

Article

SOX2OT Long Noncoding RNA Is Regulated by the UPR in Oestrogen Receptor-Positive Breast Cancer

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Abstract: Endoplasmic reticulum (ER) stress perturbs cell homeostasis and induces the unfolded protein response (UPR). In breast cancer, this process is activated by oestrogen deprivation and is associated with tamoxifen resistance. We present evidence that the transcription factor SOX2 and the long noncoding RNA SOX2 overlapping transcript (SOX2OT) are up-regulated in oestrogen receptor-positive (ER+) breast cancer and in response to oestrogen deprivation. We examined the effect of the UPR on SOX2 and SOX2OT expression, and the effect of SOX2OT on UPR pathways in breast cancer cell lines. The induction of the UPR by thapsigargin or glucose deprivation up-regulates SOX2OT expression. This up-regulation is also shown with the anti-oestrogen 4OH-tamoxifen and mTOR inhibitor everolimus in ER + breast cancer cells that are sensitive to oestrogen deprivation or everolimus treatment. SOX2OT overexpression decreased BiP and PERK expression. This effect of SOX2OT overexpression was confirmed on BiP and PERK pathway by q-PCR. Our results show that a long noncoding RNA regulates the UPR and evince a new function of SOX2OT as a participant of ER stress reprogramming of breast cancer cells.

Keywords: Breast cancer; LncRNA; SOX2OT; SOX2; UPR; 4-OH tamoxifen

1. Introduction

Despite extensive studies in breast cancer and more detailed knowledge of its molecular pathways, many aspects of breast cancer cell regulation are still enigmatic. Recent genomic and transcriptomic analyses showed that most of the transcripts are long noncoding RNAs (lncRNAs) and evidence for regulatory roles for lncRNAs continues to rise. The dysregulation of several lncRNAs in breast cancer has been reported [1–3] and the functions of this class of transcript require elucidation. Within an intronic region of the gene specifying the SOX2 overlapping transcript (SOX2OT) lncRNA lies the SOX2 gene, one of the main regulators of pluripotency (Figure 1, [4]). As described previously [4], SOX2 and SOX2OT are differentially expressed in ER+ and ER- breast

cancer. They are both up-regulated in suspension culture under conditions that prioritize spheroid formation. Hence we suggest that in breast cancer, *SOX2OT* is key to the regulation of *SOX2* expression. However, the mechanism of action of *SOX2OT* in breast cancer remains to be fully defined. Expression analysis of murine *Sox2* and *Sox2OT* in different developmental systems has also elucidated the dynamically changing expression patterns of these two RNA species, and has suggested important roles for these genes in normal development [5].

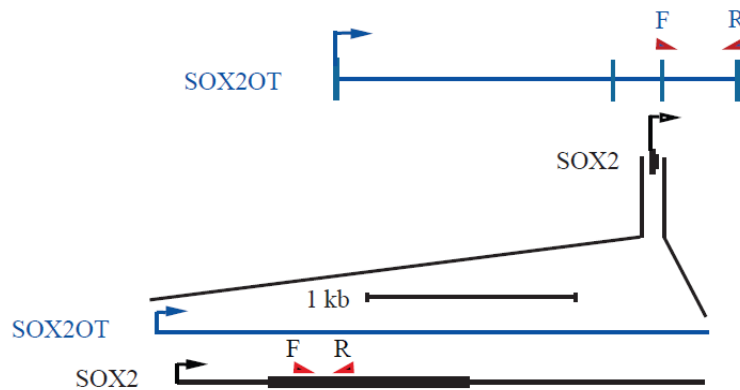


Figure 1. Schematic of the *SOX2* and *SOX2OT* genes. The *SOX2* gene lies in an intronic region of the *SOX2 Overlapping Transcript* gene (*SOX2OT*). The direction of the transcription is shown with arrows. The triangles above each gene show the forward (F) and reverse (R) primers used in PCR. The *SOX2* gene is enlarged.

The dysregulation of *SOX2OT* and *SOX2* expression has also been shown in cancers such as glioma and kidney carcinoma [5], and significant correlations in expression of these two genes were found in breast cancer [4], as well as in oesophageal [6] and lung squamous cell carcinoma [7–9]. We have previously shown in breast cancer that the differential expression of *SOX2* and *SOX2OT* is oestrogen receptor-dependent. *SOX2OT* and *SOX2* are more highly expressed in oestrogen receptor-positive (ER+) than in ER-negative (ER-) breast cancer cell lines [4]. Interestingly, high expression levels of *SOX2OT* and *SOX2* are associated with the sensitivity of breast cancer cells to tamoxifen [4]. To further investigate the mechanism of action of *SOX2OT* in breast cancer, we have examined the role of the unfolded protein response (UPR) pathway in expression of this lncRNA.

Multiple mechanisms have been described for tamoxifen resistance of breast cancer. One of those pathways is known as the unfolded protein response (UPR). Three endoplasmic reticulum (ER) stress transducers defining three distinct axes of the UPR have been identified so far and characterized as components of the UPR activation pathway (Figure 2). IRE1, PERK and ATF6 are the three transmembrane inducers of ER-stress [10]. These three regulators of UPR are controlled by the ER chaperone BiP, constitutively bound to them but dissociated under ER stress. The UPR transiently inhibits protein synthesis and induces the production of chaperone molecules in order to restore ER homeostasis and promote cell survival [11]. The failure of this rescue mechanism results in apoptotic cell death [12].

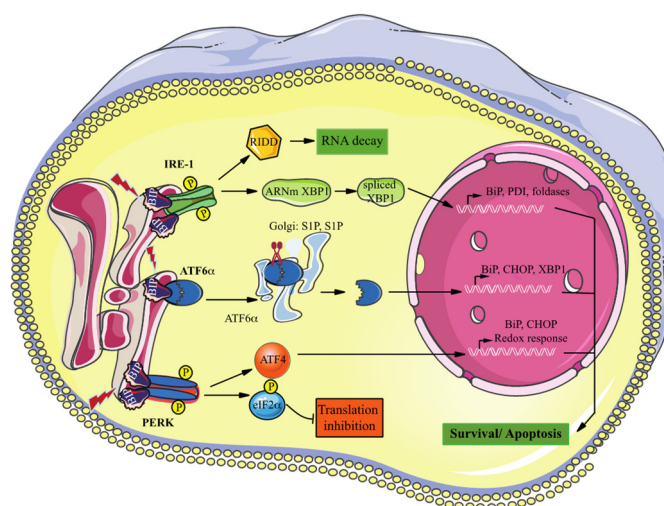


Figure 2. Schematic of pathways involved in the UPR.

UPR activation is associated with poor prognosis in breast cancer [13,14]. Breast cancer cells of all subtypes have elevated UPR signalling with elevated BiP expression [15,16]. The UPR may favour oestrogen-dependent breast cancer survival when oestrogen availability is low [17–19]. The IRE-1 pathway is activated in ER+ breast cancer: it induces the splicing of XBP1 mRNA, consequently increasing the abundance of its pro-survival target XBP1s, which are strongly correlated with ER alpha expression in breast cancer [14,20]. It has been shown that silencing of XBP1, or inhibition of IRE1 by the pharmacological inhibitor STF-80310 or MKC866, reverses resistance to anti-oestrogen therapies [21–23]. XBP1s expression promotes the survival of triple-negative breast cancer (TNBC) cells [24]. Finally, the PERK pathway is activated in breast cancer cells resistant to tamoxifen [20,21,25].

The ability of the UPR to regulate gene expression and protein synthesis and its contribution to oncogenesis have been well documented. Recent results have shown that the UPR can regulate post-transcriptional networks either by regulated IRE1-dependent decay (RIDD) of selected mRNAs [26] or by modulation of expression of micro-RNAs [27]. The UPR can also suppress the expression of several miRNAs that regulate either the PERK or the IRE1 pathway. However, an added layer of regulatory complexity that entails the role of lncRNA in the UPR pathway has been barely explored. Here, we investigate the gene expression pattern of *SOX2OT* in a UPR-induced system. Our results show that *SOX2OT* expression can be up-regulated by ER stress inducers and that this lncRNA can also down-regulate the PERK pathway and BiP expression in ER+ breast cancer cell lines.

2. Material and Methods

2.1. Cell Lines

As previously described, the MCF-7 cell line was purchased from the ATCC, grown in alpha-MEM containing 5% foetal calf serum (FCS), penicillin/streptomycin (100 U/mL and 100 µg/mL respectively), and insulin/transferrin/selenium supplement (Roche). Long-term oestrogen-deprived sub-lines TamC3 and TamC6 were generated by growing the MCF-7 cells in oestrogen-deprived medium (phenol red-free RPMI 1640 (GIBCO) with 5% charcoal-stripped foetal bovine serum) and penicillin/streptomycin (10 U/mL and 10 µg/mL), for 10 months, [28–31]. The T47D cell line was purchased from the ATCC, grown in alpha-MEM containing 5% foetal calf serum (FCS) and penicillin/streptomycin (100 U/mL and 100 µg/mL).

For glucose depletion, cells were seeded in 6-well plates and maintained 48 h under normal culture conditions, i.e., 25 mM glucose concentration, 5% FCS, 5% CO₂, 21% O₂. The medium was replaced with no glucose DMEM (GIBCO) containing 5% FCS, penicillin/streptomycin (100 U/mL and 100 µg/mL), and insulin/transferrin/selenium supplement (Roche).

2.2. Reagents

Thapsigargin was obtained from Applichem (St. Louis, MO, USA). PERK inhibitor GSK2656257 and IRE-1 inhibitor SFT-080310 were purchased from Selleckchem (Houston, TX, USA). Anti-PERK (No. 3192) antibody was purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-BIP antibody (610978) was from BD Laboratories™ (Franklin Lakes, NJ, USA). Antibody against α -tubulin was from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Protein Extraction and Western Blot Analysis

As described in detail previously [29], breast cancer cell lines were grown to log-phase, washed twice with ice-cold PBS, and lysed in SDS lysis buffer according to the manufacturer's protocol (Cell Signaling Technology, Danvers, MA, USA). Protein concentration was quantified using the bicinchoninic acid reagent (Sigma). Cell lysates containing 20 μ g of protein were separated by electrophoresis on a 4–10% SDS-PAGE gel (Life Technologies) and transferred to a polyvinylidenedifluoride membrane (PVDF) (Millipore, Billerica, MA USA). Blocking of non-specific binding was achieved in a 0.1% Tween 20 Tris-buffered saline solution containing 5% *w/v* non-fat dry milk powder for 1 h. Membranes were incubated with primary antibodies overnight at 4 °C, washed and incubated with the corresponding immunoperoxidase-conjugated secondary antibody (Santa Cruz Biotechnology) for 1 h at room temperature. Bound antibody was visualized using SuperSignal West Pico (Thermo Scientific, Waltham, MA, USA) or ECL Select (Amersham) and the chemiluminescence detection system by Fujifilm Las-3000. To avoid cross-detection between phosphorylated and total forms of the protein, membranes were stripped.

2.4. Reverse Transcription, cDNA Synthesis and Quantitative PCR and PCR

As described in detail previously [4], oligo-dT and random primers were used to reverse transcribe RNA with qScript Flex cDNA kit (Dnature) according to the manufacturer's instructions. For qRT-PCR analysis, qPCR was performed using gene-specific primers (Supplementary Materials Table S1) and Sybr Green MasterMix (Life Technologies), and expression values normalized relative to *GAPDH* and *HPRT* mRNA expression.

2.5. Ectopic Expression of SOX2 and SOX2OT

This has been described in detail previously [4]. Constructs overexpressing SOX2 (NM_003106) and SOX2OT (NR_004053) and control empty plasmid (vector), Ex-NEG-M95 and EX-hLUC-M90 respectively, were purchased from GeneCopoeia. Both plasmids express SV40-mCherry-IRES-puromycin resistance, allowing detection of transfected cells. Breast cancer cells (MDA-MB-231) were chosen because of the low expression of SOX2OT and SOX2 as we have previously shown [4]. The cells were transfected with 5 μ g of DNA and Lipofectamine Plus (Invitrogen) according to the manufacturer's instructions. Three biological replicates for each construct were made, the transfected cells treated with puromycin and selected on the basis of mCherry expression by fluorescence-activated cell sorting (FACS), as previously described [4]. The sorted cells were maintained in the presence of puromycin.

2.6. Statistical Analysis

Results are presented as mean \pm SEM. As described in detail previously [4], t-tests or Mann-Whitney Rank Sum Tests was used for comparison between two groups. Correlation analysis was performed with Pearson's rank correlation coefficient (R) and statistical significance (P) using SigmaPlot. $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***) were indications of statistical significance.

3. Results

3.1. Relationship between Expression of SOX2OT and ER Stress-Inducible Genes in Breast Cancer

SOX2OT and SOX2 transcripts are upregulated in tamoxifen-resistant cell lines [4,32]. The activation of the UPR in ER+ breast cancer cell lines following tamoxifen treatment has also been shown previously [33–35]. We initially analysed the genome wide-RNA transcript profile of breast cancer samples from The Cancer Genome Atlas (TCGA) by RNAseq dataset (TGCA8BRCA_exp_HiSeqV2-2015-02-24-160222) including 1025 samples from breast cancer patients. Interestingly, we found different patterns of expression of UPR-inducible genes relative to SOX2OT, but not SOX2 (Figure 3). As shown in Table 1, expression of XBP1 and SOX2OT were positively correlated (Pearson's correlation coefficient $r = 0.34$, $p = 3.6 \times 10^{-34}$). This analysis showed no correlation between the expression of BiP and SOX2OT. A weak positive correlation was found for PDIA4 ($r = 0.156$, and $p = 4.5 \times 10^{-8}$), while the expression of the other genes examined was negatively correlated with that of SOX2OT. However, we found no correlation of ER stress-inducible genes with SOX2 with the exception of PDIA4 ($r = 0.13$, $p = 8.6 \times 10^{-6}$). We next examined the expression of SOX2 and SOX2OT relative to ER stress-inducible genes, i.e., the transcription factors XBP1, ATF4, and CHOP/GADD153, as well as the genes coding for the chaperone protein BiP/Hsp78, GADD34/pp1r15a and PDIA4.

Table 1. Pearson correlation coefficients of relative gene expression for SOX2OT and SOX2 compared to the expression of genes of the UPR pathways in TGCA samples (n = 1025).

	ATF4	CHOP	GADD	PDIA4	XBP1	BiP
SOX2OT	-0.194	-0.172	-0.192	0.156	0.34	0.0165
<i>p</i> value	8.62×10^{-12}	1.49×10^{-9}	1.62×10^{-11}	4.49×10^{-8}	3.59×10^{-34}	0.565
SOX2	0.00301	0.0437	-0.0522	0.127	0.051	0.0517
<i>p</i> value	0.917	0.128	0.0689	8.56E-6	0.0756	0.0714

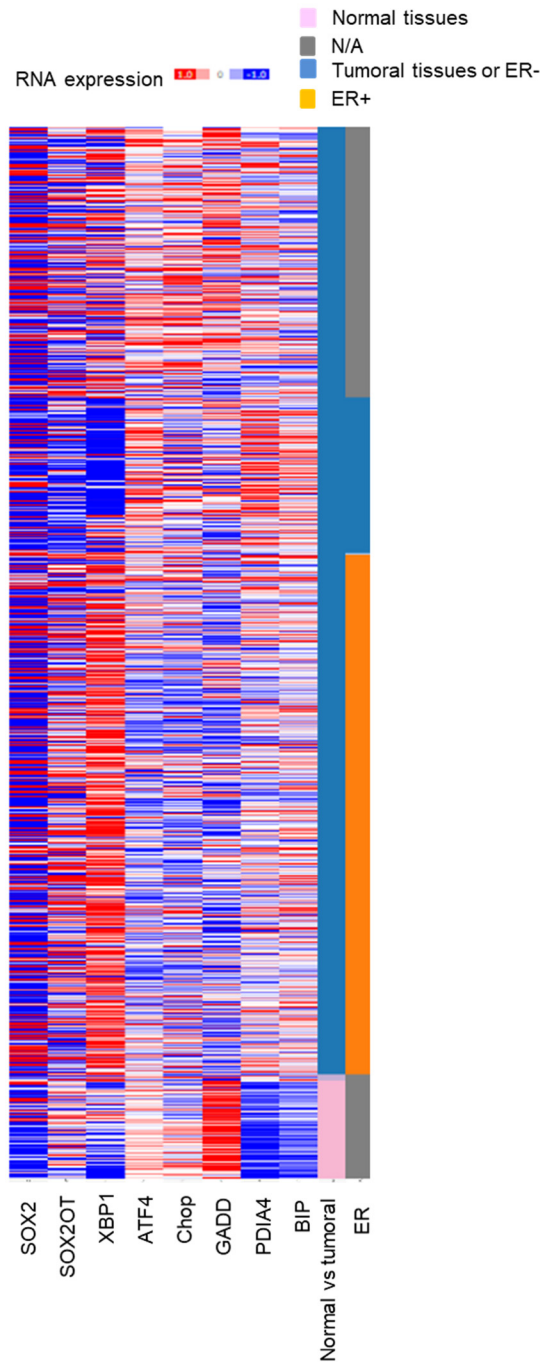


Figure 3. Heat map showing the expression of *SOX2*, *SOX2OT* and UPR-related genes. It shows the expression of genes in the PERK pathway (*ATF4*, *CHOP/ddit3*, *GADD/pp1r15a*), ER-associated degradation (*BiP/hspa5*, *PDIA4*) and the IRE1 pathway (*XBP1*) in breast cancer samples. Data were from TCGA invasive breast carcinoma (TCGA8BRCA_exp_ HiSeqV2-2015-02-24-160222). The samples were classified based on their receptor status (oestrogen receptor (ER), HER2 or progesterone receptor (PR)).

3.2. *SOX2OT* Expression Is up-Regulated in ER + Stressed Cells

To investigate whether lncRNA *SOX2OT* or *SOX2* is differentially expressed by ER stress, two breast cancer cell lines (MCF-7 and T47D) were treated with the UPR inducer thapsigargin (Tg). We also studied the effect of two other drugs used to treat ER+ breast cancer cells: the anti-oestrogen 4OH-Tam and the mTOR inhibitor everolimus, both of which have been shown to induce the UPR in breast cancer cell lines [36] and in MCF-7 and T47D (Supplementary Materials Figure S1). Cells were

assessed for PERK phosphorylation and BiP induction as markers of UPR activation using western blot analysis (Supplementary data S1). After 16h of treatment with Tg, a significant upregulation of *SOX2OT* expression was detected in both cell lines (9.3 ± 2.26 fold in MCF-7 cells and 3.8 ± 0.96 fold in T47D cells) (Figure 4). This induction was also observed for *SOX2* expression in both cell lines. Treatment with 4OH-Tam and everolimus also up-regulated *SOX2OT* in MCF-7 cells, while everolimus significantly induced *SOX2OT* in T47D cells. The *SOX2* level was significantly up-regulated in MCF-7 and T47D cells treated with Tg or 4OH-Tam but not with everolimus.

MCF-7 cells were also grown under conditions of glucose deprivation, an intrinsic inducer of the UPR. After 48h of cultivation in glucose-depleted medium, significant increases in the expression of *SOX2OT* and *SOX2* were detected (Figure 4C).

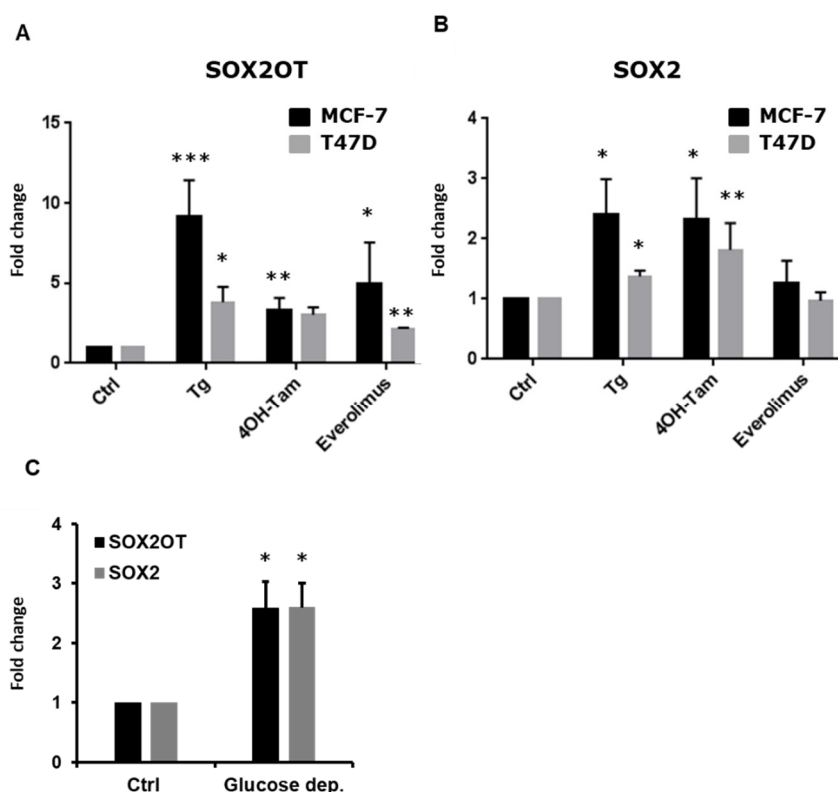


Figure 4. Expression of *SOX2OT* and *SOX2* following UPR induction in ER+ breast cancer cell lines. MCF-7 and T47D cells were incubated in control medium (Ctrl) or in medium containing the ER stress-inducing agents thapsigargin (Tg, 300 nM), 4OH-Tam (0.1 μ M) or everolimus (10 nM) for 16 h. A, B: The effects of Tg, 4OH-Tam and everolimus on the expression of *SOX2OT* and *SOX2* were measured by qRT-PCR and are relative to the house keeping genes *HPRT* and *GAPDH*. C: MCF-7 cells were cultivated in media containing 25 mM glucose (Ctrl), or lacking glucose (Glucose dep) for 48h. The expression of *SOX2OT* and *SOX2* was measured by qRT-PCR and is relative to *HPRT* and *GAPDH*. Error bars represent standard deviations of at least 3 independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

As the UPR is up-regulated in tamoxifen-resistant cells, we analysed the effects of Tg, 4OH-Tam and everolimus in two oestrogen-deprived and -resistant breast cancer cell lines derived from MCF-7 cells: TamC3 and TamC6 [28–31]. We have previously shown that the sensitivity to mTOR inhibition of these two cell lines is different: TamC6 cells are highly sensitive to everolimus as compared to TamC3 cells [29–31]). Here we show that Tg up-regulated the expression of *SOX2OT* in both cell lines while *SOX2* is up-regulated only in the TamC6 cell line (Figure 5). 4OH-Tam did not induce the expression of these genes in TamC6 cells whereas in TamC3 *SOX2OT* expression was increased

(Figure 5). Everolimus, on the other hand, induced a significant increase of *SOX2OT* expression in TamC6 cells but not in TamC3 cells, while *SOX2* was upregulated by everolimus in both cell lines.

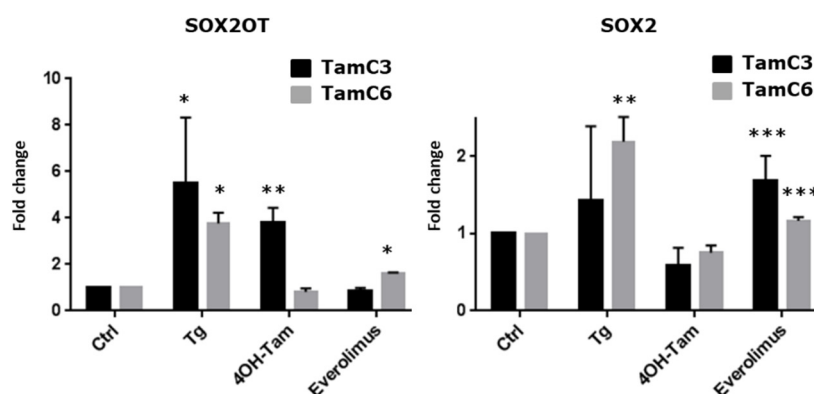


Figure 5. Expression of *SOX2OT* and *SOX2* following UPR induction in oestrogen-deprived cancer cells. TamC3 and TamC6 were incubated in control medium (Ctrl) or in medium containing the ER stress-inducing agents thapsigargin (Tg, 300 nM), 4OH-Tam (0.1 μ M) or everolimus (10 nM) for 16h. The expression of *SOX2OT* and *SOX2* was measured by qRT-PCR and is relative to that of *HPRT* and *GAPDH*. Error bars represent standard deviations of 3 independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.3. *SOX2OT*, but not *SOX2*, Regulates *BiP* Expression and *PERK* Activation

To further study the role of the *SOX2OT* transcript in the UPR in breast cancer, we used the MDA-MB-231 cell line, which has low expression of *SOX2OT*, and in which we had induced ectopic expression of *SOX2OT* lncRNA [4]. The activation of the UPR pathway in cells overexpressing either *SOX2OT* or *SOX2* was studied at a transcriptional level, quantifying *BiP* and the *PERK* targets *ATF4* and *CHOP* by RT-qPCR. When *SOX2OT* was overexpressed, *BiP* and *CHOP* expression was significantly reduced, to 50% of control values (Figure 6A) while *XBP1s* mRNA expression was not significantly changed (Figure 6B). However, in *SOX2* overexpressing cells *ATF4* was upregulated while *CHOP* and *BiP* showed no significant change (Figure 6A). *XBP1s* mRNA was quantified by RT-qPCR and was not modified by *SOX2* overexpression (Figure 6B). We further investigated whether *SOX2OT* could inhibit *BiP* and the *PERK* by western blot analysis for *BiP* and *PERK* expression. Our results show that the expression of *BiP* and *PERK* decreased in *SOX2OT* overexpressing cells (Figure 6C).

A positive correlation for the expression of *SOX2OT* and *SOX2* has been reported [4,5]. Therefore, we examined whether the down-regulation of *PERK* and *BiP* was dependent on the *SOX2* transcription factor or whether *SOX2OT* reduced the expression of these proteins independently of *SOX2*. In *SOX2*-overexpressing cells (Supplementary Materials Figure S2), when *BiP*, *CHOP* and *ATF4* were quantified at the mRNA level by RT-qPCR, *ATF4* was slightly but significantly up-regulated compared to the control cells (Figure 6A) (Fold change = 1.32, $p < 0.001$).

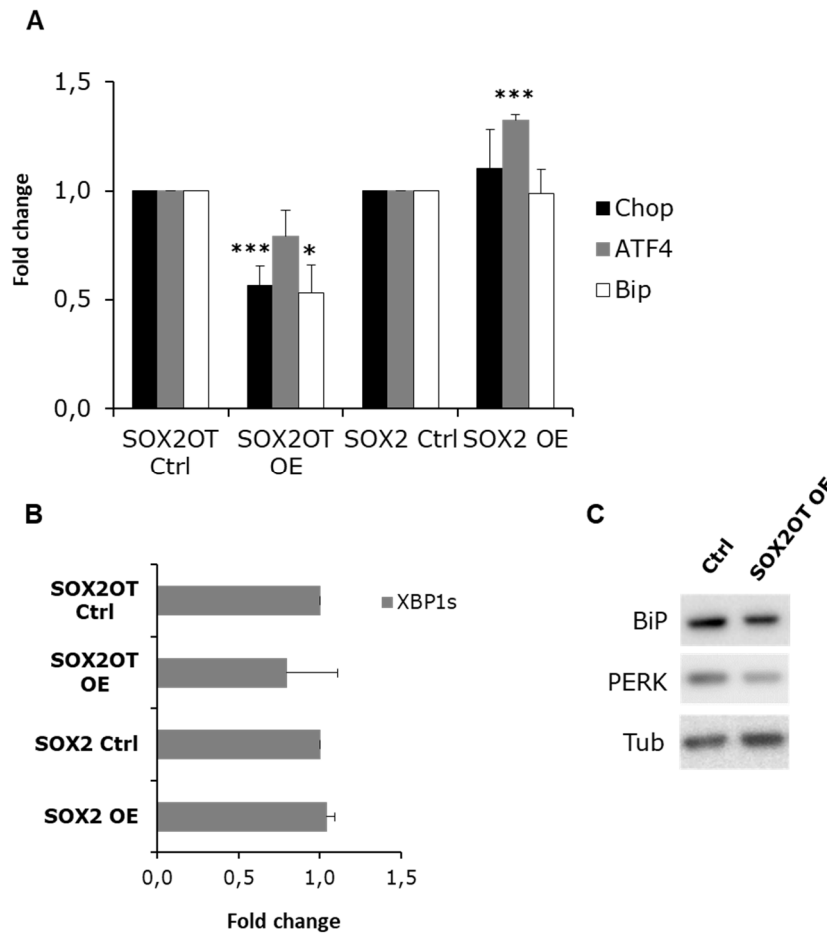


Figure 6. Effect of ectopic expression of *SOX2OT* or *SOX2* on the UPR signalling pathway. MDA-MB-231 cells were transfected with plasmids containing the *SOX2OT* gene (NR_004053.3) or the *SOX2* gene (NM_003106), or with their respective control vectors. **A:** Expression of *BiP*, *ATF4* and *CHOP* relative to that of *HPRT* and *GAPDH* was measured by qRT-PCR. **B:** Relative expression of *XBP1s* was measured by qRT-PCR relative to that of *HPRT* and *GAPDH*. **C:** Protein expression levels of *BiP*, and *PERK* were examined using Western Blot analysis. α -tubulin was used as loading control. Error bars represent standard deviations of 3 independent clones. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

Since the discovery of the adaptive response to the disruption of endoplasmic reticulum homeostasis, the UPR has emerged as having a major role in modulating the expression of cancer-related genes, notably through transcriptional or post-transcriptional changes. Here we describe a new layer of regulatory mechanisms of the UPR, effected by the lncRNA *SOX2OT*.

Thousands of lncRNAs have been identified in cancer cells [37], but very little is known about their functions and mechanisms of action. Their localization in varying subcellular compartments (nucleus or cytoplasm), and their ability to bind to a variety of targets suggest that they should not only regulate gene expression but also have an effect on post-transcriptional regulation or structural interaction. *SOX2OT* was first described in 2009 [3] and little is known about its role and the regulation of its expression. *SOX2OT* is expressed in different cancers, including breast [4,38,39], oesophagus [6] and lung [7,9]. Its effect on proliferation depends on the cell line studied. In lung cancer cell lines *SOX2OT* inhibits cell cycle progression by regulating expression of the histone-lysine N-methyltransferase enzyme *EZH2* [7]. Conversely in breast cancer cell lines, *SOX2OT* overexpression reduced proliferation and increased anchorage-independent growth [4].

In this study we have shown that two ER-stress inducers —the decrease of glucose availability or the inhibition of the endoplasmic reticulum Ca^{2+} -ATPase by Tg— induced the expression of *SOX2OT*. Everolimus and 4OH-Tam, two drugs that are used to treat breast cancer and that are known to induce the UPR in breast cancer cell lines [21,40], also induced an up-regulation of the *SOX2OT* transcript, suggesting that *SOX2OT* and the UPR are related. This was confirmed using the MDA-MB-231 TNBC (triple negative breast cancer) cell line that has a very low expression of *SOX2OT* and a strong expression of BiP. Here, we have shown that the overexpression of *SOX2OT* down-regulates BiP expression and PERK pathway activity.

SOX2 lies in an intron of the *SOX2OT* gene and is positively correlated with *SOX2OT* expression in breast cancer [4]. Therefore *SOX2OT* is proposed to contribute to the transcriptional regulation of *SOX2* [6,7]. We observed that when MCF-7 or T47D breast cancer cells were treated with 4OH-Tam, *SOX2* expression and *SOX2OT* expression increased concordantly. However, *SOX2* over-expression neither decreased BiP expression nor affected the PERK pathway. These results show that the effect of *SOX2OT* on BiP or PERK pathway (measured by *ATF4* and *CHOP* RNA expression level) is independent of *SOX2*. Thus, *SOX2OT* has other targets yet to be identified. Furthermore, when the UPR was activated by Tg or glucose depletion, we observed that the correlation between *SOX2* and *SOX2OT* abundance was lost, suggesting that other factors regulate *SOX2* expression. This dissociation of *SOX2OT* and *SOX2* expression is also observed in TNBCs that have down-regulated *SOX2OT* but still expressed *SOX2* [4]. Feng et al. [41] have demonstrated that in TNBC, cells that are prone to EMT have a high level of expression of BiP. This high expression may arise in part from *SOX2OT* down-regulation and may promote the selection of more aggressive phenotypes or stem cell differentiation.

Other lncRNA molecules are known to be regulated by the UPR including *MALAT1* [42]. Its splicing is enhanced by PERK during infection by flavivirus [41]. These results suggest that the activation of the PERK pathway not only enhances the translation of specific mRNAs or miRNA but also the expression of lncRNAs. The analysis of TCGA breast cancer samples showed a positive correlation between *XPB1* and *SOX2OT*, suggesting that the IRE1 pathway could also regulate lncRNA.

Numerous studies have demonstrated that oestrogen deprivation induced an UPR [33–35]. In ER+ breast cancer cell lines, ER alpha-targeted therapy increased aggregation of ER alpha in the cytoplasm and increased UPR signalling [43]. The activation of UPR contributes to the development of resistance to oestrogen deprivation as demonstrated by the overexpression of *XPB1s* [23] and BiP [44,45] or the activation of the PERK pathway [20,21,25]. As *SOX2OT* is expressed early during UPR activation, it can participate in the reprogramming of gene expression by the UPR during the acute phase of oestrogen deprivation-mediated stress and lead to the emergence of resistant phenotypes. When treated with 4OH-Tam, MCF-7, T47D or TamC3 cells increased the expression of *SOX2OT* whereas in TamC6 cells, 4OH-Tam has no effect on *SOX2OT* expression, suggested that in some resistant cell lines, upon chronic induction of the UPR, the mechanisms of regulation of *SOX2OT* expression are lost. The same observation was made following treatment with the mTOR inhibitor everolimus that has no effect on *SOX2OT* expression when cells are resistant to mTOR inhibition.

Together our results show that long non-coding RNA can be considered a regulator of the UPR and provide evidence for a new function of *SOX2OT* as a participant of ER stress reprogramming of breast cancer cells. Therefore, overcoming *SOX2OT* overexpression and its effect on the PERK pathway or on *SOX2* expression could result in the adaptation of the cells to ER stress and consequently in the resistance to anti-oestrogen treatment, and/or the promotion of EMT (Figure 7). Further investigations are needed to determine whether *SOX2OT* may provide a basis for new therapeutics to modify UPR in breast cancer.

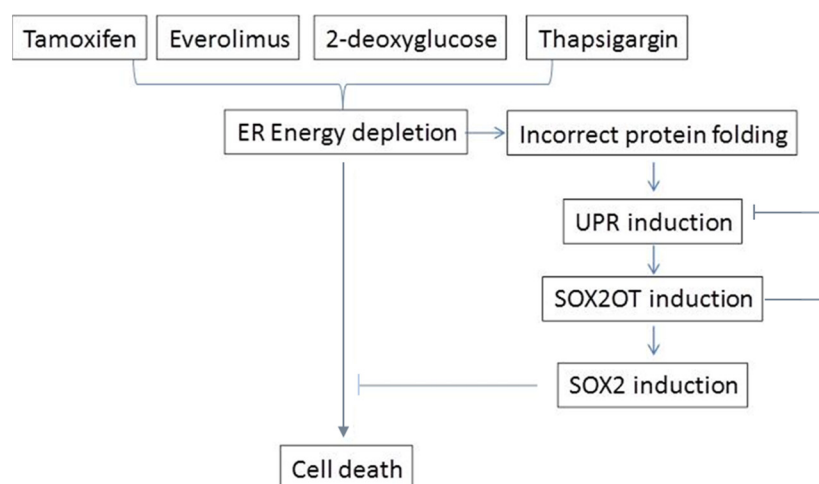


Figure 7. Hypothetical schema of *SOX2OT* lncRNA and UPR interrelation.

Supplementary Materials: The following are available online at www.mdpi.com/2413-4155/2/2/24/s1.

Availability of data and materials: Data and materials are available on request from the corresponding authors.

Authors contributions: C.F.P., E.Y.L. and M.A.A: designed the experiments; C.F.P., D.S. and W.J. performed the experiments; C.F.P., E.Y.L., H.H. and M.A.A. provided reagents, materials, or analysis tools; C.F.P., D.S. analyzed the data; C.F.P., E.Y.L., M.A.A. and B.B.: wrote the paper. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

References

- Hansji, H.; Leung, E.Y.; Baguley, B.C.; Finlay, G.J.; Askarian-Amiri, M.E. Keeping abreast with long non-coding RNAs in mammary gland development and breast cancer. *Front. Genet.* **2014**, *5*, 379.
- Su, X.; Malouf, G.G.; Chen, Y.; Zhang, J.; Yao, H.; Valero, V.; Weinstein, J.N.; Spano, J.-P.; Meric-Bernstam, F.; Khayat, D.; et al. Comprehensive analysis of long non-coding RNAs in human breast cancer clinical subtypes. *Oncotarget* **2014**, *5*, 9864–9876.
- Youness, R.A.; Gad, M.Z. Long non-coding RNAs: Functional regulatory players in breast cancer. *NonCoding RNA Res.* **2019**, *4*, 36–44.
- Askarian-Amiri, M.E.; Seyfoddin, V.; Smart, C.E.; Wang, J.; Kim, J.E.; Hansji, H.; Baguley, B.C.; Finlay, G.J.; Leung, E.Y. Emerging Role of Long Non-Coding RNA *SOX2OT* in *SOX2* Regulation in Breast Cancer. *PLoS ONE* **2014**, *9*, e102140.
- Amaral, P.P.; Neyt, C.; Wilkins, S.J.; Askarian-Amiri, M.E.; Sunkin, S.M.; Perkins, A.C.; Mattick, J.S. Complex architecture and regulated expression of the *Sox2ot* locus during vertebrate development. *RNA* **2009**, *15*, 2013–2027.
- Shahryari, A.; Rafiee, M.R.; Fouani, Y.; Olliae, N.A.; Samaei, N.M.; Shafiee, M.; Semnani, S.; Vasei, M.; Mowla, S.J. Two novel splice variants of *SOX2OT*, *SOX2OT-S1*, and *SOX2OT-S2* are coregulated with *SOX2* and *OCT4* in esophageal squamous cell carcinoma. *Stem Cells* **2014**, *32*, 126–134.
- Hou, Z.; Zhao, W.; Zhou, J.; Shen, L.; Zhan, P.; Xu, C.; Chang, C.; Bi, H.; Zou, J.; Yao, X.; et al. A long noncoding RNA *Sox2ot* regulates lung cancer cell proliferation and is a prognostic indicator of poor survival. *Int. J. Biochem. Cell Biol.* **2014**, *53*, 380–388.

8. Saghaeian Jazi, M.; Samaei, N.M.; Ghanei, M.; Shadmehr, M.B.; Mowla, S.J. Identification of new SOX2OT transcript variants highly expressed in human cancer cell lines and down regulated in stem cell differentiation. *Mol. Biol. Rep.* **2016**, *43*, 65–72.
9. Saghaeian Jazi, M.; Samaei, N.M.; Ghanei, M.; Shadmehr, M.B.; Mowla, S.J. Overexpression of the non-coding SOX2OT variants 4 and 7 in lung tumors suggests an oncogenic role in lung cancer. *Tumour Biol.* **2016**, *37*, 10329–10338.
10. Ron, D.; Walter, P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 519–529.
11. Ma, Y.; Hendershot, L.M. ER chaperone functions during normal and stress conditions. *J. Chem. Neuroanat.* **2004**, *28*, 51–65.
12. Medigeschi, G.R.; Lancaster, A.M.; Hirsch, A.J.; Briese, T.; Lipkin, W.I.; DeFilippis, V.; Früh, K.; Mason, P.W.; Nikolich-Zugich, J.; Nelson, J.A. West Nile Virus Infection Activates the Unfolded Protein Response, Leading to CHOP Induction and Apoptosis. *J. Virol.* **2007**, *81*, 10849–10860.
13. Andruska, N.; Zheng, X.; Yang, X.; Helferich, W.G.; Shapiro, D.J. Anticipatory estrogen activation of the unfolded protein response is linked to cell proliferation and poor survival in estrogen receptor α -positive breast cancer. *Oncogene* **2015**, *34*, 3760–3769.
14. Davies, M.P.A.; Barraclough, D.L.; Stewart, C.; Joyce, K.A.; Eccles, R.M.; Barraclough, R.; Rudland, P.S.; Sibson, D.R. Expression and splicing of the unfolded protein response gene XBP-1 are significantly associated with clinical outcome of endocrine-treated breast cancer. *Int. J. Cancer* **2008**, *123*, 85–88.
15. Dong, D.; Ni, M.; Li, J.; Xiong, S.; Ye, W.; Virrey, J.J.; Mao, C.; Ye, R.; Wang, M.; Pen, L.; et al. Critical Role of the Stress Chaperone GRP78/BiP in Tumor Proliferation, Survival, and Tumor Angiogenesis in Transgene-Induced Mammary Tumor Development. *Cancer Res.* **2008**, *68*, 498–505.
16. Livezey, M.; Kim, J.E.; Shapiro, D.J. A New Role for Estrogen Receptor α in Cell Proliferation and Cancer: Activating the Anticipatory Unfolded Protein Response. *Front. Endocrinol.* **2018**, *9*, 325.
17. Cook, K.L.; Clarke, R. Role of GRP78 in promoting therapeutic-resistant breast cancer. *Future Med. Chem.* **2015**, *7*, 1529–1534.
18. Fu, Y.; Li, J.; Lee, A.S. GRP78/BiP Inhibits Endoplasmic Reticulum BIK and Protects Human Breast Cancer Cells against Estrogen Starvation-Induced Apoptosis. *Cancer Res.* **2007**, *67*, 3734–3740.
19. Zhou, H.; Zhang, Y.; Fu, Y.; Chan, L.; Lee, A.S. Novel Mechanism of Anti-apoptotic Function of 78-kDa Glucose-regulated Protein (GRP78) endocrine resistance factor in breast cancer, through release of b-cell lymphoma 2 (bcl-2) from bcl-2-interacting killer (BIK). *J. Biol. Chem.* **2011**, *286*, 25687–25696.
20. Sengupta, S.; Sharma, C.G.N.; Jordan, V.C. Estrogen regulation of X-box binding protein-1 and its role in estrogen induced growth of breast and endometrial cancer cells. *Horm. Mol. Biol. Clin. Investig.* **2010**, *2*, 235–243.
21. Gomez, B.P.; Riggins, R.B.; Shajahan, A.N.; Klimach, U.; Wang, A.; Crawford, A.C.; Zhu, Y.; Zwart, A.; Wang, M.; Clarke, R. Human X-box binding protein-1 confers both estrogen independence and antiestrogen resistance in breast cancer cell lines. *FASEB J.* **2007**, *21*, 4013–4027.
22. Logue, S.E.; McGrath, E.P.; Cleary, P.; Greene, S.; Mnich, K.; Almanza, A.; Chevet, E.; Dwyer, R.M.; Oommen, A.; Legembre, P.; et al. Inhibition of IRE1 RNase activity modulates the tumor cell secretome and enhances response to chemotherapy. *Nat. Commun.* **2018**, *9*, 1–14.
23. Ming, J.; Ruan, S.; Wang, M.; Ye, D.; Fan, N.; Meng, Q.; Tian, B.; Huang, T. A novel chemical, STF-083010, reverses tamoxifen-related drug resistance in breast cancer by inhibiting IRE1/XBP1. *Oncotarget* **2015**, *6*, 40692–40703.
24. Chen, X.; Iliopoulos, D.; Zhang, Q.; Tang, Q.; Greenblatt, M.B.; Hatzia Apostolou, M.; Lim, E.; Tam, W.L.; Ni, M.; Chen, Y.; et al. XBP1 promotes triple-negative breast cancer by controlling the HIF1 α pathway. *Nature* **2014**, *508*, 103–107.
25. Del Vecchio, C.A.; Feng, Y.; Sokol, E.S.; Tillman, E.J.; Sanduja, S.; Reinhardt, F.; Gupta, P.B. De-differentiation confers multidrug resistance via noncanonical PERK-Nrf2 signaling. *PLoS Biol.* **2014**, *12*, e1001945.
26. Hollien, J.; Weissman, J.S. Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response. *Science* **2006**, *313*, 104–107.
27. Chevet, E.; Hetz, C.; Samali, A. Endoplasmic reticulum stress-activated cell reprogramming in oncogenesis. *Cancer Discov.* **2015**, *5*, 586–597.

28. Leung, E.; Kannan, N.; Krissansen, G.W.; Findlay, M.P.; Baguley, B.C. MCF-7 breast cancer cells selected for tamoxifen resistance acquire new phenotypes differing in DNA content, phospho-HER2 and PAX2 expression, and rapamycin sensitivity. *Cancer Biol. Ther.* **2010**, *9*, 717–724.
29. Leung, E.; Kim, J.E.; Askarian-Amiri, M.; Finlay, G.J.; Baguley, B.C. Evidence for the existence of triple-negative variants in the MCF-7 breast cancer cell population. *Biomed. Res. Int.* **2014**, *2014*, 836769.
30. Leung, E.Y.; Kim, J.E.; Askarian-Amiri, M.; Joseph, W.R.; McKeage, M.J.; Baguley, B.C. Hormone Resistance in Two MCF-7 Breast Cancer Cell Lines is Associated with Reduced mTOR Signaling, Decreased Glycolysis, and Increased Sensitivity to Cytotoxic Drugs. *Front. Oncol.* **2014**, *4*, 221.
31. Leung, E.Y.; Askarian-Amiri, M.; Finlay, G.J.; Rewcastle, G.W.; Baguley, B.C. Potentiation of Growth Inhibitory Responses of the mTOR Inhibitor Everolimus by Dual mTORC1/2 Inhibitors in Cultured Breast Cancer Cell Lines. *PLoS ONE* **2015**, *10*, e0131400.
32. Leung, E.Y.; Askarian-Amiri, M.E.; Sarkar, D.; Ferraro-Peyret, C.; Joseph, W.R.; Finlay, G.J.; Baguley, B.C. Endocrine Therapy of Estrogen Receptor-Positive Breast Cancer Cells: Early Differential Effects on Stem Cell Markers. *Front. Oncol.* **2017**, *7*, 184.
33. Andres, S.A.; Wittliff, J.L. Relationships of ESR1 and XBP1 expression in human breast carcinoma and stromal cells isolated by laser capture microdissection compared to intact breast cancer tissue. *Endocrine* **2011**, *40*, 212–221.
34. Raina, K.; Noblin, D.J.; Serebrenik, Y.V.; Adams, A.; Zhao, C.; Crews, C.M. Targeted protein destabilization reveals an estrogen-mediated ER stress response. *Nat. Chem. Biol.* **2014**, *10*, 957–962.
35. Scriven, P.; Coulson, S.; Haines, R.; Balasubramanian, S.; Cross, S.; Wyld, L. Activation and clinical significance of the unfolded protein response in breast cancer. *Br. J. Cancer* **2009**, *101*, 1692–1698.
36. Cook, K.L.; Soto-Pantoja, D.R.; Clarke, P.A.G.; Cruz, M.I.; Zwart, A.; Wärrri, A.; Hilakivi-Clarke, L.; Roberts, D.D.; Clarke, R. Endoplasmic Reticulum Stress Protein GRP78 Modulates Lipid Metabolism to Control Drug Sensitivity and Antitumor Immunity in Breast Cancer. *Cancer Res.* **2016**, *76*, 5657–5670.
37. Chen, Q.; Wei, C.; Wang, Z.; Sun, M. Long non-coding RNAs in anti-cancer drug resistance. *Oncotarget* **2016**, *8*, 1925–1936.
38. Iranpour, M.; Soudyab, M.; Geranpayeh, L.; Mirfakhraie, R.; Azargashb, E.; Movafagh, A.; Ghafouri-Fard, S. Expression analysis of four long noncoding RNAs in breast cancer. *Tumour Biol.* **2016**, *37*, 2933–2940.
39. Tang, X.; Gao, Y.; Yu, L.; Lu, Y.; Zhou, G.; Cheng, L.; Sun, K.; Zhu, B.; Xu, M.; Liu, J. Correlations between lncRNA-SOX2OT polymorphism and susceptibility to breast cancer in a Chinese population. *Biomark. Med.* **2017**, *11*, 277–284.
40. Martín-Pérez, R.; Palacios, C.; Yerbes, R.; Cano-González, A.; Iglesias-Serret, D.; Gil, J.; Reginato, M.J.; López-Rivas, A. Activated ERBB2/HER2 licenses sensitivity to apoptosis upon endoplasmic reticulum stress through a PERK-dependent pathway. *Cancer Res.* **2014**, *74*, 1766–1777.
41. Feng, Y.-X.; Sokol, E.S.; Del Vecchio, C.A.; Sanduja, S.; Claessen, J.H.L.; Proia, T.A.; Jin, D.X.; Reinhardt, F.; Ploegh, H.L.; Wang, Q.; et al. Epithelial-to-mesenchymal transition activates PERK-eIF2 α and sensitizes cells to endoplasmic reticulum stress. *Cancer Discov.* **2014**, *4*, 702–715.
42. Quan, H.; Fan, Q.; Li, C.; Wang, Y.-Y.; Wang, L. The transcriptional profiles and functional implications of long non-coding RNAs in the unfolded protein response. *Sci. Rep.* **2018**, *8*, 4981.
43. Clarke, R.; Shajahan, A.N.; Wang, Y.; Tyson, J.J.; Riggins, R.B.; Weiner, L.M.; Bauman, W.T.; Xuan, J.; Zhang, B.; Facey, C.; et al. Endoplasmic reticulum stress, the unfolded protein response, and gene network modeling in antiestrogen resistant breast cancer. *Horm. Mol. Biol. Clin. Investig.* **2011**, *5*, 35–44.
44. Clarke, R.; Cook, K.L.; Hu, R.; Facey, C.O.B.; Tavassoly, I.; Schwartz, J.L.; Baumann, W.T.; Tyson, J.J.; Xuan, J.; Wang, Y.; et al. Endoplasmic reticulum stress, the unfolded protein response, autophagy, and the integrated regulation of breast cancer cell fate. *Cancer Res.* **2012**, *72*, 1321–1331.
45. Cook, K.L.; Clarke, R. Heat shock 70 kDa protein 5/glucose-regulated protein 78 “AMP” ing up autophagy. *Autophagy* **2012**, *8*, 1827–1829.

