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ORIGINAL ARTICLE / ÖZGÜN MAKALE

INHIBITION OF IRE1α/XBP-1 BRANCH OF UPR BY GSK2850163 DRIVES THE SENSITIVITY TO TAMOXIFEN IN BREAST CANCER CELLS

UPR'NİN IRE1α/XBP-1 DALININ GSK2850163 ARACILI İNHİBİSYONU MEME KANSERİ HÜCRELERİNDE TAMOKSİFENE DUYARLILIĞI ARTIRIR

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ABSTRACT

Objective: *Tamoxifen is used as the leading treatment against breast cancer and has been broadly applied for the last 40 years. However, resistance development against tamoxifen is one of the major limitations in the effective treatment of breast cancer. The aim of our study was to investigate whether blockage of the IRE1α/XBP-1 branch of UPR by GSK2850163 efficiently limited the carcinogenic ability of tamoxifen-resistant MCF-7 cells.*

Material and Method: *Firstly, tamoxifen-resistant breast cancer cells were obtained by regularly exposing MCF-7 cells to tamoxifen. The biochemical activity of GSK2850163 was confirmed by immunoblotting and qRT-PCR. The possible effect of combined treatment of GSK2850163 and tamoxifen on proliferation, invasion, migration, and colony formation abilities of tamoxifen-resistant breast cancer cells were evaluated by using WST-1 based proliferation assay, Boyden-chamber invasion test, wound-healing assay, and plate colony formation methods, respectively.*

Result and Discussion: *Here, we showed that specific blockage of the IRE1α/XBP-1 by GSK2850163 efficiently limited the carcinogenic ability of tamoxifen-resistant MCF-7 cells. Moreover, co-treatment with*

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tamoxifen and GSK2850163 significantly reduced the invasion, migration, and colony formation abilities of breast cancer cells through improved the anti-cancer property of tamoxifen. Our results strongly suggested that IRE1α/XBP-1 inhibitors may be potent therapeutics in breast cancer treatment.

Keywords: *Breast Cancer, GSK2850163, IRE1α/XBP-1, Tamoxifen, UPR*

ÖZ

Amaç: *Tamoksifen meme kanserine karşı önde gelen tedavi olarak kullanılmaktadır ve son 40 yıldır yaygın olarak uygulanmaktadır. Ancak tamoksifene karşı direnç gelişimi meme kanserinin etkin tedavisindeki en büyük sınırlamalardan birisidir. Çalışmamızın amacı, UPR'nin IRE1α/XBP-1 dalının GSK2850163 tarafından bloke edilmesinin, tamoksifene dirençli MCF-7 hücrelerinin kanserojen yeteneğini verimli bir şekilde sınırlayıp sınırlamadığını araştırmaktır.*

Gereç ve Yöntem: *İlk olarak, MCF-7 hücrelerinin düzenli olarak tamoksifene maruz bırakılmasıyla tamoksifene dirençli meme kanseri hücreleri elde edildi. GSK2850163'ün biyokimyasal aktivitesi, immünoblotlama ve qRT-PCR ile doğrulandı. GSK2850163 ve tamoksifenin kombine tedavisinin tamoksifene dirençli meme kanseri hücrelerinin proliferasyon, invazyon, migrasyon ve koloni oluşturma yetenekleri üzerindeki olası etkileri sırasıyla WST-1 tabanlı proliferasyon testi, Boyden-chamber invazyon testi, yara iyileştirme testi ve plaka koloni oluşturma yöntemleri ile değerlendirildi.*

Sonuç ve Tartışma: *Çalışmamızda IRE1α/XBP-1'in GSK2850163 tarafından spesifik blokajının, tamoksifene dirençli MCF-7 hücrelerinin kanserojen yeteneğini verimli bir şekilde sınırladığını gösterdik. Ayrıca, tamoksifen ile GSK2850163'ün eş uygulaması tamoksifenin anti-kanser özelliğini geliştirerek meme kanseri hücrelerinin istila, göç ve koloni oluşturma yeteneklerini önemli ölçüde azalttı. Sonuçlarımız, IRE1α/XBP-1 inhibitörlerinin meme kanseri tedavisinde güçlü terapötikler olabileceğini önermektedir.*

Anahtar Kelimeler: *Meme Kanseri, GSK2850163, IRE1α/XBP-1, Tamoksifen, UPR*

INTRODUCTION

Breast cancer is the second reason of cancer-related deaths among women around the globe [1]. The receptor expression profiles of breast cancer cells directly affect the developmental profile in the carcinogenesis process. Today, we know that up to 75% of breast cancer cells possess estrogen receptors (Er) and/or progesterone receptors (PR) [2]. Selective estrogen modulator, tamoxifen behaves as an estrogen antagonist and prevents the binding of estrogens to the Er in breast tissue. In hormone receptor-positive breast tumors, tamoxifen is one of the most frequently used and effective chemotherapeutic agents [3]. It is used as a leading treatment against breast cancer and has been broadly applied for the last 40 years [4]. However, 20-30% of breast cancer patients develop de novo or acquired resistance to tamoxifen for various reasons [3,4]. Today, patients can have the chance of hormonal treatment according to the receptor status of the primary tumor. However, resistance development against tamoxifen is the main limitation in the effective treatment of breast cancer.

Recent studies have pointed out that Endoplasmic reticulum (ER) stress and the Unfolded Protein Response (UPR) mechanism are crucial key regulators in cancer progression and acquired drug resistance [5]. ER comprises 30% of the newly synthesized proteins in eukaryotic cells, therefore maturation processes of newly synthesized polypeptide chains are tightly controlled by protein quality mechanisms organized in the ER. Additionally, ER coordinates a variety of metabolic processes, such as synthesis of lipids, phospholipids, and steroids, biogenesis of autophagosomes and peroxisomes, and

gluconeogenesis [6,7]. Under altered physiological conditions, ER directly responds to adapt and protect the eukaryotic cells. Besides, various stressful conditions such as malformed protein accumulation, imbalance protein synthesis, and hypoxic conditions are caused by the disruption of the ER homeostasis, which is termed ER stress [6]. To readjust the ER homeostasis, the UPR signaling mechanism is activated, which is regulated through the ER-membrane localized three transmembrane proteins, activating transcription factor 6 alpha (ATF6α), protein kinase RNA-like ER kinase (PERK) and inositol requiring-enzyme 1 alpha (IRE1α) [6]. Recent studies have pointed out that IRE1α/XBP-1 branch of UPR is an important key regulator in the process of carcinogenesis, including prostate and breast cancer [8–10]. Notably, increased IRE1α/XBP-1 activity has been associated with the resistance of breast cancer cells to tamoxifen [11].

X-box binding protein-1 (XBP-1) (also known as unspliced XBP-1, XBP-1u) is an effector protein of IRE1α, which is activated through excising a 26-nucleotide-long intron in the XBP1 mRNA by the endoribonucleolytic activity of IRE1α. Activated XBP-1 termed spliced XBP1 (XBP-1s) function as a transcription factor and induces a specialized transcriptional program of UPR target genes under ER stress [12]. Previous studies have shown elevated expression levels of XBP-1 in therapy-resistant breast cancer cells [13,14]. Moreover, XBP-1s overexpression in Er (+) breast cancer enables estrogen-independent growth and less sensitivity to growth inhibition caused by tamoxifen and Faslodex independent of p53 [15]. Furthermore, activated XBP-1 has been shown to play a critical role in the tumorigenicity and progression of triple-negative breast cancer and also other cancer types [9,16–19]. Furthermore, higher ratios of XBP-1s/XBP-1u mRNA were determined in 100 primary breast cancer patients who received tamoxifen treatment [16]. Lastly, Ming et al. have determined that the modulation of IRE1α by STF083010 which inhibits the endonuclease activity of IRE1α, significantly increased tamoxifen sensitivity of MCF-7 cells, and progressively reduced breast carcinogenesis [11].

In this study, we evaluate the effect of the highly selective inhibitor of IRE1α, GSK2850163 effect on tamoxifen sensitivity and tumorigenic abilities of tamoxifen-resistant MCF-7 cells (termed MCF-7(R) hereafter). Our results suggest that GSK2850163 efficiently reverses tamoxifen resistance of MCF-7(R) cells. Moreover, tumorigenic abilities including proliferation rate, invasion-migration, and colony formation capability of MCF-7(R) cells are strongly limited by GSK2850163.

MATERIAL AND METHOD

Materials

Cell culture grade reagents including Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), and additional growth requirements were obtained from Biological Industries. Tamoxifen was obtained from Santa Cruz Biotechnology. GSK2850163 was obtained from Sigma Aldrich. Rabbit

polyclonal antibodies XBP-1u (25997-1-AP) and XBP-1s (24868-1-AP) were obtained from Proteintech and Mouse monoclonal beta-actin (#A5316) and polyclonal anti-phospho-IRE1α (#SAB5700519) were purchased from Sigma Aldrich. Rabbit monoclonal anti-IRE1α (#3294) was purchased from Cell Signaling Technology. HRP-conjugated anti-mouse or anti-rabbit IgG was purchased from Pierce.

Cell culture and treatments

MCF-7, a human breast cancer cell line was obtained from American Type Culture Collection (ATCC, USA). The MCF-7 cell lines were cultured and passaged in DMEM media supplemented with 10% FBS at 37°C and 5% CO_2. All the treatment compounds were prepared as 1000x in the DMSO or ethanol. The final concentration of solvent did not exceed 0.1%.

Establishment of Tamoxifen-resistant MCF-7 cells

To develop the tamoxifen-resistant MCF-7 cells were cultured at conventional cell culture conditions and integrities. Additionally, cell culture media was supplemented with 1 μM tamoxifen and then cells were cultured for 30 days.

Reverse Transcription PCR (RT-PCR)

Total RNA was extracted from cell pellets using Aurum™ Total RNA Mini Kit (Bio-Rad Laboratories, Hercules, California). Total RNA (1 μg) was subjected to reverse transcription reaction using iScript cDNA Synthesis kit (Bio-Rad Laboratories, Hercules, California). Specific primers were designed against XBP-1u/s and RPLP0. Sequence information of PCR primers is available upon request. The expression of RPLP0 was used for normalization. Target regions were amplified by Polymerase Chain Reaction (PCR). PCR products were separated by 3% agarose gel and visualized by Gel-Doc UV-transilluminator (Bio-Rad).

Western blot analysis

MCF-7 cell lysates were prepared by homogenizing cultured cells in RIPA buffer. After removal of the insoluble phase by centrifugation at 14.000 r.p.m. for 20 minutes at 4 °C, protein concentrations were determined using the Bicinchoninic acid assay (BCA) kit (Takara). Samples were resolved by SDS-PAGE and electroblotted to a PVDF membrane. Protein bands were visualized using enhanced chemiluminescence (Bio-Rad) by ChemiDoc XRS+ System (Bio-Rad). Protein bands were densitometrically analysed by Image Studio™ Lite (LI-COR®).

Proliferation assay

The cell proliferation rate of MCF-7(R) cells was evaluated in triplicates by WST-1 cell proliferation assay according to the manufacturer's protocol (Takara). 5000 cells/well were seeded into a 96-well plate. After 24h incubation period compounds were applied to the cells for 48h. Following 20

µl of WST-1 were added to the cells, and the cells were incubated under conventional cell culture conditions for 2h. The plate was read using a microplate reader (BioTek, Epoch 2) by measuring the absorbance of the dye at 450nm, with 600nm set as the reference wavelength. Averages of the absorbance values were calculated.

Colony formation assay

MCF-7(R) cells were seeded in a 6-well plate (1000 cells/ml). 24h later cells were treated with compounds and kept at 37 °C in a CO_2 incubator for 72h. The media was removed, and the plate was washed with 1xPBS solution twice. Colonies were fixed and stained with 0,05% crystal violet (Alpha Chemika)

Invasion assay

Matrigel (BD Biosciences) and serum-free DMEM were gently mixed (1:8) in a tube. A volume of 45 µl mixed Matrigel was used to cover the bottom of the transwell insert with an 8-µm pore size (Sarstedt). All chambers were placed at 37 °C in a CO_2 incubator for 3h. The residual liquid in the upper chamber was removed, 100 µl serum-free DMEM was added, and the chamber was placed in the incubator for 30 min hydration. MCF-7(R) cells (10000 cells) were seeded into the Transwell basket before the addition of 600 µl complete medium containing 20% FBS to the lower chamber. The cells were kept at 37 °C in a CO_2 incubator for 72h. Invaded cells were fixed and stained with crystal violet. Invaded cells were observed using Sunny SopTop microscope and camera system. The invasion was quantified by counting stained cells.

Wound-healing assay

MCF-7(R) cells were seeded in a 12-well plate ($3,5x10^5$ cells/well) and propagated at 37 °C in a CO_2 incubator for 24h. The following scratch was made in the 12-well plate with a 200 µl micropipette tip. Cell culture media was removed and cells were washed off for removing the detached cells with preheated PBS. Cells were treated with compounds for 72h. Scratch width ratios were monitored, and images were taken by microscope. Wound closure (%) was analysed by ImageJ software (http://imagej.nih.gov/ij/).

Statistics

Results were presented as means ± standard deviation (SD). The statistical significance of differences between groups was determined by a two-tailed equal variance Student's t-test with a confidence interval, minimum, of 95% using GraphPad Prism 5 software. Values of $p < 0.05$ were considered significant.

RESULT AND DISCUSSION

Obtaining Tamoxifen-resistant MCF-7 cells

To achieve tamoxifen-resistant MCF-7, conventional MCF-7 cells were continuously exposed to 1μM tamoxifen for 30 days and then cell viability of these cells and non-exposed MCF-7 cells were compared at 2μM, 5μM, and 10μM tamoxifen doses. Our data indicated that the MCF-7(R) cells exhibited significantly less sensitivity to tamoxifen at all three concentrations compared to the conventional MCF-7 cells (Figure 1A). Besides, tamoxifen-resistant MCF-7 cells are known to exhibit higher XBP-1s expression [15,16] thus we evaluated the endogenous XBP-1s levels by qRT-PCR and immunoblotting. To analyze the mRNA level of XBP-1s, XBP1s/ XBP1u ratio was used as a measure of XBP1 splicing activity. Our results showed that MCF-7(R) cells have significantly higher mRNA expression levels of XBP-1s compared to conventional MCF-7 cells (Figure 1B). Consistently with these data, XBP-1s protein levels were higher in MCF-7(R) compared to conventional MCF-7 cells (Figure 1B). Moreover, cell proliferation assay data confirmed the resistance of MCF-7(R) cells to tamoxifen compared to regularly propagated MCF-7 cells (Figure 1C).

Inhibition of the IRE1α branch of UPR by GSK2850163 sensitizes the tamoxifen-resistant MCF-7 cells to tamoxifen

GSK2850163 is a novel IRE1α inhibitor, which is specifically inhibited IRE1α kinase activity at 20nM and it also blockage IRE1α RNase activity affecting its endonuclease activity at 200nM [20]. In this assay system, we used well-known ER stress inducer, Thapsigargin as a positive control. Our results show that GSK2850163 treatment efficiently reversed Thapsigargin-induced splicing of XBP-1 at 20nM and 200nM doses (Figure 2A). In addition, kinase activity blockage of IRE1α at 20nM dose compared to 200nM treatment is less efficient for inhibition on XBP-1s (Figure 2A).

GSK2850163 significantly decreased the proliferation rate of MCF-7(R) cells

To evaluate the anticarcinogenic ability of IRE1α inhibition by GSK2850163 in breast cancer, we tested the tumorigenic features of MCF-7(R) cells by proliferation rate. Our results showed that GSK2850163 significantly decreased the proliferation rate of MCF-7(R). A combination of tamoxifen and GSK2850163 showed a more efficient regressive effect in MCF-7(R) ($p < 0.05$ for all tested groups) (Figure 2C).

GSK2850163 and tamoxifen synergistically reduce the invasion-migration and colony formation capability of MCF-7(R) cells

In these assay systems, we specifically aimed to investigate the effects of IRE1α inhibition on breast cancer cells by colony formation, invasion, and migration abilities. To evaluate the effect of

GSK2850163 on the invasion capability of MCF-7(R) cells, we modelized the invasion assay by using a Boyden-chamber assay. Our data showed that GSK2850163 and tamoxifen applied groups more effectively delimitate the invasion of breast cancer cells than only the GSK2850163 treated group (Figure 3B). Furthermore, we tested the migration ability of MCF-7 (R) cells by scratching assay, our data indicated that similar to the invasion assay, GSK2850163 significantly decreased the migration capacity of breast cancer cells (Figure 3C). Similar to migration assay results, the colony formation abilities of MCF-7(R) cells were significantly limited by GSK2850163 (Figure 3A). Moreover, more effective results were obtained with the combined application of GSK2850163 and tamoxifen for all cell-based assays (Figure 3A, B, C).

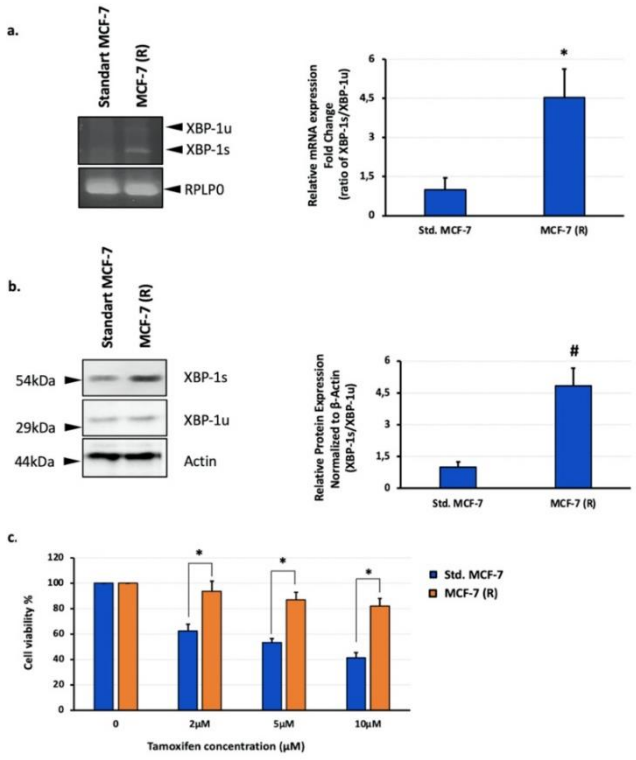

Figure 1. Confirmation of high expression level of XBP-1s in obtained tamoxifen-resistant MCF-7 cells. MCF-7 cells were exposed to 1μM tamoxifen for 30 days. a. Endogenous XBP-1s mRNA levels were detected by RT-PCR. RPLP0 gene is used as a housekeeping gene. b. Protein expression levels of XBP-1s were determined by immunoblotting and beta-actin was used as a loading control. Representative results are shown. p-values were calculated concerning vehicle-treated cells by two-tailed equal variance Student's t-test (*$p < 0.05$). c. The efficiency of tamoxifen resistance was evaluated with a WST-1 based cell growth assay of three biological and six technical replicates. Cells were treated with tamoxifen (2, 5, 10μM) for 48h. p-values were calculated concerning control group cells by two-tailed equal variance Student's t-test (*$p < 0.05$).

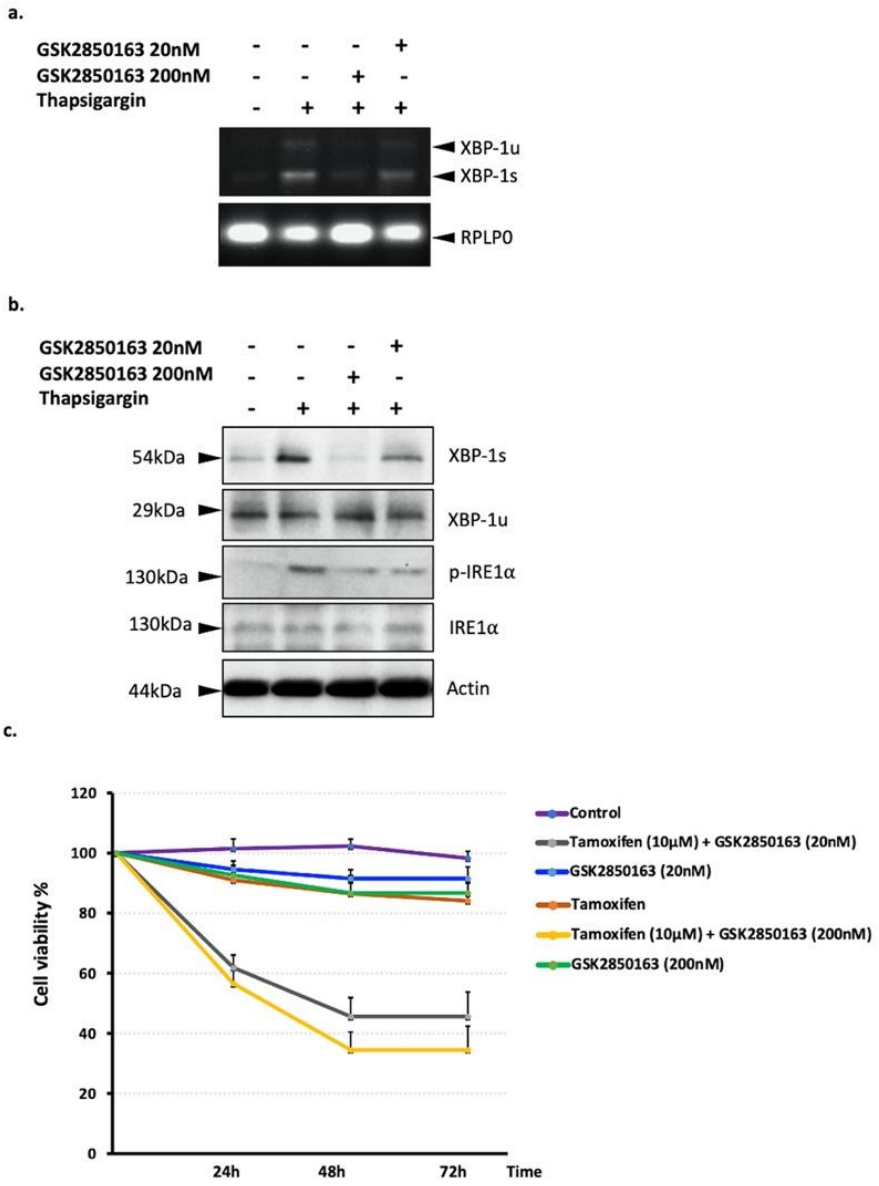

Figure 2. IRE1α/XBP-1 inhibitor GSK2850163 reverses the tamoxifen resistance of MCF-7(R). Cells were treated with 20 or 200nM GSK2850163 and Thapsigargin or combined with Thapsigargin and indicated doses of GSK2850163. a. XBP-1s levels were analyzed as in Fig. 1a. b. The expression levels of the related proteins were evaluated by immunoblotting. Beta-actin was used as a loading control. Representative results are shown. Thapsigargin was used as a positive control. c. The effect of GSK2850163 on tamoxifen sensitivity of MCF-7(R) cells was determined with cell viability assay, WST-1. Cells were treated with GSK2850163, tamoxifen, and co-treatment of GSK2850163 and tamoxifen as indicated doses. Results from three different experiments are presented in a graph.

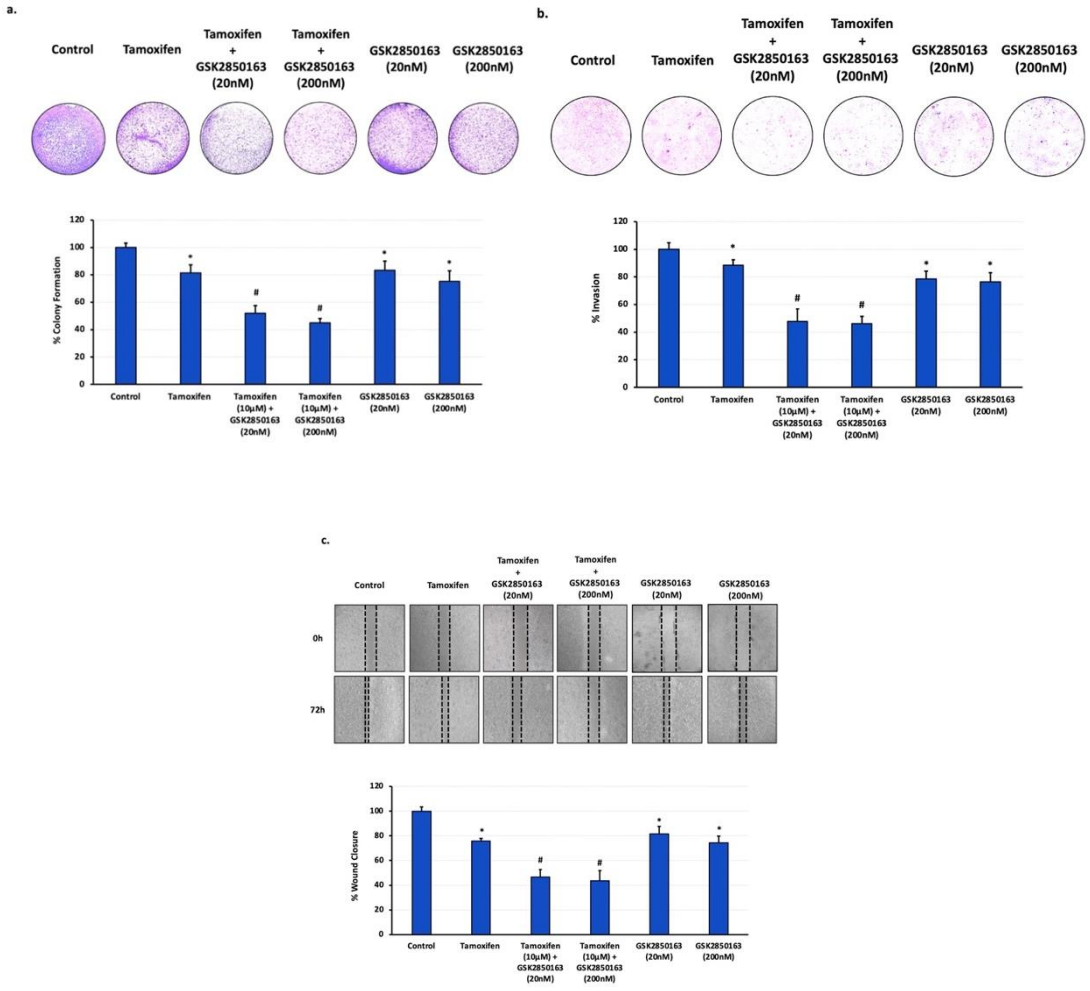

Figure 3. GSK2850163 significantly suppresses the tumorigenic properties of MCF-7(R) cells by acting synergistic effect with tamoxifen. a. MCF-7(R) cells were treated with 20 or 200nM GSK2850163 and tamoxifen or combined with tamoxifen and indicated doses of GSK2850163. Representative microscope images are presented. Quantification was performed with Image J software (*$p < 0.05$, #$p < 0.005$). b. The invaded MCF-7(R) cells on the lower surface of the membrane filter were fixed and stained with 0.2% crystal violet. Representative images are shown. The efficiency of invasion was quantified by counting stained cells with an inverted microscope. The mean percentage of invaded cells compared to control groups was given using the data obtained from two independent biological replicates in triplicates (*$p < 0.05$, #$p < 0.005$). c. A wound-healing assay was performed using MCF-7(R) cells. The closure of the gap created by the removal of the insert was monitored for 72h. The analysis of wound closure % was determined using the ImageJ software. Representative images are presented. p-values were calculated with respect to control group cells by two-tailed equal variance Student's t-test (*$p < 0.05$, #$p < 0.005$).

UPR is a key adaptive cytoprotective mechanism and mediates the adaptation of cells to changing physiological conditions [7]. In mammals, ER-resident three transmembrane proteins; IRE1α, PERK, and ATF6 are controlled the UPR [17]. Several researchers reported that the highly active IRE1α/XBP-1 is correlated with hepatocellular carcinoma, prostate, pancreatic, as well breast cancer [17–19]. Moreover, previous studies have shown that UPR activity is strictly associated with various features of tumor cells including invasion, migration, and angiogenesis. In particular, the IRE1α/XBP-1 has a critical role in implicated in the development of drug resistance in breast cancer [9]. Here, we indicated that GSK2850163, IRE1α dual inhibitor molecule which specifically inhibited IRE1α kinase activity RNase activity, is an important therapeutic molecule against drug-resistance development in breast cancer cells.

Firstly, we established tamoxifen-resistance MCF-7 cells by regularly exposing cells to a low dose of tamoxifen for 30 days. To validate the tamoxifen resistance, we performed cell viability tests and our results showed that MCF-7 (R) cells have less sensitivity to tamoxifen than standard MCF-7 cells by up to 60%. The previous study has demonstrated that tamoxifen-resistant MCF-7 cells exhibit a higher XBP-1s expression profile than standard cells [11]. Next, we investigated the expression level of XBP-1s and phospho IRE1α in MCF-7(R) and standard cells by immunoblotting assay, our results confirmed higher expression level of interested proteins in MCF-7(R) cells (Figure 1A, B). Collectively, these results showed that MCF-7(R) cells successfully mimic tamoxifen-resistant breast cancer.

GSK2850163 was discovered in an attempt to identify IRE1α-selective inhibitors of XBP-1 splicing that could regulate multiple myeloma cancer cells. It selectively inhibits the kinase and RNase activities of IRE1α [20]. In studies where we tested the efficiency of GSK2850163 treatment, we showed that thapsigargin-induced phospho-Ire1α and XBP-1s levels were significantly reduced compared to the thapsigargin group. As expected, we determined that 20nM dose of GSK2850163, which blocks the kinase activity of IRE1α, suppresses XBP-1s levels less efficiently than 200nM GSK2850163 administration (Figure 2B).

To investigate the limiting role of IRE1α/XBP-1 in tamoxifen-resistant breast carcinogenesis, we disrupted the IRE1α/XBP-1 by a specific inhibitor of IRE1α, which has a dual role inhibit IRE1α kinase activity and RNase activity for dose-dependent manner and evaluated the cell proliferation, invasive, migrative, and colony formation ability of tamoxifen-resistant breast cells. To evaluate the possible effect of the combined treatment of GSK2850163 and tamoxifen, MCF-7(R) cells were exposed and sensitivity to tamoxifen was determined by WST-1 based proliferation assay. Cell viability assay data showed that the GSK2850163 for both doses significantly reverses the tamoxifen resistance of MCF-7(R) cells. Besides, we observed that a higher dose of GSK2850163 treatment, which inhibits the

ribonuclease activity of IRE1α, is more efficient than a low dose of GSK2850163 in improving the tamoxifen sensitivity (Figure 2C).

Recent work has associated the IRE1α/XBP-1 branch of UPR with promoting tumor growth [19–22]. IRE1α/XBP-1 signaling is proposed as a functional mechanism of survival and adaptation for cancer cells. To investigate the possible effects of GSK2850163 on the tumorigenic abilities of tamoxifen-resistance breast cancer cells, we treated the MCF-7(R) cells either with the GSK2850163 at 20nM and tamoxifen, or GSK2850163 at 200nM and tamoxifen. Our data showed that combined treatment of GSK2850163 and tamoxifen synergistically affected the colony formation capability of MCF-7(R) cells. Continuity of clonogenic formation ability is an important limitation of cancerous tissues and cell-based assays evaluate the adhesion-independent cell proliferation of cancer cells [22,23]. Our results suggested that the administration of GSK2850163 in tamoxifen-resistant breast cancer cells significantly suppresses the colony-forming ability (Figure 3A).

Highly active migration and invasion ability are recognized as hallmarks of aggressive cancer [24]. We analyzed the limiter effect of GSK2850163 on the invasion of MCF-7(R) cells by using the matrigel-modified Boyden Chamber assay. Our data indicated that reducing IRE1α/XBP-1 significantly decreased the invasion ability of MCF-7(R) cells (Figure 3B). We also drastically obtained similar results in the wound-healing assay (Figure 3C).

The downstream target of IRE1α, XBP-1 has been suggested as a candidate oncogenic gene and is overexpressed in various cancers including prostate, oral squamous cell carcinoma, chronic lymphocytic leukemia as well as breast cancer [10,25–27]. Previous studies have shown that ectopically overexpressed XBP-1 resulted in metastasis in breast cancer [28,29]. Moreover, XBP-1 was reported to be significantly correlated with clinical outcomes in various tumors, such as breast carcinoma [28]. In addition, XBP-1s has been suggested as a critical regulator in drug resistance in certain subtypes of breast cancers [28].

The previous study has demonstrated that co-treatment with STF083010 inhibits the endonuclease activity of the IRE1α/XBP-1, without affecting its kinase activity, and tamoxifen significantly reduced breast cancer progression in a xenograft mammary tumor model [11]. Consistently with these results, our data suggest that targeting of kinase or endonuclease activity of IRE1α by GSK2850163 can restore the tamoxifen sensitivity of MCF-7(R) cells.

In conclusion, our study suggests that therapeutic approaches involving GSK2850163 may offer a potential therapeutic approach against breast cancer by overcoming tamoxifen resistance.

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AUTHOR CONTRIBUTIONS

Conception: *Y.E.;* Design: *Y.E.;* Supervision: *Y.E.;* Resources: *Y.E.;* Materials: *-;* Data Collection and/or processing: *Y.E., H.K.D., D.C.;* Analysis and/or interpretation: *Y.E.;* Literature search: *Y.E., H.K.D., D.C.;* Writing manuscript: *Y.E.;* Critical review: *Y.E., H.K.D., D.C.;* Other: -

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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