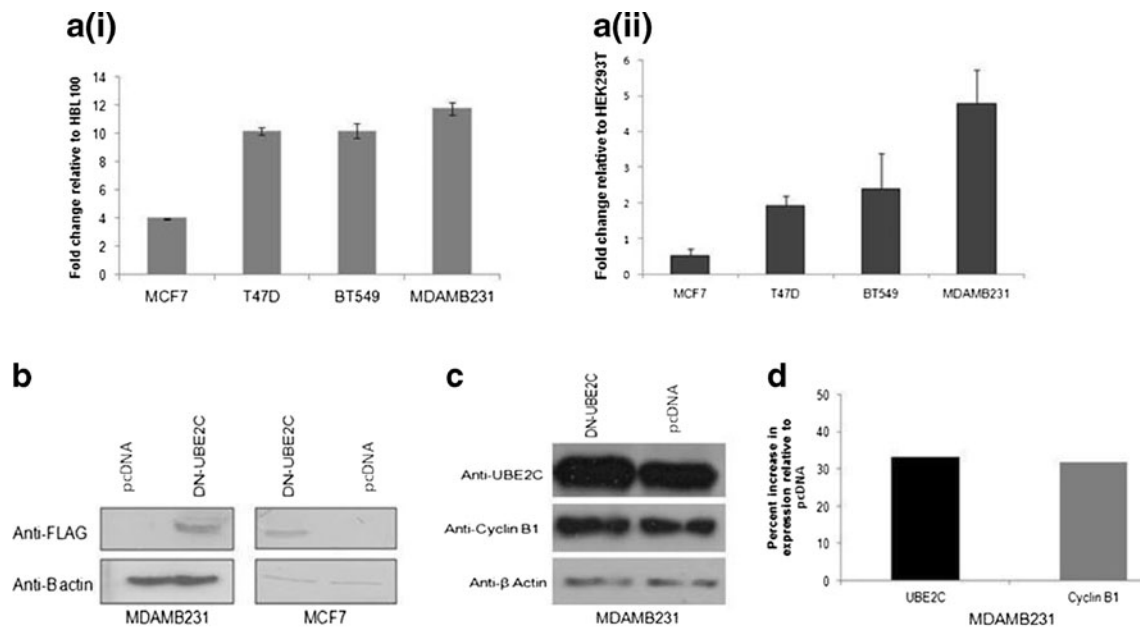
Fig. 1 **a(i)** UBE2C levels in breast cancer cell lines relative to HBL100 were measured by quantitative real-time PCR. The fold change represents the means of three replicates. *Error bar* represents mean standard deviation. **a(ii)** UBE2C levels in breast cancer cell lines relative to HEK293T were measured by quantitative real-time PCR. The fold change represents the means of three replicates. *Error bar* represents mean standard deviation. **b** MDAMB231 and MCF7 cells stably expressing DN-UBE2C was confirmed by Western blotting using an anti-FLAG antibody. β actin was used as internal loading control. **c** Cyclin B1 was measured in MDAMB231 transfected DN–UBE2C and pcDNA / FLAG alone transfected cells by Western blotting. **d** Densitometric analysis of Western blot film showing higher UBE2C and cyclin B1 levels in MDAMB231 transfected DN–UBE2C cells compared to pcDNA / FLAG alone transfected cells

2.7 Soft agar assay

Growth in soft agar was assessed as described before [9], with slight modifications. About 3×10^3 cells were suspended in 0.2 % low-melting point agarose (Becton, Dickinson and company, MD, USA) dissolved in DMEM containing 10 % FBS with 20 μg / ml of Hygromycin, and seeded in a 6-well plate containing DMEM supplemented with 10 % FBS, solidified in 0.5 % agarose. The plates were incubated for 4–5 weeks after which colony counts were performed.

2.8 Densitometric and statistical analyses

Densitometric analyses of Western blot films were performed using ImageJ version 1.43 (NIH, USA) and statistical analyses were performed using a one-way ANOVA and multiple-comparison correction by the Holm-Sidak method using Sigmaplot (version 11.0) (Systat Software, Inc., USA).
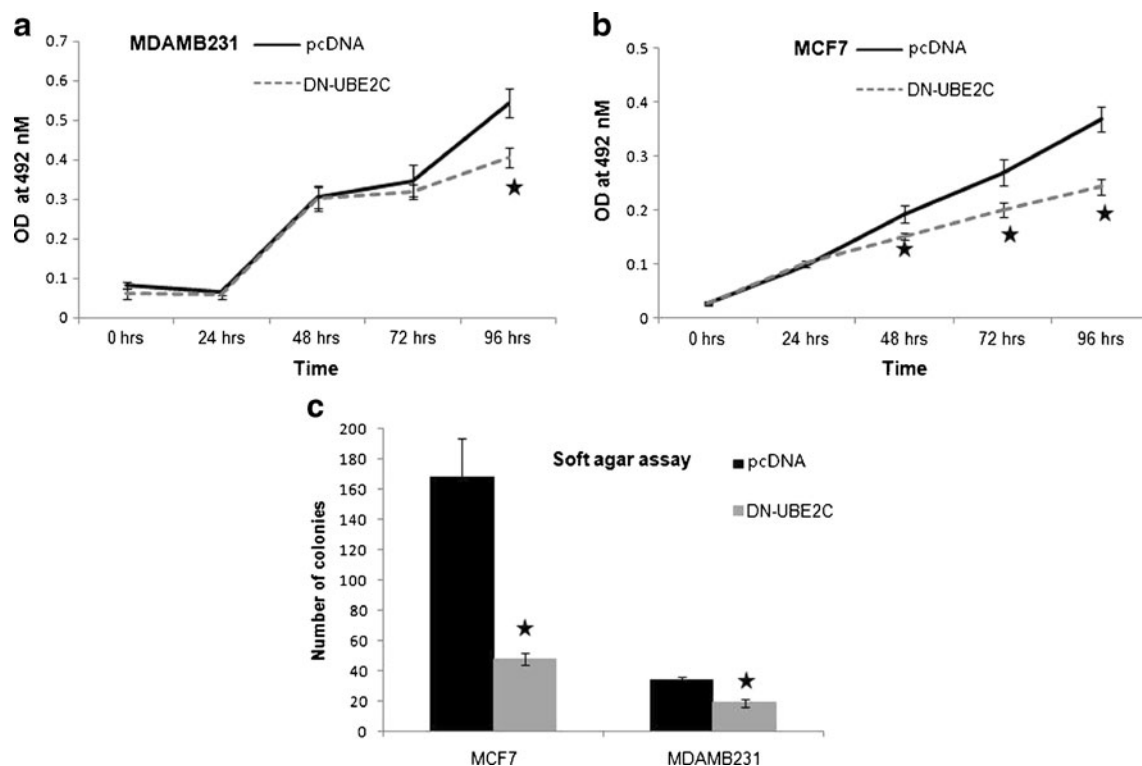
3 Results

3.1 UBE2C is overexpressed in breast cancer cell lines

We determined the expression of UBE2C by Real time RT-PCR in breast cancer cell lines relative to its expression in the immortalized breast epithelial cell line HBL100 and the transformed human embryonic kidney cell line HEK293T. As shown in Fig. 1a(i), a more than eleven-fold higher expression of UBE2C mRNA was observed in MDAMB231, and about a ten-fold higher expression in T47D and BT549, whereas MCF7 showed a moderate expression (4-fold higher), when compared to the expression levels of UBE2C in HBL100. Similarly, in comparison to HEK293T a more than four-fold higher expression of UBE2C mRNA was observed in MDAMB231 and about a two-fold higher expression in T47D and BT549, whereas MCF7 showed only a slightly higher level (Fig. 1a(ii)).

3.2 Dominant negative UBE2C represses proliferation and anchorage independent growth

Next, we aimed at studying the effects of UBE2C inhibition on breast cancer cells. To this end, we used a dominant negative form of UBE2C (DN-UBE2C). This DN-UBE2C was transfected into MCF7 and MDAMB231 cells and its expression was confirmed using an anti-FLAG antibody in conjunction with Western blotting (Fig. 1b). As the UBE2C-APC complex controls cyclin B1 degradation, the expression level of cyclin B1 was also measured in MDAMB231 cells transfected with DN-UBE2C or pcDNA / FLAG alone by Western blotting (Fig. 1c). A densitometric analysis of the Western blot film showed around 30 % higher UBE2C and cyclin B1 levels in MDAMB231 cells transfected with DN-UBE2C compared to pcDNA / FLAG alone transfected cells

Fig. 2 **a** DN-UBE2C decreases the proliferation of MDAMB231 cells (*p <0.05 at 96 h) and **b** MCF7 cells (*p <0.05 at 48, 72 and 96 h) as determined by MTS assay. Cells transfected with pcDNA alone served as controls. Absorbance was measured at 492 nm and the results were obtained from quadruplicate wells. **c** Decrease in colony formation in soft agar of MCF7 and MDAMB231 cells after transfection of DN-UBE2C (*p <0.05)

(Fig. 1d). The DN-UBE2C transfected MDAMB231 cells, which originally exhibited an eleven-fold increase in UBE2C level, showed a significant decrease in proliferation at 96 h (p < 0.05), when compared to pcDNA alone transfected MDAMB231 cells (Fig. 2a). Similarly, MCF7 cells transfected with DN-UBE2C showed a significant decrease in proliferation at 48, 72 and 96 h, respectively (p <0.05), when compared to vector alone transfected MCF7 cells (Fig. 2b). In the soft agar assay, performed to assess the anchorage independent growth of cells, MCF7-pcDNA formed on average 170 colonies, whereas MCF7-DNUBE2C on average gave rise to around 50 colonies. Similarly, MDAMB231-pcDNA formed on an average 35 colonies, whereas MDAMB231-DNUBE2C on average gave rise to around 19 colonies (Fig. 2c).

3.3 Dominant negative UBE2C sensitizes breast cancer cells to radiation and doxorubicin

Differences in response to radiation were observed in all the four breast cancer cell lines studied. Figure 3a shows the radiation responses of the different cell lines to single doses of 2, 4, 8 and 12 Gy. The data are expressed as mean percent survival of the individual cell lines at different dose points. Among the cell lines, MCF7 was found to be most resistant, showing about 35 % cell survival at 12 Gy of radiation, followed by T47D, MDAMB231 and BT549.

The radiation sensitivity of the MCF7 and MDAMB231 cell lines transfected with DN-UBE2C was also assessed using the MTS assay. At doses of 2, 4, 8 and 12 Gy, MCF7-pcDNA showed percent survival rates of 86.44, 63.07, 40.36 and 26.12, respectively, whereas at the same doses MCF7-DNUBE2C showed percent survival rates of 42.70, 24.47, 11.70 and 6.27 (Fig. 3b). A significant decrease in resistance to radiation at doses of 2, 4, 8 and 12 Gy by DN-UBE2C transfected MCF7 cells was observed compared to pcDNA vector alone transfected MCF7 cells (p <0.05). Similarly, at doses of 2, 4, 8 and 12 Gy, MDAMB231-pcDNA showed percent survival rates of 91.8, 70.71, 51.25, and 20.99, respectively, whereas at the same doses MDAMB231-DNUBE2C showed percent survival rates of 59.46, 48.48, 21.26, and 7.25 (Fig. 3c). MDAMB231 cells transfected with DN-UBE2C showed a significant decrease in their resistance to radiation at doses of 2, 8 and 12 Gy (p <0.05) when compared to pcDNA vector alone transfected MDAMB231 cells. The radiation sensitivity was further assessed using a clonogenic assay performed on MCF7 (Fig. 4a) and MDAMB231 (Fig. 4b) cells transfected with DN-UBE2C. This assay showed a significant decrease in resistance to radiation at the tested doses of 2 and 4 Gy compared to pcDNA vector alone transfected cells. MCF7-pcDNA formed on average 452 colonies at 2 Gy, whereas MCF7-DNUBE2C formed 287 colonies at the same dose (Fig. 4c). Similarly, MDAMB231-pcDNA formed 243 colonies at 2 Gy,
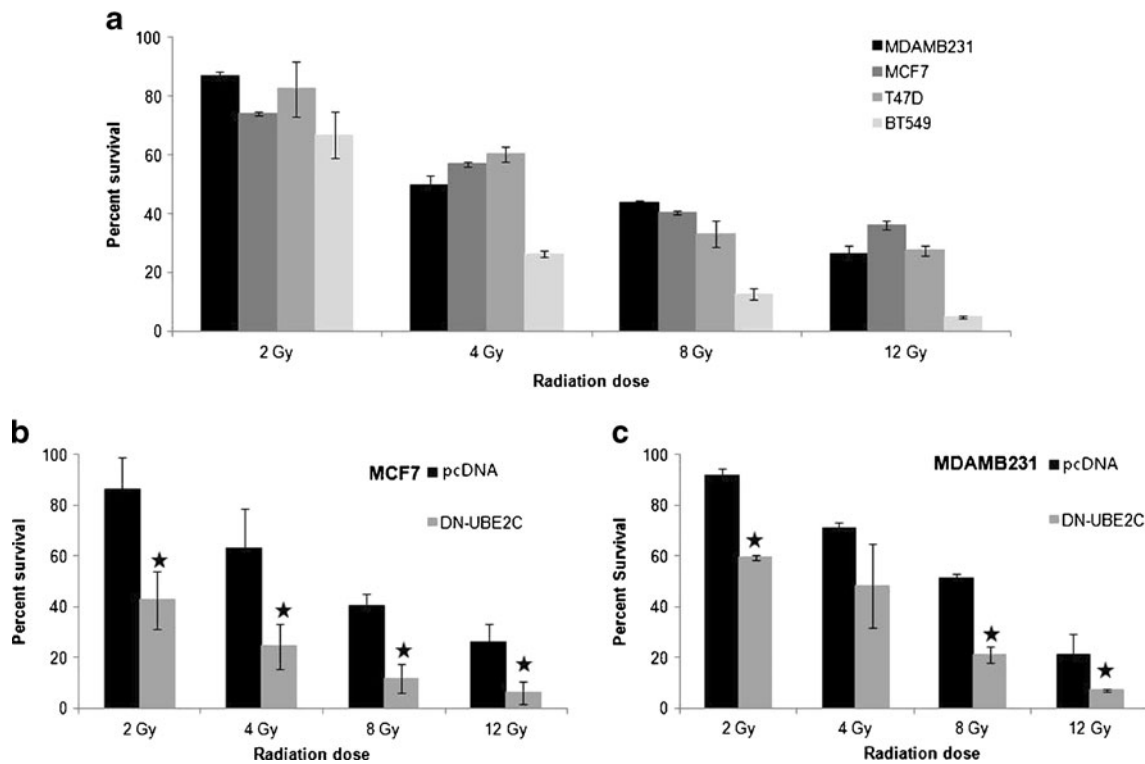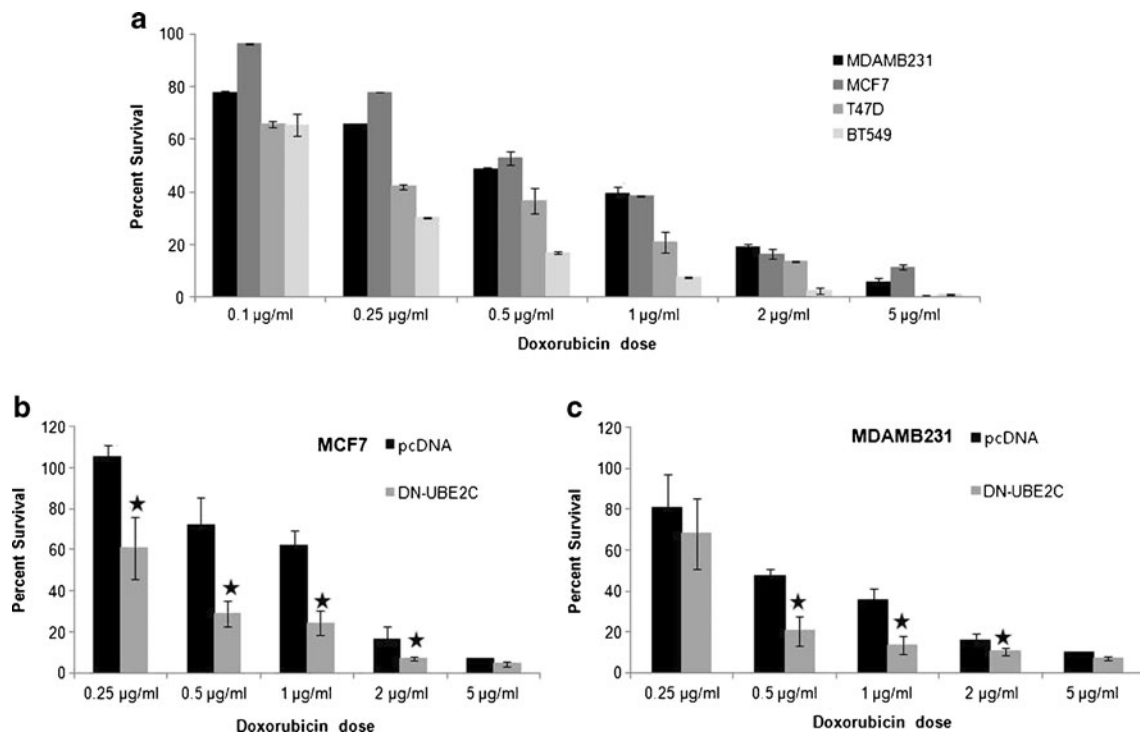
Fig. 3 **a** Radiation sensitivity of breast cancer cells (MDAMB231, MCF7, T47D and BT549) to different doses. An MTS assay was used to generate the percent survival compared to untreated controls, with assays run in quadruplicate twice. *Error bar* represents standard deviation ($n=4$). DN-UBE2C sensitizes cells to radiation. **b** MCF7 at 2, 4, 8 and 12 Gy and **c** MDAMB231 at 2,8 and 12 Gy as determined by MTS assay. Cells transfected with pcDNA alone served as controls. Absorbance was measured at 492 nm and the assays were performed twice in quadruplicate. *Error bar* represents standard deviation ($n=4$), (*$p<0.05$)

Fig. 4 Radiation sensitivity of MCF7 and MDAMB231 cells transfected with DN– UBE2C were also assessed by clonogenic assay. Significant decreases in colony formation at 2 and 4 Gy in DN-UBE2C transfected **a** MCF7 and **b** MDAMB231 cells compared to vector control were observed. **c** MCF7 clonogenic assay at 2 Gy radiation dose. **d** MDAMB231 clonogenic assay at 2 Gy radiation dose. Assays were performed twice in duplicate
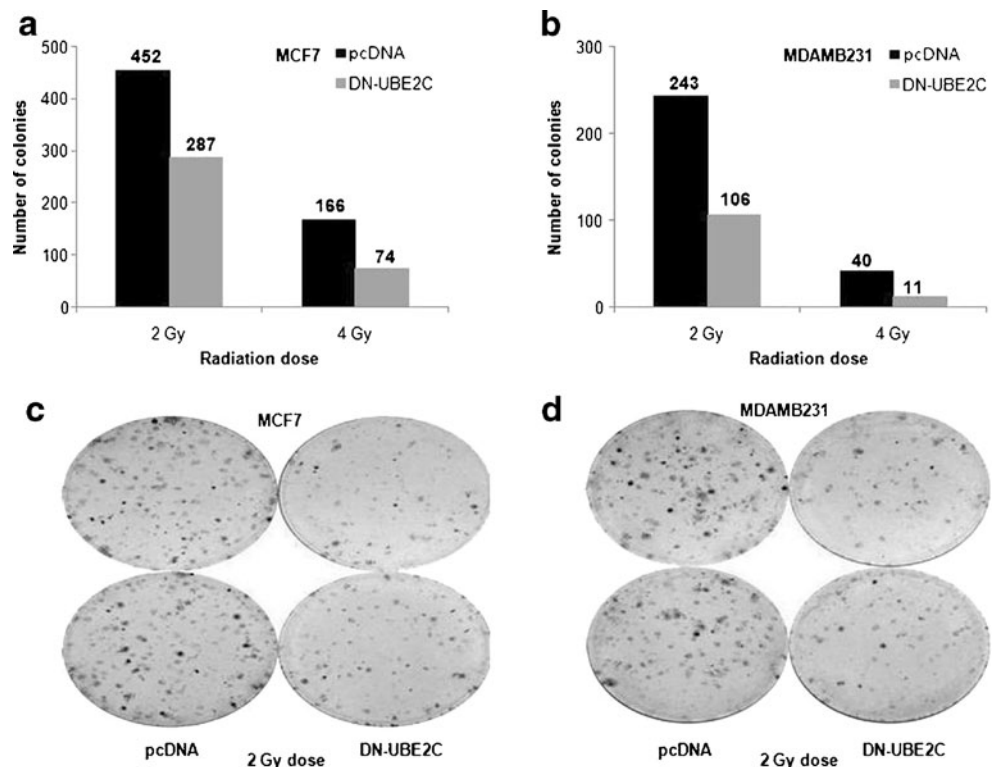
Fig. 5 a Doxorubicin sensitivity of breast cancer cells (MCF7, MDAMB231, T47D and BT549) to different doses. An MTS assay was used to generate the percent survival compared to untreated controls, with assays run in quadruplicate twice. *Error bar* represents standard deviation ($n=4$). DN-UBE2C sensitizes cells to doxorubicin. b MCF7 at 0.25, 0.5, 1 and 2 μg/ml. c MDAMB231 at 0.5, 1 and 2 μg/ml as determined by MTS assay. Cells transfected with pcDNA alone served as controls (*$p < 0.05$)

whereas MDAMB231-DNUBE2C formed 106 colonies at the same dose (Fig. 4d).

Figure 5a shows the responses of various cell lines to the different doses of doxorubicin. MCF7 was found to be the

Fig. 6 a Tamoxifen and letrozole sensitivity of MCF7 cells to different doses. MTS assay was used to generate the percent survival compared to untreated controls, with assays run in quadruplicate twice. *Error bar* represents standard deviation ($n = 4$). b DN-UBE2C sensitizes cells to tamoxifen at doses of 1, 5 and 10 μg/ml. c DN-UBE2C sensitizes cells to letrozole at doses of 1, 5 and 10 μg/ml as determined by MTS assay. Cells transfected with pcDNA alone served as controls (*$p < 0.05$)
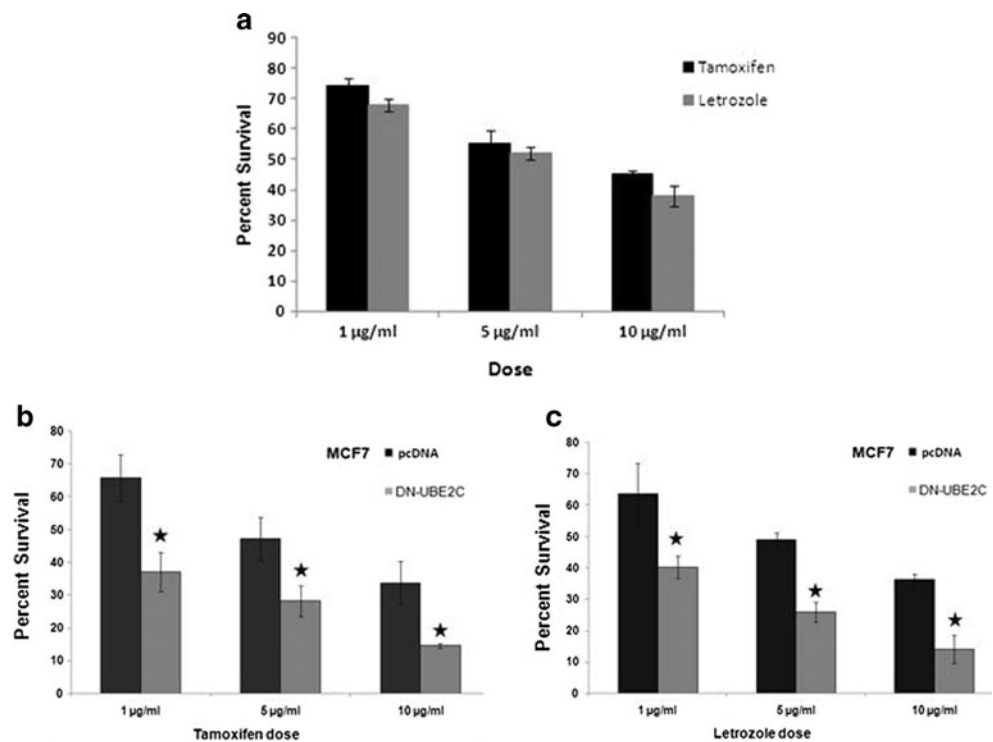
least sensitive, followed by MDAMB231, T47D and BT549. MCF7 showed 80 % survival at a concentration of 0.25 μg/ml of doxorubicin, whereas BT549 cells were the most sensitive as indicated by less than 30 % survival at the same concentration. In the case of cells transfected with pcDNA and DN-UBE2C, MCF7-pcDNA showed percent survival rates of 100, 72.31, 67.96, and 16.63 at doses of 0.25, 0.5, 1 and 2 μg/ml, respectively, whereas at the same doses MCF7-DNUBE2C showed percent survival rates of 60.91, 29.08, 24.36, and 6.97 (Fig. 5b). A significant decrease in resistance was observed at doses of 0.25, 0.5, 1 and 2 μg/ml in DN-UNE2C transfected MCF7 cells. Similarly, at doses of 0.25, 0.5, 1 and 2 μg/ml, MDAMB231-pcDNA showed percent survival rates of 80.07, 47.52, 35.8 and 15.9, respectively, whereas at the same doses MDAMB231-DNUBE2C showed percent survival rates of 68.12, 20.62, 13.69, 10.5 and 7.25 (Fig. 5c). A significant increase in sensitivity was observed at doses of 0.5, 1 and 2 μg/ml for DN-UBE2C transfected MDAMB231 cells at an overall significance level set at a p-value of 0.05 for comparison between groups. Together, our results indicate both a radio-sensitization and a chemo-sensitization effect upon inhibition of UBE2C in breast cancer cells.

3.4 Dominant negative UBE2C sensitizes MCF7 cells to anti-hormonal agents

Figure 6a shows the response of wild type MCF7 cells to different doses of tamoxifen and letrozole and Fig. 6b and c show the responses of vector alone (pcDNA) and DN-UBE2C transfected MCF7 cells to different doses of tamoxifen and letrozole, respectively. A significant increase ($p < 0.05$) in sensitivity to tamoxifen and letrozole was observed for the various doses in DN-UBE2C transfected MCF7 cells compared to pcDNA vector alone transfected cells. In case of tamoxifen, MCF7-pcDNA showed percent survival rates of 65.08, 47.23 and 35.67 at doses of 1, 5 and 10 μg/ml, respectively, whereas at the same doses MCF7-DNUBE2C showed percent survival rates of 37.21, 28.13 and 14.96 (Fig. 6b). Similarly, in case of letrozole MCF7-pcDNA showed percent survival rates of 62.08, 48.97 and 38.01 at doses of 1, 5 and 10 μg/ml, respectively, whereas at the same doses MCF7-DNUBE2C showed percent survival rates of 38.92, 25.84 and 13.12 (Fig. 6c).

4 Discussion

In recent years, interest in the ubiquitin-proteasome proteolysis pathway and the role of UBE2C in cancer development has shown a dramatic increase. Upregulation of UBE2C has been reported in cancers of the liver, lung, uterus, ovary, breast, bladder, gastric, thyroid, colon, and cervix [10–19]. In breast cancer, UBE2C has been reported to serve as a prognostic marker [15, 20] and potential therapeutic target [13]. The importance of UBE2C in the regulation of cell proliferation and transformation has been reported by Okamoto et al. [11] and Fujita et al. [9]. In the current study, overexpression of UBE2C in breast cancer cell lines was observed. A similar overexpression has also been reported by Berlingieri et al. [13] and Fujita et al. [9]. To evaluate whether inhibition of UBE2C may be therapeutically relevant in breast cancer, we employed a mutant version of UBE2C whose dominant negative effect was previously demonstrated. Townsley et al. [6] reported that mutation of a UBE2C active site cystine to a serine results in a dominant negative phenotype. This mutant protein (DN-UBE2C) competitively inhibits cyclin B ubiquitination and the degradation of both cyclin A and B. The UBE2C-APC complex is known to control cyclin B1 degradation and, concordantly, Wagner et al. [14] reported that UBE2C knockdown by siRNAs coincides with an increase in cyclin B1 expression. We observed an increase in cyclin B1 expression in MDAMB231 cells transfected with DN-UBE2C relative to control cells, indicating that the DN-UBE2C protein indeed inhibits cyclin B1 degradation.

In our experiments, we observed that $ER^+/PR^+/Her2^-$ MCF7 and T47D cells showed lower UBE2C expression levels compared to $ER^-/PR^-/Her2^-$ MDAMB231 and BT549 cells. Considering the difference in the metastatic potential of MCF7 and T47D (non metastatic) and MDAMB231 and BT549 (highly metastatic) cells, it is plausible to assume that highly aggressive tumor cells express higher levels of UBE2C. Concordantly, we found that ectopic expression of DN-UBE2C inhibits the growth of both MCF7 and MDAMB231 cells. This effect was more prominent in MCF7 cells than in MDAMB231 cells, which express UBE2C at a much higher level than MCF7. Previous studies have shown that DN-UBE2C competitively inhibits wild type UBE2C, based on which it is plausible to surmise that the effects of DN-UBE2C at the expression levels could result in an increased competitive inhibition in MCF7 cells as compared to MDAMB231 cells. Comparison of anchorage independent growth in soft agar and proliferation between vector alone transfected cells and stably transfected DN-UBE2C cells showed a significant decrease in number of colonies and cell proliferation rates of DN-UBE2C transfected cells in both MCF7 and MDAMB231. Our results are consistent with findings reported by Lin et al. [22] and Bose et al. [19] on Seg-1, an esophageal adenocarcinoma cell line, and SiHa and HeLa, both cervical cancer cell lines. In both studies, cell lines expressing high levels of UBE2C, transfection with a dominant-negative form of UBE2C resulted in a significant decrease in cell proliferation. Our results are also consistent with findings of Fujita et al. [9] and Berlingieri et al. [13] in which knockdown of UBE2C through RNA interference in MCF7 and T47D cells significantly suppressed the proliferation and anchorage-independent growth of the cells. Therefore, it appears evident that inhibition of UBE2C in breast

cancer cells represses their tumorigenic properties and, hence, that UBE2C may serve as a potential therapeutic target in breast cancer.

The current treatment of breast cancer entails a multimodality therapeutic approach combining cytotoxic chemotherapy, radiation therapy, hormonal therapy and targeted therapies. To investigate whether combining the inhibition of UBE2C with radiation or chemotherapeutic agents could enhance radio-sensitivity and cytotoxicity in breast cancer, UBE2C levels, radiation sensitivity and doxorubicin sensitivity of different breast cancer cell lines were compared. Among the cell lines studied, MCF7 was found to be most resistant to radiation and doxorubicin, whereas BT549 was most sensitive. The observed doxorubicin sensitivities were similar to those previously reported [21]. We could not find any direct correlation between levels of UBE2C expression and radiation and chemo sensitivity in the breast cancer cell lines tested. These results indicate that also other networks, independent of UBE2C, might contribute to radiation and chemo sensitivity. Furthermore, to explore the role of UBE2C in radiation and chemo responses, we studied the sensitivity of MCF7 and MDAMB231 cells transfected with DN-UBE2C to different doses of radiation, doxorubicin and anti-hormonal agents. Significant decreases in resistance to radiation and doxorubicin in MCF7 and MDAMB231 cells transfected with DN-UBE2C were observed when compared to vector alone transfected controls. To further validate our findings, we performed a clonogenic assay, which also revealed that DN-UBE2C transfected MCF7 and MDAMB231 cells showed an increased sensitivity to radiation compared to vector controls. A similar increase in sensitivity to radiation due to functional inhibition of UBE2C in cervical cancer cell lines has been reported by Bose et al. [19]. We also studied the sensitivity of MCF7 cells transfected with DN-UBE2C to different doses of tamoxifen and letrozole. By doing so, an increase in sensitivity was observed in cells transfected with DN-UBE2C compared to vector controls. These effects may be explained by competitive inhibition of functional wild type UBE2C by the dominant negative mutant of UBE2C.

In this study we show that functional inhibition of UBE2C inhibits breast cancer cell proliferation and growth. Furthermore, in spite of their diverse mechanisms of action, functional inhibition of UBE2C sensitizes the breast cancer cell to radiation and significantly enhances the cytotoxic response of the chemotherapeutic agent doxorubicin and the anti-hormonal agents tamoxifen and letrozole. Therefore, UBE2C could serve as a therapeutic target in a subset of patients whose tumors show an overexpression of UBE2C. The precise molecular mechanisms by which DN-UBE2C sensitizes cells to radiation, doxorubicin and anti-estrogenic agents remain to be resolved.

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