

Targeting Endoplasmic Reticulum Stress to Attenuates Cisplatin Resistance in Non-Small Cell Lung Cancer

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Abstract

Cisplatin-based chemotherapy remains a cornerstone of treatment for advanced non-small cell lung cancer (NSCLC); however, the emergence of chemoresistance severely limits its clinical efficacy. Endoplasmic reticulum stress and adaptive unfolded protein response have been implicated in cancer cell survival and therapy resistance, highlighting modulation of this signalling as a potential therapeutic strategy. In this study, we investigated whether pharmacological induction of endoplasmic reticulum stress via sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) inhibition could attenuate cisplatin resistance in NSCLC.

Using parental and cisplatin-resistant cell lines, we demonstrate that thapsigargin induces robust endoplasmic reticulum stress responses characterised by dynamic regulation of *GRP78*, *PERK*, *XBP1*, and *ATF4* expression. Notably, thapsigargin pre-treatment significantly reduced cisplatin IC_{50} values and decreased resistance indices in resistant cells, indicating attenuation of the resistant phenotype. In addition, SERCA inhibition enhanced apoptotic cell death in selected models and markedly suppressed clonogenic survival and migratory capacity across all cell lines examined.

Distinct UPR activation patterns were observed between parental and resistant cells, suggesting adaptive remodelling of ER stress signalling during acquisition of cisplatin resistance. Collectively, these findings identify ER calcium homeostasis as a modifiable determinant of platinum responsiveness and support targeting ER stress pathways as a potential adjunct strategy to improve therapeutic efficacy in chemoresistant NSCLC.

Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) accounting for approximately 85% of all cases [1, 2]. Despite advances in treatment, chemotherapy remains the standard approach for advanced-stage disease, with cisplatin widely used across multiple solid tumours. However, the development of cisplatin resistance represents a major obstacle to therapeutic success [3, 4].

The endoplasmic reticulum (ER) is the largest organelle in the cell and plays a central role in protein and lipid synthesis as well as Ca^{2+} homeostasis [5]. Despite the highly regulated nature of protein synthesis, various physiological and pathological stimuli can disrupt ER function, leading to the accumulation of misfolded or unfolded proteins and the induction of ER stress [6]. When adaptive capacity is exceeded, cells activate the unfolded protein response (UPR), a conserved signalling network designed to restore ER homeostasis [7].

The UPR is initiated by three principal ER-resident sensor proteins: inositol-requiring enzyme 1 α (IRE1 α), protein kinase R-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6). Under basal conditions, these sensors are maintained in an inactive state through their association with the ER chaperone binding immunoglobulin protein/glucose-regulated protein 78 (BiP/GRP78). Upon ER

stress, GRP78 preferentially binds misfolded or unfolded proteins, thereby dissociating from the sensor proteins and permitting their activation [8].

Activated IRE1 α promotes the expression of X-box binding protein 1 (XBP1), a transcription factor that regulates genes involved in ER protein translocation, folding, secretion, and the degradation of misfolded proteins. PERK activation leads to phosphorylation of eukaryotic translation initiation factor 2 α (eIF2 α), resulting in a transient attenuation of global protein synthesis and a reduction in ER protein load, while selectively inducing the expression of activating transcription factor 4 (ATF4). ATF4 contributes to cellular adaptation by regulating amino acid metabolism, protein secretion, and redox homeostasis. In parallel, ATF6 undergoes proteolytic cleavage in the Golgi apparatus, after which it translocates to the nucleus and functions as a transcription factor controlling genes involved in ER protein folding, growth regulation, and cell survival [9].

The sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA) pump maintains intracellular Ca²⁺ homeostasis by transporting Ca²⁺ ions from the cytoplasm into the sarco/endoplasmic reticulum. Disruption of SERCA function results in elevated cytosolic Ca²⁺ levels and depletion of Ca²⁺ within the ER lumen, leading to ER stress and the activation of apoptotic signalling pathways. Owing to this central role in ER homeostasis, SERCA has emerged as an attractive therapeutic target in cancer [10].

Thapsigargin is the most extensively characterised SERCA inhibitor and exerts its anticancer effects by irreversibly blocking the SERCA pump. This inhibition induces sustained ER stress and triggers cancer cell death independently of cell proliferation. Notably, thapsigargin has attracted considerable interest in oncology due to its reported synergistic effects when combined with conventional chemotherapeutic agents. By promoting ER stress, thapsigargin has been proposed as a strategy to overcome resistance mechanisms that limit the efficacy of standard chemotherapy [11]. However, whether this therapeutic potential extends to cancers that have already acquired chemoresistance remains largely unexplored.

Based on these considerations, the present study aimed to investigate whether pharmacological induction of ER stress by thapsigargin can overcome cisplatin resistance in non-small cell lung cancer, with the hypothesis that SERCA inhibition restores chemosensitivity by promoting ER stress-mediated apoptotic signalling.

Materials and Methods

Cell lines and chemicals

Calu1 (RRID: CVCL_0608) and H2030 (RRID: CVCL_1517) non-small cell lung cancer cell lines were obtained from the American Type Culture Collection (ATCC). Calu1 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), while H2030 cells were maintained in RPMI-1640 medium. All culture media were supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and 1% L-glutamine. Cisplatin (DDP) was prepared at a stock concentration of 50 mg/50 mL (Cipintu).

Thapsigargin (#HY-13433, MedChemExpress) was dissolved in dimethyl sulfoxide (DMSO) to obtain a 10 mM stock solution.

Generation of cisplatin-resistant NSCLC cell lines

Cisplatin-resistant Calu1 cells (Calu1/DDP) used in this study were previously generated and characterised, as described in our earlier publication [12]. Briefly, the cisplatin-sensitive NSCLC cell line Calu1 was subjected to a dose-escalation protocol involving initial cisplatin exposure followed by recovery periods in drug-free complete medium.

Cisplatin-resistant H2030 cells were newly generated in the present study using a similar dose-escalation approach. Cells were initially exposed to 2.5 μ M cisplatin, and the drug concentration was gradually increased to a final concentration of 25 μ M. The resulting resistant subline was designated as H2030/DDP and maintained under selective pressure prior to experimental use.

Cytotoxicity assay

The cytotoxic effects of DDP and thapsigargin in Calu1, Calu1/DDP, H2030, and H2030/DDP cells were assessed using the Thiazolyl Blue/MTT assay (#HY-15924, MedChemExpress) to determine half-maximal inhibitory concentrations (IC_{50}). Cells were seeded in 96-well plates and allowed to attach for 24 h, followed by treatment with increasing concentrations of DDP or thapsigargin for 24, 48, and 72 h. After treatment, MTT solution was added and incubated for 4 h. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured using a microplate reader. Mean absorbance values were normalised to untreated controls, and IC_{50} values were calculated using GraphPad Prism 9.3.1 (GraphPad Software, Inc.). The cisplatin resistance index (RI) was calculated as $IC_{50}(\text{Calu1/DDP})/IC_{50}(\text{Calu1})$ and $IC_{50}(\text{H2030/DDP})/IC_{50}(\text{H2030})$.

qRT-PCR

mRNA expression levels of target genes were analysed by quantitative real-time PCR (qRT-PCR). Total RNA was isolated using the Aurum™ Total RNA Mini Kit (#7326820, Bio-Rad), and complementary DNA (cDNA) was synthesised using the iScript™ cDNA Synthesis Kit (#1708891, Bio-Rad). qRT-PCR was performed using iTaq Universal SYBR Green Supermix (#1725120, Bio-Rad) on a LightCycler 480 system. Gene expression levels were normalised to the reference gene *GAPDH* and calculated relative to untreated controls using the comparative $2^{-\Delta\Delta C_t}$ method. Primer sequences are listed in Table 1.

Table 1
Primer sequences used in the qRT-PCR analysis
(5'...3')

GRP78	F: CTGTCCAGGCTGGTGTGCTCT
	R: CTTGGTAGGCACCACTGTGTTC
PERK	F: GTCCCAAGGCTTTGGAATCTGTC
	R: CCTACCAAGACAGGAGTTCTGG
XBP1	F: CTGCCAGAGATCGAAAGAAGGC
	R: CTCCTGGTTCTCAACTACAAGGC
ATF4	F: TTCTCCAGCGACAAGGCTAAGG
	R: CTCCAACATCCAATCTGTCCCG
GAPDH	F: GTCCCTCTGACTTCAACAGCG
	R: ACCACCCTGTTGCTGTAGCCAA

Annexin V staining

Annexin V-FITC (#630109, Takara) staining was performed to distinguish modes of cell death. Cells were washed twice with cold PBS and resuspended in 1× Annexin V binding buffer. The cell suspensions were then incubated with Annexin V-FITC for 15 min at room temperature in the dark, followed by the addition of propidium iodide (PI). Samples were analysed by flow cytometry using a BD Accuri C6 instrument, and data were processed with CFlow software (v1.0.264.15).

Colony formation assay

Calu1 (1×10^3 cells/well), Calu1/DDP (5×10^2 cells/well), H2030 (2×10^2 cells/well) and H2030/DDP (2.5×10^2 cells/well) cells were seeded into 6-well plates and incubated in drug-containing medium for 72 h. Following thapsigargin treatment (499.4 nM for Calu-1, 2468 nM for Calu-1/DDP, 16.46 nM for H2030, and 8.05 nM for H2030/DDP), cells were maintained in drug-free complete medium for 14 days to allow colony formation. Colonies were then fixed with cold methanol and stained with crystal violet. Excess stain was removed at room temperature, plates were washed with distilled water, and allowed to air-dry at 25°C.

Wound healing assay

Calu1 and Calu1/DDP (5×10^4 cells/ml), H2030 and H2030/DDP (2×10^4 cells/ml) cells were seeded into 6-well plates and incubated for 24 h to reach confluence. A linear scratch was introduced in each well using a sterile pipette tip, after which debris was removed by washing twice with PBS. In thapsigargin-

treated cells (499.4 nM for Calu-1, 2468 nM for Calu-1/DDP, 16.46 nM for H2030, and 8.05 nM for H2030/DDP), images were acquired at 0, 6, 12, 24, 36, and 48 hours. Wound closure was quantified by measuring the relative open wound area using TScratch software (v2) and normalised to the initial time point.

Data analysis

All experiments were performed in triplicate. Data are presented as mean $\pm$ standard deviation (SD). Statistical significance was determined using Student's t-test or analysis of variance (ANOVA), as appropriate. $p < 0.05$ was considered statistically significant.

Results

Generation of DDP-resistant NSCLC cell lines

To investigate the effect of thapsigargin on resistance in NSCLC, two cisplatin-resistant cell lines, Calu1/DDP and H2030/DDP, were established. Parental and resistant cell lines were treated with increasing concentrations of DDP, and cell viability was assessed using the MTT assay to determine IC_{50} values. For Calu1 cells, IC_{50} values at 24, 48, and 72 h were 65.94 μ M, 29.94 μ M, and 9.08 μ M, respectively (Fig. 1A), whereas corresponding values for Calu1/DDP cells were markedly higher at 573 μ M, 196.1 μ M, and 67.85 μ M (Fig. 1B). In H2030 cells, IC_{50} values were 28.21 μ M, 14.04 μ M, and 6.38 μ M at 24 h, 48 h, and 72 h, respectively (Fig. 1C), while H2030/DDP cells exhibited increased IC_{50} values of 79.75 μ M, 38.91 μ M, and 24.77 μ M (Fig. 1D).

The resistance index (RI) for Calu1/DDP cells was calculated as 8.7, 6.5, and 7.4 at 24 h, 48 h, and 72 h, respectively, whereas RI values for H2030/DDP cells were 2.8, 2.8, and 3.9 at the corresponding time points. The significantly elevated IC_{50} and RI values in Calu1/DDP and H2030/DDP cells compared with their parental counterparts confirm the successful establishment of DDP-resistant NSCLC cell lines (Fig. 1E-F).

Thapsigargin reduces cisplatin resistance in NSCLC cells

To determine the cytotoxic effects of thapsigargin, parental and resistant cell lines were treated with increasing concentrations of thapsigargin, and IC_{50} values were calculated using the MTT assay. In Calu1 cells, thapsigargin IC_{50} values at 24, 48, and 72 h were 1585 nM, 986.5 nM, and 499.4 nM, respectively (Fig. 1G), whereas substantially higher IC_{50} values were observed in Calu1/DDP cells (18686 nM, 5597 nM, and 2468 nM, respectively; Fig. 1H). In contrast, thapsigargin IC_{50} values for H2030 cells were 123.4 nM, 53.7 nM, and 16.46 nM at 24, 48, and 72 h, respectively (Fig. 1I), while comparable or slightly lower values were observed in H2030/DDP cells (128.7 nM, 48.82 nM, and 8.05 nM; Fig. 1J). These results indicate that Calu1/DDP cells, but not H2030/DDP cells, exhibit reduced sensitivity to thapsigargin.

To assess the effect of thapsigargin on cisplatin responsiveness in resistant cells, Calu1/DDP and H2030/DDP cells were pre-treated with thapsigargin at IC_{50} concentration for 72 h, followed by exposure to increasing concentrations of DDP and subsequent MTT analysis. In thapsigargin-treated Calu1/DDP cells, DDP IC_{50} values were reduced to 232.8 μ M, 99.18 μ M, and 31.05 μ M at 24 h, 48 h, and 72 h, respectively (Fig. 1K), corresponding to a 2.46-, 1.98-, and 2.18-fold decrease in DDP resistance (Fig. 1E). Similarly, in thapsigargin-treated H2030/DDP cells, cisplatin IC_{50} values decreased to 49.53 μ M, 23.07 μ M, and 12.42 μ M at 24 h, 48 h, and 72 h, respectively (Fig. 1L), representing 1.61-, 1.69-, and 1.99-fold reductions in DDP resistance (Fig. 1F).

Changes in the RI were calculated following thapsigargin treatment. The RI values for Calu-1/DDP were 3.5, 3.3, and 3.41 at 24, 48, and 72 h, respectively (Fig. 1E), while the RI values for H2030/DDP were 1.75, 1.6, and 1.9 at the corresponding time points (Fig. 1F). The observed reduction in RI indicates that thapsigargin effectively attenuates cisplatin resistance in DDP-resistant cells.

Thapsigargin induces endoplasmic reticulum stress in NSCLC cell lines

To evaluate the induction of ER stress, Calu1, Calu1/DDP, H2030, and H2030/DDP cells were treated with thapsigargin at the IC_{50} concentration for 72 h, and time-dependent changes in the mRNA expression of ER stress markers *GRP78*, *PERK*, *XBP1*, and *ATF4* were analysed by qRT-PCR.

In Calu1 cells, *GRP78* mRNA expression was significantly upregulated, reaching a peak increase of 14.31-fold at 8 h ($p < 0.0001$), followed by a gradual decline to basal levels by 72 h (Fig. 2A). In Calu1/DDP cells, *GRP78* expression peaked at 12 h with a 5.5-fold increase ($p = 0.0048$) and remained elevated at 72 h (5.04-fold, $p = 0.0105$), although the magnitude of induction was lower compared with Calu1 cells (Fig. 2A). *PERK* mRNA expression did not show significant changes in Calu1 cells; however, Calu1/DDP cells exhibited significant increases at 4 h (8.05-fold, $p = 0.0215$) and 8 h (9.6-fold, $p = 0.0049$) (Fig. 2B). *XBP1* expression began to increase in Calu1 cells at 4 h ($p = 0.0138$), whereas a significant induction was observed only at 72 h in Calu1/DDP cells ($p = 0.0363$) (Fig. 2C). *ATF4* mRNA levels remained largely unchanged in Calu1 cells but were markedly increased in Calu1/DDP cells at 72 h (6.6-fold, $p < 0.0001$) (Fig. 2D).

In H2030 cells, *GRP78* mRNA expression increased to 3.2-fold at 8 h ($p = 0.0278$), peaked at 12 h with a 4.4-fold increase ($p = 0.0026$), and subsequently declined (Fig. 2E). In contrast, *GRP78* expression in H2030/DDP cells began to increase at 4 h and remained elevated up to 48 h (Fig. 2E). *PERK* mRNA expression showed no significant variation in H2030 cells, whereas a significant 4-fold increase was detected in H2030/DDP cells at 48 h ($p = 0.0143$) (Fig. 2F). *XBP1* mRNA expression in H2030 cells was significantly upregulated at 4 h (3.3-fold, $p = 0.0016$) and 8 h (2.6-fold, $p = 0.0192$), while no significant changes were observed in H2030/DDP cells (Fig. 2G). *ATF4* expression increased significantly in H2030 cells at 12 h (4.2-fold, $p < 0.0001$) and in H2030/DDP cells at 48 h (6.1-fold, $p = 0.0427$) (Fig. 2H).

Thapsigargin induces apoptosis in NSCLC cell lines

To assess the effects of thapsigargin on cell death, Calu1, Calu1/DDP, H2030, and H2030/DDP cell lines were treated with thapsigargin at the IC₅₀ concentration for 72 h, and apoptotic cell populations were analysed by Annexin V/PI staining followed by flow cytometry.

In Calu1 cells, the basal apoptotic rate was 3.9%, which increased to 6.1% following thapsigargin treatment. In Calu1/DDP cells, apoptosis increased from 5.0% in untreated cells to 12.6% after thapsigargin exposure (Fig. 3A). In H2030 cells, thapsigargin treatment resulted in a marked increase in apoptosis from 8.3% to 25.4%. In contrast, H2030/DDP cells exhibited only a modest change in apoptotic rate, increasing from 5.8% to 6.9% following thapsigargin treatment (Fig. 3B). Overall, thapsigargin significantly increased apoptosis in Calu1, Calu1/DDP, and H2030 cell lines, whereas no significant change in apoptotic cell death was observed in H2030/DDP cells (Fig. 3C).

Thapsigargin reduces colony formation in NSCLC cell lines

To evaluate the effect of thapsigargin on clonogenic potential, colony formation assays were performed in parental and resistant cells. Relative colony formation efficiency was reduced to $39.73 \pm 0.38\%$ in Calu1 cells, $10.36 \pm 2.01\%$ in Calu1/DDP cells, $48.54 \pm 1.48\%$ in H2030 cells, and $44.04 \pm 3.23\%$ in H2030/DDP cells following thapsigargin treatment (Fig. 3D). These findings demonstrate that thapsigargin significantly suppressed colony formation across all cell lines compared to control ($p < 0.0001$) (Fig. 3E).

Thapsigargin reduces cell migration in NSCLC cell lines

The effects of thapsigargin on cell migration were assessed using a wound healing assay, and migratory capacity was quantified by calculating the relative open wound area over time. In Calu1 cells, the relative open wound area at 12 h, 24 h, and 48 h was $63.48 \pm 9.54\%$, $50.85 \pm 5.41\%$, and $0.71 \pm 1.23\%$, respectively, whereas thapsigargin-treated Calu1 cells exhibited significantly larger open wound areas of $89.18 \pm 3.64\%$, $84.9 \pm 6.76\%$, and $66.70 \pm 7.78\%$ at the corresponding time points (Fig. 4A). Similarly, in Calu1/DDP cells, relative open wound areas were $85.20 \pm 5.87\%$, $61.49 \pm 4.73\%$, and $28.57 \pm 12.38\%$ at 12, 24, and 48 h, respectively, while thapsigargin treatment resulted in values of $93.28 \pm 4.33\%$, $77.19 \pm 5.36\%$, and $75.3 \pm 1.19\%$ (Fig. 4B).

In H2030 cells, relative open wound areas were $79.24 \pm 8.01\%$, $53.06 \pm 4.86\%$, and $0.73 \pm 1.27\%$ at 12, 24, and 48 h, respectively, compared with $80.16 \pm 4.56\%$, $71.96 \pm 13.77\%$, and $85.23 \pm 14.48\%$ in thapsigargin-treated cells (Fig. 4C). Likewise, H2030/DDP cells displayed relative open wound areas of $77.81 \pm 7.44\%$, $15.03 \pm 1.45\%$, and $2.84 \pm 4.93\%$ at the same time points, which increased to $88.01 \pm 0.83\%$, $79.29 \pm 6.87\%$, and $70.06 \pm 7.46\%$ following thapsigargin treatment (Fig. 4D). Collectively, these results demonstrate that thapsigargin markedly suppresses cell migration across all NSCLC cell lines analysed.

Discussion

In the present study, we investigated whether pharmacological induction of endoplasmic reticulum stress through SERCA inhibition modulates cisplatin responsiveness in non-small cell lung cancer. Our findings demonstrate that thapsigargin attenuates the cisplatin-resistant phenotype in established resistant cells, reduces IC₅₀ values, and suppresses clonogenic survival and migration. These observations support the concept that ER calcium homeostasis represents a modifiable determinant of platinum sensitivity in NSCLC.

Disruption of ER calcium homeostasis is a central regulator of cellular stress responses. ER calcium release independent of plasma membrane influx has been highlighted as a critical mechanism in stress signalling [13]. Thapsigargin, one of the best-characterised SERCA inhibitors, induces ER stress by depleting ER calcium stores and activating the UPR [14]. In our study, coordinated transcriptional regulation of canonical UPR markers, including *GRP78*, *PERK*, *XBP1* and *ATF4*, was observed following thapsigargin exposure, consistent with previous reports describing thapsigargin-mediated ER stress activation [15–18].

GRP78 upregulation across all cell lines confirms ER stress induction, as dissociation of *GRP78* from ER stress sensors is essential for activation of *PERK*, *IRE1* and *ATF6* branches. In Calu1 cells, *GRP78* and *XBP1* were significantly upregulated, whereas *PERK* and *ATF4* were not markedly altered, suggesting preferential activation of the *IRE1*–*XBP1* axis. A previous study demonstrated that increased *XBP1* activity can drive *GRP78* upregulation even in the absence of significant *ATF6* activation [19], supporting our findings.

In H2030 cells, *GRP78*, *XBP1* and *ATF4* were elevated despite no significant change in *PERK* expression. Although *ATF4* is classically downstream of *PERK*, *PERK*-independent regulation of *ATF4* has been described, particularly in ER stress-induced autophagy [20]. Moreover, *ATF4* has been implicated in tumour progression and metastasis [21], suggesting that ER stress signalling may intersect with pro-adaptive and pro-migratory pathways.

In cisplatin-resistant H2030/DDP cells, *GRP78*, *PERK* and *ATF4* were upregulated, whereas *XBP1* did not significantly increase. Differential activation of UPR branches in response to thapsigargin has been previously reported in various cancer models [22]. These findings suggest that resistant cells may exhibit selective rewiring of ER stress pathways as part of an adaptive survival mechanism.

Cisplatin resistance is multifactorial, involving altered drug metabolism and transport [23], enhanced DNA damage repair capacity [23–25], epithelial–mesenchymal transition [26] suppression of apoptotic pathways [27], redox remodelling [28], and proteostasis regulation [29]. Dysregulated calcium signalling also contributes to chemoresistance. Reduced cytosolic calcium levels have been observed in cisplatin-resistant cancer cells, and SERCA inhibition by thapsigargin restores calcium flux and reverses resistance [30]. Furthermore, ER stress induction combined with autophagy inhibition has been shown to reduce cisplatin resistance [31]. Consistent with previous reports demonstrating reversal of cisplatin resistance through alternative strategies [32–34], our findings support SERCA inhibition as a viable sensitisation approach.

Apoptosis analysis revealed significant induction of cell death in Calu1, Calu1/DDP and H2030 cells, in agreement with studies showing thapsigargin-induced ER stress-mediated apoptosis in adrenal cortical carcinoma [17], pancreatic beta cells [35], and via mitochondrial dysfunction pathways [36]. However, apoptosis was only partially increased in H2030/DDP cells. A recent study demonstrated that thapsigargin can induce caspase-independent programmed cell death resembling autosis in basophilic leukaemia cells [37]. While we did not directly characterise alternative death pathways, the limited apoptotic response in H2030/DDP cells suggests that highly resistant cells may display elevated apoptotic thresholds or engage alternative stress-adaptive mechanisms. This heterogeneity underscores the complexity of ER stress-mediated outcomes in chemoresistant disease.

Thapsigargin significantly suppressed colony formation and migration in all cell lines. In studies aimed at reversing cisplatin resistance, decreased colony formation has been reported following low-intensity ultrasound combined with cisplatin [33], ferroptosis induction via shikonin-mediated HMOX1 upregulation [34], and SOX2 suppression affecting APE1 signalling [32]. ER stress has also been shown to impair cell migration and wound healing processes [38, 39], supporting our findings that targeting ER homeostasis limits tumour progression-related phenotypes.

From a translational perspective, cisplatin remains a cornerstone of NSCLC treatment, yet resistance severely limits therapeutic efficacy. Targeting the SERCA/ER stress axis represents a rational strategy to overcome resistance. Our data suggest that modulation of ER calcium homeostasis may enhance platinum responsiveness; however, quantitative synergy modelling and *in vivo* validation will be required to define clinical feasibility.

Several limitations of this study should be acknowledged. The current study is restricted to *in vitro* models, UPR activation was assessed primarily at the transcriptional level, and combination synergy analyses were not performed. Furthermore, the precise mode of cell death in highly resistant cells remains to be elucidated. Future investigations should integrate protein-level signalling analyses, functional perturbation of specific UPR branches, calcium flux measurements, and *in vivo* validation in resistant tumour models.

In conclusion, our findings indicate that pharmacological induction of ER stress through SERCA inhibition attenuates cisplatin resistance and suppresses aggressive phenotypes in NSCLC cell models. These results support ER calcium homeostasis as a potential therapeutic vulnerability in chemoresistant disease and provide a foundation for future mechanistic and translational investigation.

Declarations

Conflict of interest

The authors declare that they have no conflicts of interest.

Author Contributions Statement

S.C., Z.Y., S.A., B.B. and E.D. carried out the experiments; S.C. and V.B. analysed data, interpreted the results and wrote the paper; Z.E. and V.B. supervised the project. All authors reviewed the results and approved the final version of the manuscript.

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Figures

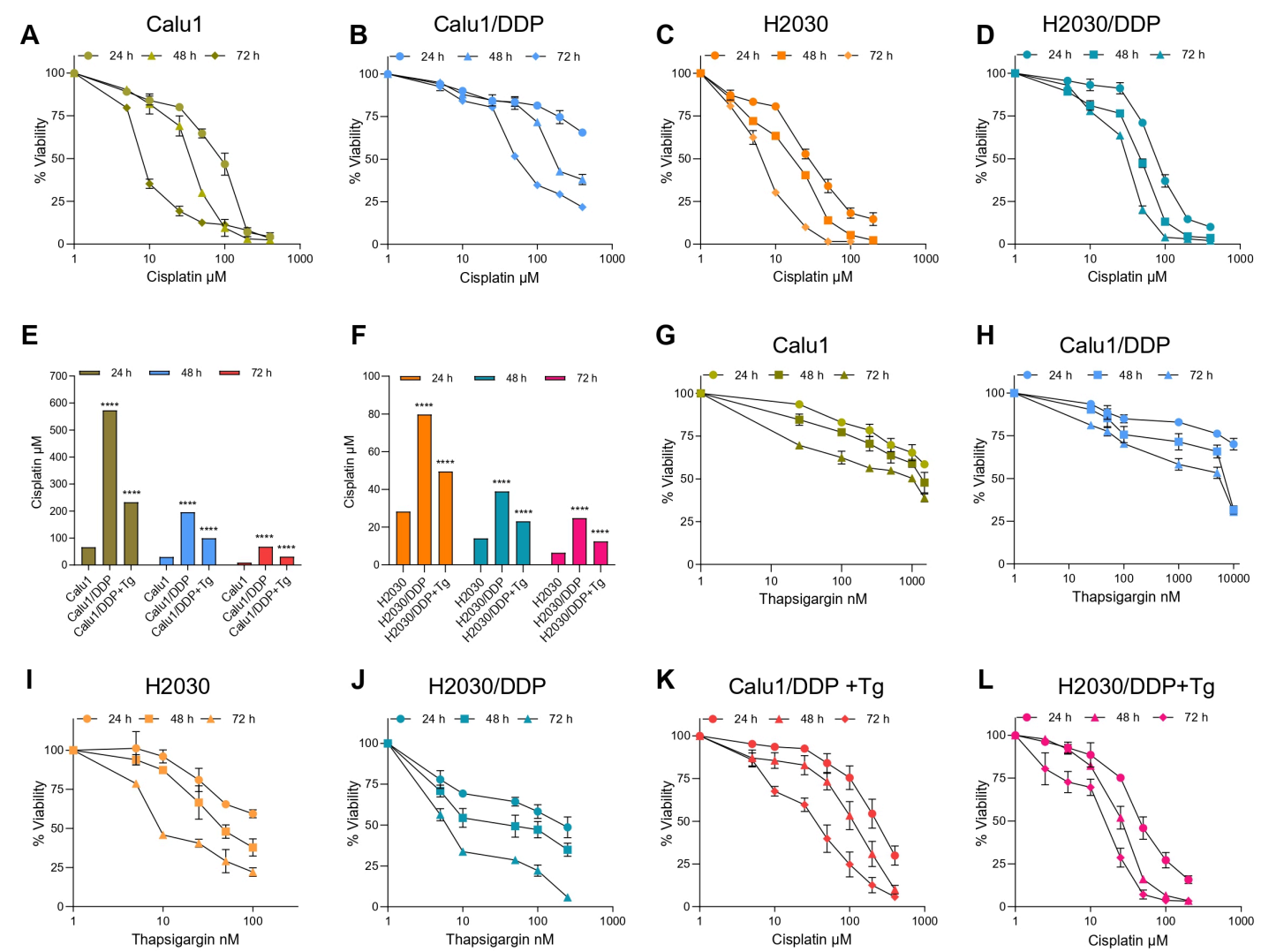


Figure 1

Thapsigargin reduces cisplatin resistance in NSCLC cells Cytotoxic effects of cisplatin in Calu1 (A), Calu1/DDP (B), H2030 (C), and H2030/DDP (D) cell lines, as determined by MTT assay. Comparison of cisplatin IC₅₀ levels of Calu1, Calu1/DDP and Thapsigargin pretreated Calu1/DDP cells (E). Comparison of cisplatin IC₅₀ levels of H2030, H2030/DDP and Thapsigargin pretreated H2030/DDP cells (F). Cytotoxic effects of thapsigargin in Calu1 (G), Calu1/DDP (H), H2030 (I), and H2030/DDP (J) cells. Effects of cisplatin on Calu1/DDP (K) and H2030/DDP (L) cells following exposure to thapsigargin. Tg: thapsigargin (**** $p < 0.0001$)

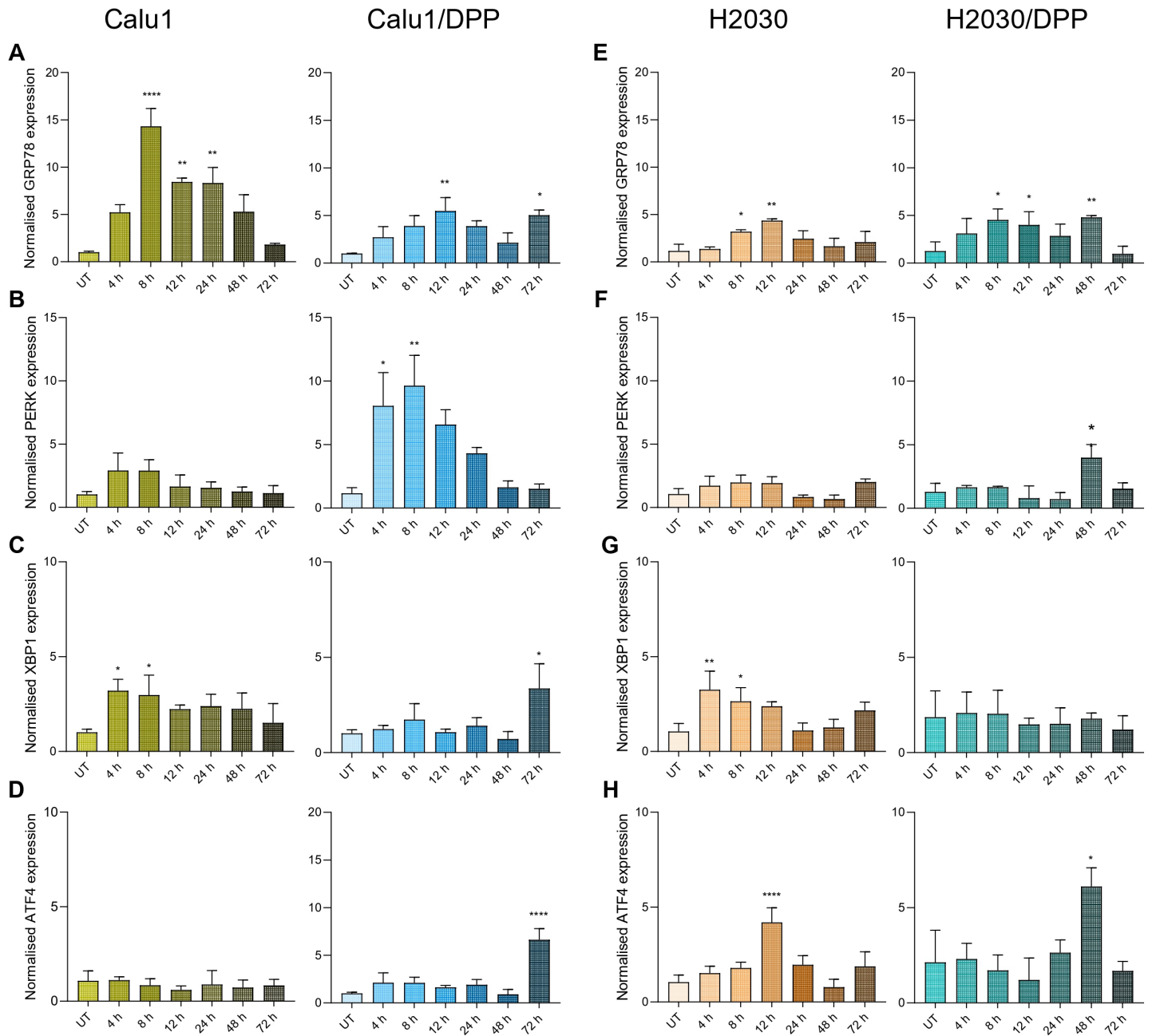


Figure 2

Thapsigargin induces endoplasmic reticulum stress Time-dependent changes in mRNA expression levels of ER stress markers GRP78, PERK, XBP1, and ATF4 were analysed by qRT-PCR following thapsigargin treatment. **(A–D)** mRNA expression of GRP78 (****: $p < 0.0001$, **: $p = 0.0033$, **: $p = 0.0039$, **: $p = 0.0048$, *: $p = 0.0105$) **(A)**, PERK (*: $p = 0.0215$, **: $p = 0.049$) **(B)**, XBP1 (*: $p = 0.0138$, *: $p = 0.0297$, *: $p = 0.0363$) **(C)**, and ATF4 (****: $p < 0.0001$) **(D)**, in Calu1 and Calu1/DDP cell lines. **(E–H)** mRNA expression of GRP78 (*: $p = 0.0278$, **: $p = 0.0026$, *: $p = 0.0150$, *: $p = 0.0434$, **: $p = 0.0084$) **(E)**, PERK (*: $p = 0.0143$) **(F)**, XBP1 (**: $p = 0.016$, *: $p = 0.0192$) **(G)**, and ATF4 (****: $p < 0.0001$, *: $p = 0.0427$) **(H)** in H2030 and H2030/DDP cell lines.

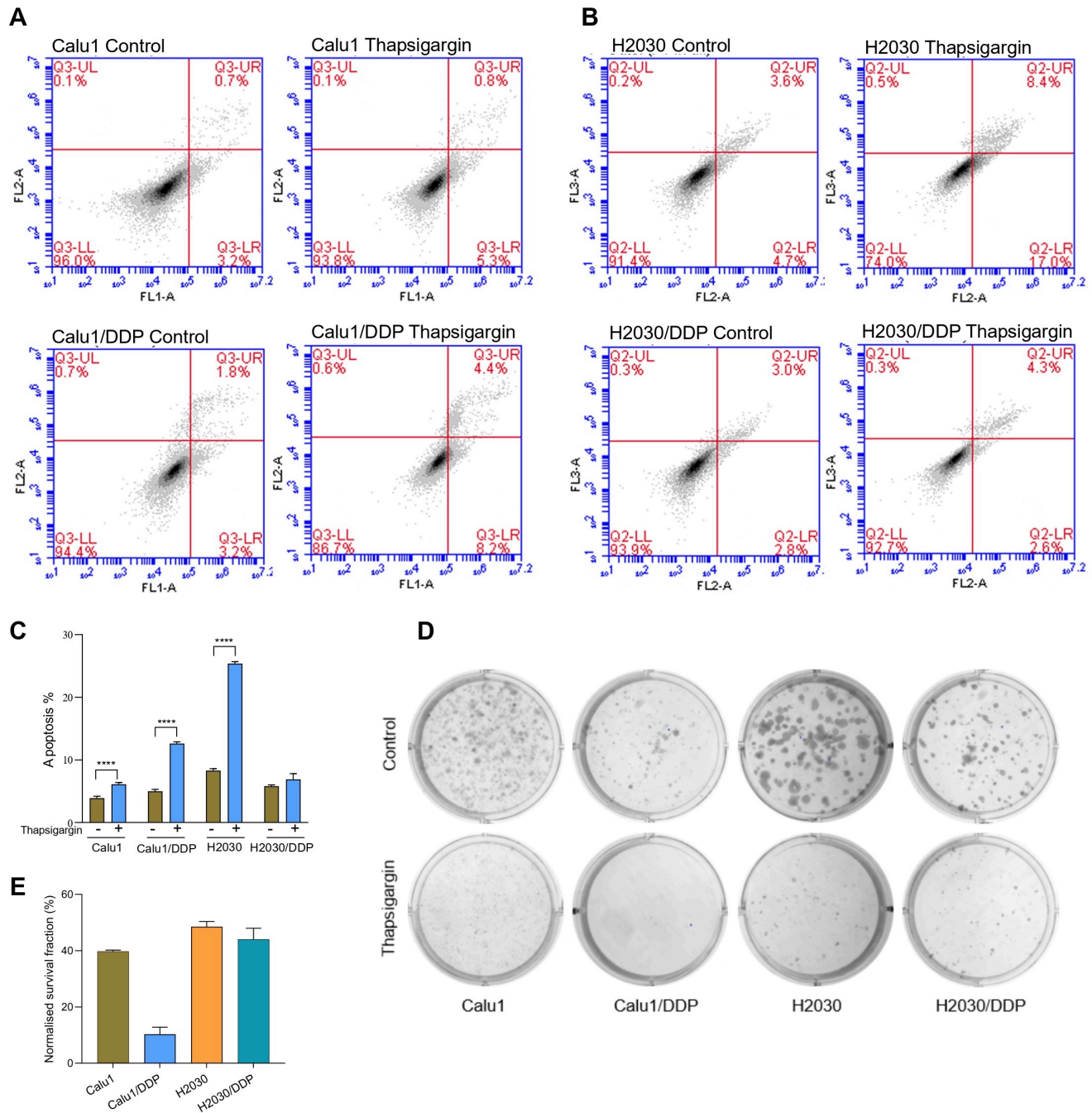


Figure 3

Thapsigargin induces apoptosis and inhibits colony formation Apoptotic cell death was evaluated by Annexin V/PI staining followed by flow cytometric analysis. **(A)** Thapsigargin treatment at the 72 h IC_{50} concentration increased apoptosis in Calu1 and Calu1/DDP cell lines. **(B)** In H2030 cells, thapsigargin markedly increased the apoptotic fraction, whereas no significant change was observed in H2030/DDP cells following treatment. **(C)** Quantification of apoptotic cell populations in Calu1, Calu1/DDP, H2030, and

H2030/DDP cell lines. (D, E) Representative images and quantification of colony formation following 14 days of thapsigargin treatment. (****: $p<0.0001$)

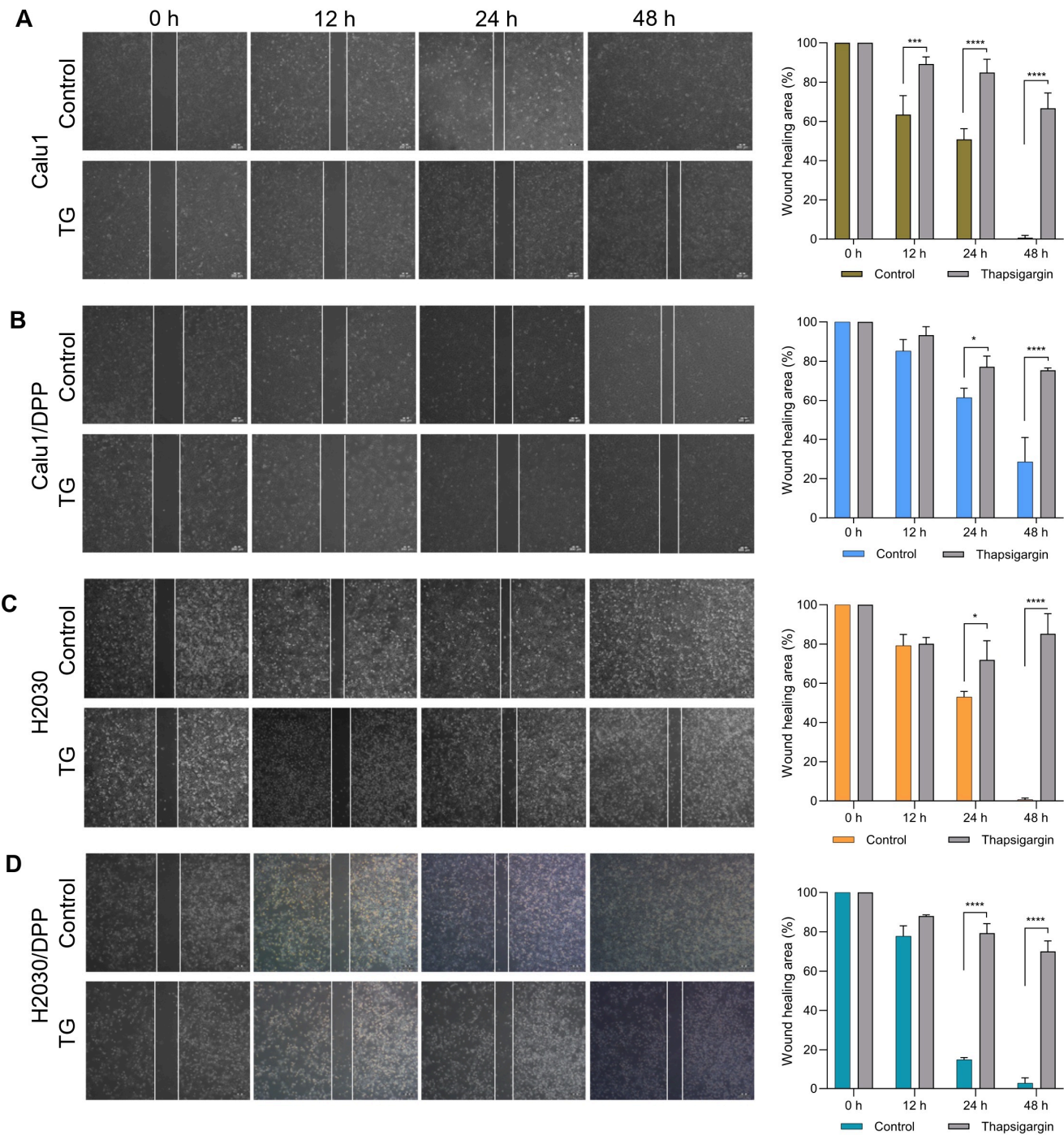


Figure 4

Thapsigargin suppresses cell migration in NSCLC cell lines Cell migration was assessed using a wound healing assay. Thapsigargin treatment significantly reduced migratory capacity in (A) Calu1(***: $p= 0.0001$,

****: $p < 0.0001$, ****: $p < 0.0001$), **(B)** Calu1/DDP (*: $p = 0.0153$, ****: $p < 0.0001$) **(C)** H2030 (*: $p = 0.0362$, ****: $p < 0.0001$) and **(D)** H2030/DDP (****: $p < 0.0001$) cell lines, as indicated by increased relative open wound areas over time. TG: Thapsigargin