Metformin triggers apoptosis via endoplasmic reticulum stress in HER2-positive breast cancer cell lines

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Abstract. The antidiabetic drug metformin has potential as an anticancer agent, particularly due to its observed efficacy in breast cancer. Metformin exerts its cytotoxic effects in the induction of endoplasmic reticulum (ER) stress, which can trigger apoptotic cell death pathways. Therefore, the present study aimed to investigate the dose-dependent effects of metformin on ER stress and apoptosis in HER2-positive breast cancer SKBR3 cells. For this purpose, SKBR3 cells were treated with 5, 10 and 20 mM metformin. Cell proliferation was assessed using real-time cell analysis, while expression levels of ER stress-associated genes [glucose-regulated protein 78 kDa (GRP78), PRKR-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6) and CHOP)] were measured by revese transcription-quantitative PCR. Apoptosis was analyzed by Annexin V-FITC/PI flow cytometry in cells treated with 10 and 20 mM metformin. Findings revealed that metformin (5, 10 and 20 mM) dose-dependently inhibited cell proliferation and activated ER stress pathways. Significant increases were observed in gene expression following treatment with 5, 10 and 20 mM metformin, respectively, including GRP78 (3.70-, 5.06- and 7.33-fold; all P<0.0001) PERK (2.48-, 4.36- and 9.11-fold; all P<0.0001), IRE1 (2.15-fold, P=0.001; 2.90-fold, P<0.001; 5.55-fold, P<0.0001), ATF6 (2.43-2.44- and 3.63-fold; all P<0.0001) and particularly in pro-apoptotic CHOP (3.31-, 27.47- and 49.85-fold; all P<0.0001). Flow cytometry revealed that 10 and 20 mM metformin significantly increased early apoptosis to 6.05% (P<0.001) and 7.28% (P<0.001) and late apoptosis to 13.24% (P<0.001) and 20.59% (P<0.001), respectively, compared with controls (early apoptosis, 0.02%; late apoptosis, 0.05%). The present findings demonstrated that metformin activates ER stress response and induces apoptosis in HER2-positive breast cancer cells in a dose-dependent manner. This supports the potential of metformin as an adjuvant therapy, though further *in vivo* studies are needed to evaluate its clinical applicability.

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Introduction

Breast cancer (BC) represents a paramount global health challenge, being the most commonly diagnosed cancer among women worldwide with an estimated 2.30 million new cases in 2022 (1). In the same year, it was the leading cause of cancer-associated mortalities among women globally and ranked first in incidence in 157 countries and in mortality in 112 countries (2). Human epidermal growth factor receptor (HER)-positive status is observed in 15-20% of patients with BC (3) and is associated with aggressive disease progression and poor prognosis (4).

Recent studies have shown that endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) are key in oncogenic transformation, survival and cancer progression (5,6). Cancer cells undergo chronic ER stress due to factors such as hypoxia, nutrient deprivation, oxidative stress and genetic mutations, adapting by activating UPR. However, severe ER stress triggers programmed cell death by activating the pro-apoptotic components of the UPR. The accumulation of misfolded proteins in the ER activates three key sensors: PRKR-like ER kinase (PERK), activating transcription factor 6 (ATF6) and inositol-requiring enzyme 1 (IRE1), thereby initiating the UPR. While this response initially promotes cell survival, prolonged or intense stress leads to divergent outcomes, whereby PERK phosphorylates eukaryotic translation initiation factor 2α (eIF2α), suppressing global translation while inducing transcription factors such as activating transcription factor 4 (ATF4) and CHOP. CHOP upregulates pro-apoptotic genes, promoting cell death. Meanwhile, ATF6 activates ER chaperones, including glucose-regulated protein 78 kDa (GRP78) and glucose-regulated protein 94 kDa, as well as X-box binding protein 1 (XBP1) (5-7). IRE1, through its endoribonuclease activity, mediates XBP1 splicing or triggers apoptosis through the JNK pathway (7). This regulatory network highlights the dual role of ER stress in maintaining cellular homeostasis or inducing apoptosis, depending on the duration and intensity of the stress signals.

The precise molecular mechanisms governing the balance between ER stress-dependent pro-survival and pro-death signals in BC pathogenesis remain incompletely understood.

XBP1 is highly expressed in estrogen receptor-positive BC (8), where estrogen activates the UPR through GRP78 to enhance cell survival and promote tumor progression (9,10). In triple-negative BC (TNBC), the spliced form of X-box binding protein 1 (XBP1s)-hypoxia-inducible factor 1-α complex has been shown to support poor prognosis (11), while the IRE1α/XBP1s pathway is activated by the MYC proto-oncogene to maintain cell survival (12). Furthermore, the PERK/eIF2α pathway initiates autophagy and redox control in TNBC and persistent ER stress activates apoptotic mechanisms through caspase-8, Noxa, CHOP and JNK (13-15). In HER2-positive BC, activation of the PERK-ATF4-CHOP axis increases cell sensitivity to apoptosis through upregulation of TNF receptor superfamily member 10b and caspase-8 (16), highlighting the complex interplay between ER stress pathways and BC subtype-specific outcomes.

The majority of chemotherapeutic agents exert therapeutic effects through either cell cycle inhibition or activation of apoptotic pathways. Apoptosis, defined as a programmed and regulated cell death mechanism, serves a key role in maintaining homeostasis (17). However, tumor cells develop resistance to chemotherapeutic agents by suppressing apoptosis (18). Therefore, triggering apoptotic processes in malignant cells represents a potential therapeutic response. Furthermore, the modulatory effect of the tumor microenvironment on ER stress-induced apoptosis may be an important factor in the emergence of adaptive mechanisms (19). Specifically, conditions within the tumor microenvironment, such as hypoxia, nutrient deprivation and stromal cell crosstalk, can activate compensatory pro-survival signaling, thereby dampening the apoptotic response to ER stress (19).

Metformin is a prescribed oral antidiabetic drug and is a first-line medication for managing type 2 diabetes (20). Beyond its glucose-lowering effects, this biguanide is a safe drug that interacts with numerous oncogenic and tumor-suppressive pathways, such as AMPK-dependent and -independent mechanisms, making it an attractive option for cancer prevention and treatment (21). Studies have shown that metformin use is associated with a 31% decrease in overall cancer risk compared with other antidiabetic drugs and insulin. Although its exact antitumor mechanisms are not fully understood, AMPK activation and the inhibition of proliferative signaling pathways may serve important roles (21,22). Current research, including both *in vivo* and *in vitro* studies, indicates that metformin may have anticancer effects in BC treatment and may be considered a therapeutic option (23-25).

The literature presents conflicting results regarding the effects of metformin, a potential anticancer agent in BC treatment, on ER stress (26-28). Modulation of the UPR is a potential strategy in cancer treatment and enhancing pro-apoptotic signals of ER stress, in particular, may allow selective targeting of cancer cells (29). Against this background, the present study aimed to investigate the dose-dependent effects of metformin on ER stress and apoptosis in SKBR3 cells, an aggressive HER2-positive BC model.

Materials and methods

Cell culture. HER2$^+$ BC SKBR3 cells (wild-type) was obtained from the American Type Culture Collection and cultured in 25 cm^2 flasks using McCoy's 5A modified medium (cat. no. 16600-082) containing 10% FBS (cat. no. 10500-064), 1% L-glutamine (cat. no. 25030-081) and 1% 100U penicillin/0.1 mg streptomycin (cat. no. 15140-122; all Gibco; Thermo Fisher Scientific, Inc.) with 5% CO_2 at 37˚C. The complete medium was removed once the cells reached 70-80% confluence and cells were rinsed with PBS. After PBS removal, the cells were detached using trypsin-EDTA (0.25% trypsin and 0.02% EDTA; cat. no. 25200-056; Gibco, Thermo Fisher Scientific, Inc.). A total of $5x10^3$ cells/well were transferred into an e-plate for a proliferation-cytotoxicity assay. For gene expression and apoptosis analysis by flow cytometry, cells were seeded in 25 cm^2 flasks.

Study groups. For the real-time cell analyzer (RTCA) and gene expression studies the following groups were used: Control and 5, 10 and 20 mM metformin. For Annexin V-FITC/PI analysis, only control and 10 and 20 mM metformin groups were included. Only complete medium was added for the control group. The administered metformin doses were selected based on the literature (24,25).

RTCA. RTCA operates based on electronic impedance readings obtained from sensor electrodes located beneath e-plates. As cells attach to or detach from the surface electrodes, the change in electronic impedance is mathematically expressed as cell index (CI) values (30). Cells were cultured in plates designed for the xCELLigence Real-Time Cell Analysis system (Agilent Technologies, Inc.) at $5x10^3$ cells/well and incubated with 5% CO_2 for 24 h at 37˚C. Furthermore, the medium containing metformin (cat. no. D15095-9; Sigma-Aldrich; Merck KGaA; 5, 10 and 20 mM) was added to different wells. Complete medium without metformin was added to the control wells. The e-plates were monitored for 6 days.

Reverse transcription-quantitative PCR. Following 24 h of treatment with metformin (5, 10 and 20 mM) at 37˚C in a 5% CO_2 incubator, the medium was removed. Using the One Step-RNA Reagent (cat. no. BS410A Bio Basic Inc.), RNA isolation was performed. The quality and quantity of obtained RNA was evaluated by a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). mRNA was converted to cDNA using the iScript™ cDNA Synthesis kit (cat. no. 1708891; Bio-Rad Laboratories, Inc.) according to the manufacturer's protocol. Using the SsoAdvanced™ Universal SYBR Green Supermix (cat. no. 1725271; Bio-Rad Laboratories, Inc.) GAPDH, GRP78, PERK, IRE1, ATF6 and CHOP gene expression was measured through qPCR, using the Biorad CFX 96 system (Bio-Rad Laboratories, Inc.) with the following cycling protocol: initial denaturation: 98˚C for 30 sec; 40 cycles of [denaturation: 98˚C for 15 sec, annealing/extension: 60˚C for 30 sec]. Primer sequences were designed using the NCBI Primer-BLAST tool. The relative gene expression was quantified using the $2^{-\Delta\Delta Cq}$ method (31). The primer sequences of the genes are detailed in Table I.

Annexin V-FITC/PI analysis. Early and late apoptotic cell populations were detected using an Annexin V-FITC/PI apoptosis detection kit (cat. no. E-CK-A211; Elabscience Bionovation

Table I. PCR primer sequences.

Gene	Forward primer (5'-3')	Reverse primer (3'-5')
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
GRP78	ACAATCAAGGTCTATGAAGGTGAAAGAC	CTCGAAGAATACCATTCACATCTATCTC
PERK	AATCATAGCTCCTTCACCACAAA	CATCTTCCACATCACAGTCTGTA
IRE1	CACAGTGACGCTTCCTGAAAC	GCCATCATTAGGATCTGGGAGA
ATF6	AGCATGTTCCTGAGGAGTTGG	AGGCTTATCTTCCTTCAGTGGC
CHOP	GGAAACAGAGTGGTCATTCCC	CTGCTTGAGCCGTTCATTCTC

GRP78, glucose-regulated protein 78 kDa; PERK, PRKR-like ER kinase; IRE1, inositol-requiring enzyme 1; ATF6, activating transcription factor 6; CHOP, C/EBP homologous protein.

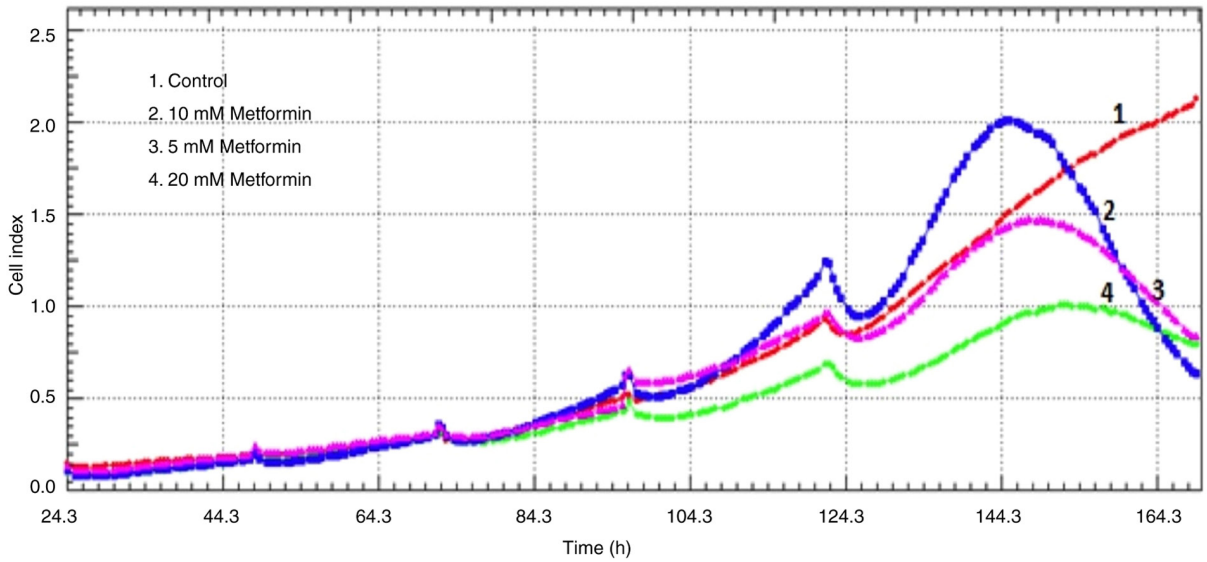

Figure 1. Cell proliferation following metformin treatment. All doses of metformin exhibited antiproliferative activity.

Inc.), according to the manufacturer's protocol. Following 24 h metformin treatment as aforementioned, cells were harvested by trypsinization, washed in PBS and stained for apoptosis analysis using an Annexin V-FITC/PI assay. The staining procedure was performed as follows: Cells were resuspended at a density of $5x10^5$ cells per sample in 500 μl of 1X Annexin V Binding Buffer. Each sample was then stained by adding 5 μl of Annexin V-FITC conjugate and 5 μl of PI directly to the cell suspension. The mixture was gently vortexed and then incubated at room temperature (~25˚C) in the dark for 15-20 min. After the incubation,cells were analyzed immediately by flow cytometry (BD FACSCalibur™; BD Biosciences) using the BD CellQuest™ acquisition and analysis software (version 5.2.1; BD Biosciences).

Statistical analysis. Statistical analyses were performed using SPSS (version 25; IBM Corp.) Data distribution normality was assessed using the Shapiro-Wilk test. For parametric data, one-way ANOVA was employed, followed by Tukey's post hoc test. Data are presented as mean ± SEM. For non-parametric data the Kruskal-Wallis test was used, followed by Dunn's test with Bonferroni correction for multiple comparisons. All experiments were performed in quadruplicate. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Metformin inhibits cell proliferation in a dose-dependent manner. The anti-proliferative effects of metformin were analyzed using RTCA via electrical impedance. While the CI in the control group continued to increase, all doses of metformin (5, 10 and 20 mM) exhibited antiproliferative and cytotoxic activity by day 6, as evidenced by a decrease in CI values based on electrical impedance signals (Fig. 1).

Metformin induces dose-dependent upregulation of ER stress marker genes. Treatment with 5, 10 and 20 mM metformin increased GRP78 gene expression by 3.70±0.11-, 5.06±0.08- and 7.33±0.08-fold (all $P<0.0001$), respectively (Fig. 2). Treatment with 5, 10 and 20 mM metformin increased PERK gene expression by 2.48±0.09-, 4.36±0.06- and 9.11±0.36-fold (all $P<0.0001$), respectively (Fig. 3). Treatment with 5, 10 and 20 mM metformin increased IRE1 gene expression levels by 2.15±0.08- ($P=0.001$), 2.90±0.03- ($P<0.001$) and

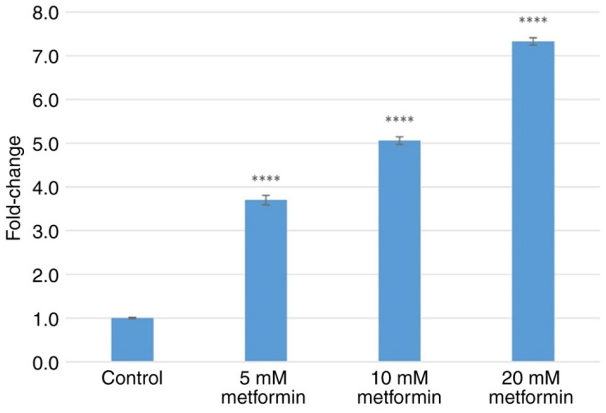

Figure 2. Gene expression changes of GRP78. Metformin increased GRP78 gene expression in a dose-dependent manner ****P<0.0001. GRP78, glucose-regulated protein 78 kDa.

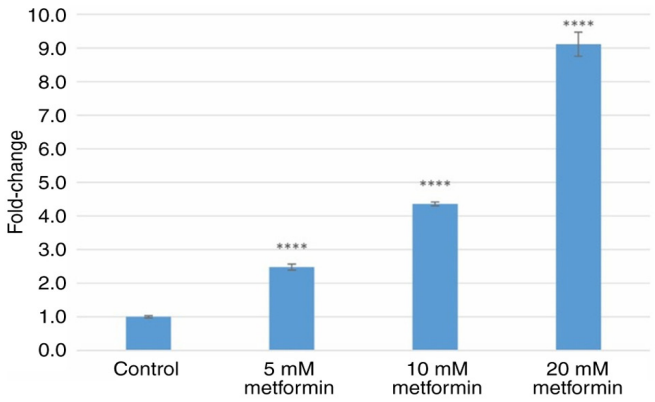

Figure 3. Gene expression changes of PERK. Metformin increased PERK gene expression in a dose-dependent manner ****P<0.0001. PERK, PRKR-like ER kinase.

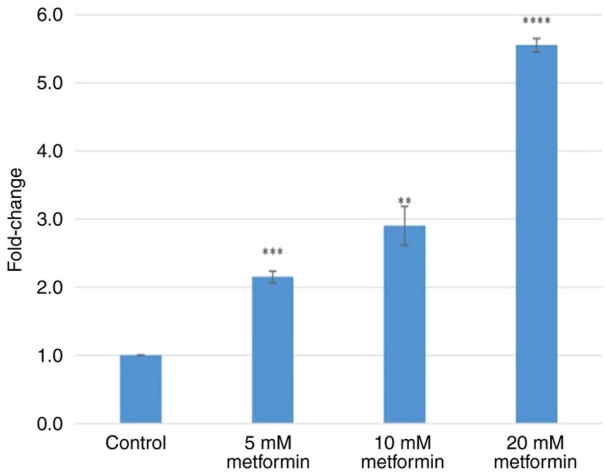

Figure 4. Gene expression changes of IRE1. Metformin increased IRE1 gene expression in a dose-dependent manner **P<0.01, ***P<0.001 and ****P<0.0001. IRE1, inositol-requiring enzyme 1.

5.55±0.10-fold (P<0.0001), respectively (Fig. 4). Treatment with 5, 10 and 20 mM metformin increased ATF6 gene expression levels by 2.43±0.01-, 2.44±0.07- and 3.63±0.05-fold

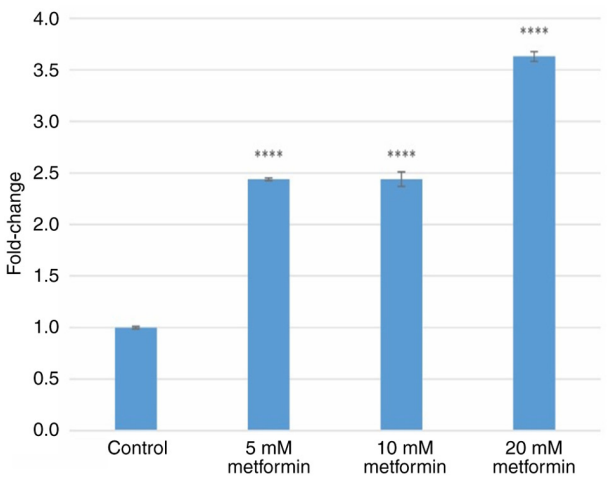

Figure 5. Gene expression changes of ATF6. Metformin increased ATF6 gene expression ****P<0.0001. ATF6, activating transcription factor 6.

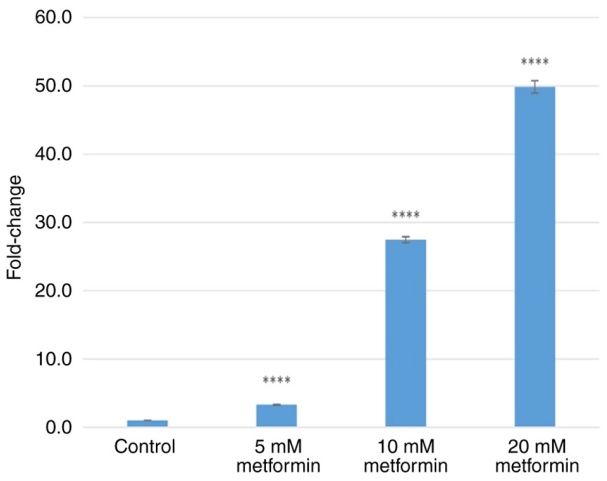

Figure 6. Gene expression changes of CHOP. Metformin increased CHOP gene expression in a dose-dependent manner. ****P<0.0001. CHOP, C/EBP homologous protein.

(all P<0.0001), respectively (Fig. 5). Treatment with 5, 10 and 20 mM metformin increased CHOP gene expression levels by 3.31±0.06-, 27.47±0.44- and 49.85±0.9-fold (all P<0.0001), respectively (Fig. 6).

Metformin triggers apoptosis in a dose-dependent manner. Treatment with 10 and 20 mM metformin significantly increased early apoptotic cells to 6.05 and 7.28, as well as late apoptotic cells to 13.24 and 20.59% (all P<0.001), respectively, compared with the control group (Fig. 7). These results demonstrated dose-dependent induction of apoptosis (Fig. 8).

Discussion

Within the present study, the multifaceted anticancer effects of metformin on the HER2-positive aggressive BC cell line SKBR3 were investigated. RTCA revealed that metformin (5, 10 and 20 mM) exhibited notable antiproliferative activity on day 6. This proliferation inhibition was functionally associated with dose-dependent increases in ER stress markers and

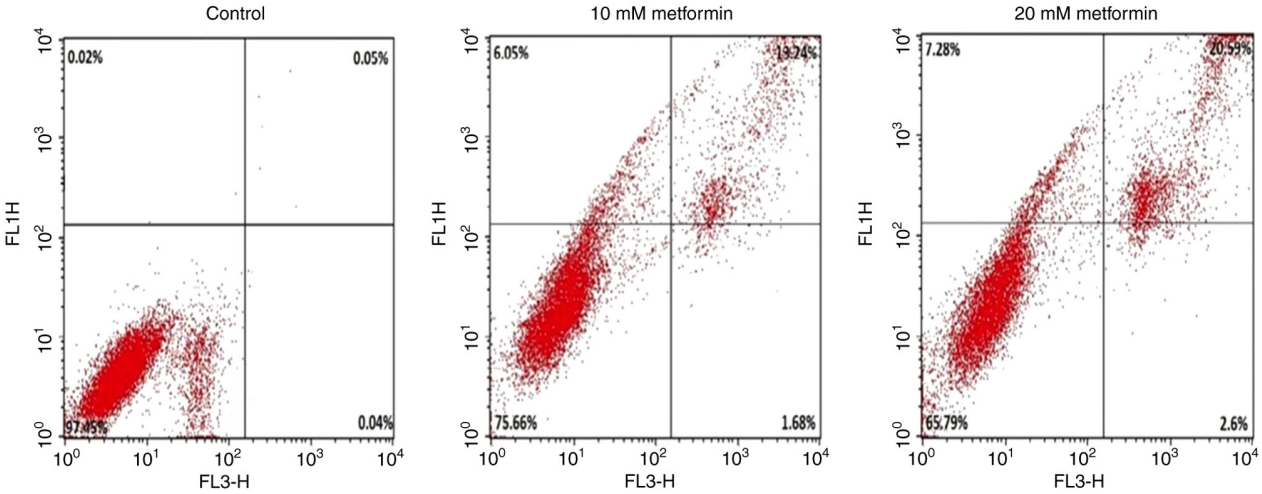

Figure 7. Flow cytometry analysis of metformin-induced apoptotic death. Treatment with 10 and 20 mM metformin significantly increased early apoptotic cells to 6.05 and 7.28 and late apoptotic cells to 13.24 and 20.59% (all P<0.001), respectively, compared with the control group.

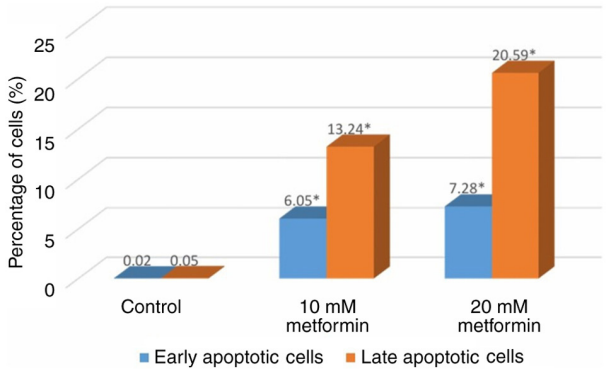

Figure 8. Percentage of early and late apoptotic cells following metformin treatment. Metformin increased the percentage of early and late apoptotic cells in a dose-dependent manner. *P<0.001.

apoptosis. Administration of 5, 10 and 20 mM metformin led to a significant dose-dependent increase in the gene expression of GRP78, PERK, IRE1, ATF6 and CHOP. Percentages of early and late apoptotic cells significantly increased in a dose-dependent manner in the 10 and 20 mM metformin groups. Collectively, these results support the hypothesis that metformin induces ER stress-mediated apoptosis by upregulating the ER chaperone GRP78 and key components of the UPR - namely PERK, IRE1 and ATF6 - which in turn activates the pro-apoptotic CHOP pathway.Despite the antitumor role of metformin having been widely reported, its molecular mechanism in inhibiting tumor progression remains unclear (21,32). Both *in vitro* and *in vivo* studies demonstrate that metformin exerts anticancer effects on BC through both AMPK-dependent and -independent mechanisms. The AMPK-dependent mechanisms include liver kinase B1-mediated AMPK phosphorylation, inhibition of mTOR through AMPK, forkhead box O3 activation and the regulation of histone deacetylases. AMPK-independent effects occur through numerous pathways, including microRNA modulation, increased oxidative stress, antifolate activity, inhibition of angiogenesis, NF-κB activation by suppressing proinflammatory cytokines such as IL-6 and IL-17, downregulation of specificity protein (SP)-1/SP3/SP4 transcription factors, upregulation of caveolin-1 and the modulation of cell cycle regulatory proteins (21,32). A potential anticancer mechanism of metformin may involve activation of ER stress-induced apoptotic cascades. The literature has consistently demonstrated the cytotoxic effects of metformin across a number of BC cell lines (24-28,33-35). In MCF-7 cells (BC cell line, ER positive), 10 μM metformin markedly decreases viability and migration compared with controls (27). Li *et al* (28) reported that 0.125 mg/ml metformin promotes death in glucose-deprived MDA-MB-231 cells (BC cell line, triple-negative). A number of studies using SKBR3 cells have shown dose- and time-dependent effects (24,25,33-36). For example, Chen *et al* (33) observed that 0.5-8.0 mM metformin inhibits proliferation and induces apoptosis, while Neamati *et al* (34) found that 30-100 mM treatment increases the number of apoptotic cells by 48 h. Xu *et al* (35) demonstrated that 20-120 μM metformin caused time-dependent proliferation, inhibition and G_1-phase arrest, with 96.25 μM treatment increasing early and late apoptosis to 4.48 and 17.13%, respectively, at 48 h. Ahmadpour *et al* (24) and Amaral *et al* (25) demonstrated that 10-80 and 0.01-5.00 mM metformin concentrations, respectively, dose-dependently decrease viability at numerous timepoints. Notably, metformin exhibits enhanced efficacy against trastuzumab-resistant cells (36). The present findings of increased early/late apoptosis and reduced viability with 10-20 mM metformin treatment are consistent with the aforementioned effects.

The dichotomous role of ER stress in promoting cancer cell survival or triggering apoptosis remains subject to debate (37), although sustained or severe ER stress leads to cell death (38). While numerous studies using various cell lines, mostly consistent with the present findings, have demonstrated that metformin causes ER stress-induced apoptosis (26-28,39-45), there are also studies suggesting it reduces ER stress (46-48). However this may be due to the cell lines used as well as the metformin dose. According to studies in non-cancerous cell lines, low-dose metformin suppresses ER stress-induced apoptosis (46-48). For example, in a colitis model, 1 mM metformin decreases

GRP78, CHOP, caspase-12, PERK and eIF2α expression levels and apoptosis (46). Furthermore, in pancreatic β cells, 0.05 mM metformin suppresses palmitate-induced ER stress induction in an AMPK-independent manner (47), and in ovarian granulosa cells, 1 mM metformin inhibits testosterone-induced ER stress and UPR activation by suppressing mitogen-activated protein kinase P38-α phosphorylation (48). In studies with cancer cell lines, metformin causes ER stress-induced apoptosis, supporting the present findings (39-45); in a study using prostate cancer cells, application of 5 mM metformin increased the expression of ER stress-associated CHOP, phosphorylated (p)-eIF2α, calreticulin, GRP78 and SR Ca^{2+}-ATPase 1 genes through miR-708-5p, thereby inducing apoptosis (39). In addition, a study of colon cancer cells demonstrated that the application of 1, 5 and 25 mM metformin induces dose-dependent increases in PERK, p-eIF2α, ATF4 and CHOP levels (40). In colon cancer cells, 5 mM metformin activates CHOP and inhibites cell proliferation by causing cell cycle arrest (41) and in thyroid cancer cells, metformin application inhibits proliferation and induces apoptosis in a concentration- (1.25, 2.50, 5, 10 or 20 mM) and time-dependent (24-48 h) manner. Furthermore, 5, 10 and 20 mM metformin activate ER stress by increasing GRP78, CHOP and caspase-12 expression (42). Similarly, metformin (0-20 mM and 0-48 h) decreases the cell proliferation index and increases the expression of GRP78, CHOP and caspase-12 in papillary thyroid carcinoma cells (43). Furthermore, in endometrial cancer cells, metformin increases CHOP expression levels while decreasing GRP78 expression levels (44). Similarly, metformin induces UPR-mediated cell death by decreasing GRP78 expression and upregulating IRE1α and CHOP in acute lymphoblastic leukemia cells (45).

Studies demonstrating metformin-induced ER stress-mediated apoptosis in BC remain limited (26-28). In MCF-7 cells, Huang *et al* (26) reported that metformin (0-40 mM) dose-dependently increases CHOP expression, inhibits proliferation, promotes apoptosis and induces cell cycle arrest. Alizade *et al* (27) found that 10 μM metformin decreases viability and migration after 48 h, upregulating apoptosis-related caspase-3, Bax, apoptosis regulator, apoptosis-inducing factor mitochondria associated 1, CHOP and GRP78 expression while downregulating Bcl-2 and WEE1 G_2 checkpoint kinase. In MDA-MB-231 cells, Li *et al* (28) demonstrated that 0.125 mg/ml metformin under glucose deprivation enhances UPR through the ATF4/ATF3/CHOP pathway while inhibiting anti-apoptotic Bcl-2 and BCL-xl effects. To the best of our knowledge, no studies have investigated the association between metformin and ER stress-induced apoptosis in aggressive HER2-positive BC cells. In the present study, a dose-dependent induction of endoplasmic reticulum stress was suggested, as increases in the expression of ER stress markers GRP78, PERK, IRE1, ATF6 and CHOP following treatment with 5, 10 and 20 mM metformin was observed. The upregulation of chaperone GRP78 and the three primary UPR sensors (PERK, IRE1 and ATF6) demonstrated ER stress induction. Notable increases in CHOP gene expression at 10 and 20 mM metformin doses were detected, mediated by PERK and ATF6 activation, an indicator of ER stress-induced apoptosis. This was supported by Annexin V-FITC/PI assays showing significant increases in early and late apoptotic cells at these concentrations. IRE1 upregulation may promote apoptosis through JNK or caspase-12 pathways. Furthermore, elevated IRE1, PERK and ATF6 levels may sustain the ER stress response by maintaining GRP78 activation.

The present study provided functional and transcriptional evidence that metformin induces ER stress and triggers apoptosis in HER2-positive BC cells. However, certain limitations must be acknowledged. Activation of ER stress pathways could not be directly demonstrated at the protein level. Secondly, the present study was not conducted in multiple HER2-positive cell lines to strengthen the generalizability of the findings. However, the present results in SKBR3 cells are consistent with the literature, which reports that metformin reduces viability in a dose-dependent manner in HER2-positive BT-474 cells (BC cell line, ER/PR/HER2-positive). Finally, the present study employed high metformin concentrations (49), consistent with preclinical literature, to elucidate ER stress and apoptosis mechanisms. However, these concentrations are markedly higher than those of therapeutic plasma levels (10-40 μM) achieved with standard oral dosing in diabetes treatment (50). Reasons behind the high *in vitro* dose requirement may include continuous drug exposure, the absence of serum protein binding and non-physiological conditions of the cell culture environment, such as high glucose and growth factor levels. These conditions may maximize cell proliferation and survival signals, necessitating higher drug concentrations to suppress oncogenic mechanisms (51). This represents a key limitation for the direct clinical translation of the present results, which require validation through *in vivo* animal experiments. The effects of metformin on ER stress and apoptosis are context-dependent. The literature reports varying and contradictory outcomes depending on cell type, metabolic profile, microenvironmental conditions and the dose (21,25,39,48,52,53). Therefore, validating the present findings in other cancer models and *in vivo* systems is key to define the therapeutic potential of metformin.

In conclusion, the present findings demonstrated that metformin treatment inhibited proliferation in a time- and dose-dependent manner in SKBR3 cells, activated the ER stress response to potentiate apoptotic signaling and disrupted cell survival mechanisms. These results suggest that the ER stress-apoptosis axis may represent a therapeutic target in HER2-positive BC. However, further *in vivo* investigations are warranted to evaluate the clinical translatability of metformin.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Authors' contributions

EÇ and BB conceived and designed the study. EÇ conducted data analysis and wrote the manuscript. All authors have read and approved the final manuscript. EÇ and BB confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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