Submitted: 09 August, 2025 Accepted: 17 September, 2025 Published: 30 October, 2025 DOI:10.22514/jomh.2025.128

ORIGINAL RESEARCH

JOMH Journal of Men's Health

The combination of Xuefu Zhuyu decoction and osimertinib inhibits the proliferation of non-small cell lung cancer cells by activating endoplasmic reticulum stress

Mingling Sun[1], Yong Liu[1], Qiong Zhang[1], Zonglang Lai[1], Yancheng He[1], Dandan Zheng[1,*]

[1]Department of Oncology, Chongqing Hospital of Traditional Chinese Medicine, 400021 Chongqing, China

***Correspondence**
dandan20221111@163.com
(Dandan Zheng)

Abstract

Background: To investigate the effects of the combination of XueFu ZhuYu (XFZY) decoction and osimertinib on the proliferation, apoptosis, migration, and endoplasmic reticulum stress (ERS) of non-small cell lung cancer (NSCLC) cell lines. **Methods**: Eight Sprague-Dawley (SD) rats were used to prepare XFZY serum. XFZY serum and osimertinib (separately or in combination) were used to treat NSCLC cells (H1975 cells and National Cancer Institute (NCI)-H1299 cells). Next, 5 mmol/L *N*-acetylcysteine (NAC) was treated with the XFZY serum + Osimertinib-induced NSCLC cells. Cell Counting Kit-8 (CCK8), flow cytometry, Transwell, and scratch assays were performed to detect the cell proliferation, apoptosis, migration, and invasion. Flow cytometry, Transmission Electron Microscopy (TEM), and Western Blot (WB) were performed to detect ERS levels in the cells. **Results**: Compared with the XFZY serum group or Osimertinib group, the XFZY serum + Osimertinib group significantly inhibited cell proliferation, migration, and invasion and promoted the apoptosis of NSCLC cells. Additionally, XFZY serum + Osimertinib induced endoplasmic reticulum (ER) expansion and mitophagy, significantly increasing Reactive oxygen species (ROS) accumulation, and upregulating the expression of ERS-related proteins, including Activating Transcription Factor 4 (ATF4), C/EBP Homologous Protein (CHOP), and phosphorylated PKR-like ER kinase (p-PERK). Interestingly, these responses were alleviated by NAC treatment. Limitations of this study arise from its focus on *in vitro* cell models data, without validation in clinical samples or *in vivo* environments. **Conclusions**: XFZY decoction increased the Osimertinib sensitivity of NSCLC cells by enhancing the ERS response and damaging the protection of the unfolded protein response (UPR) by activating the PERK-ATF4-CHOP signaling pathway.

Keywords

Non-small cell lung cancers; Xuefu Zhuyu decoction; Osimertinib; Reactive oxygen species; Endoplasmic reticulum stress

1. Introduction

Lung cancer has the highest mortality rate among all malignancies worldwide, with approximately 2.48 million incidences and 1.82 million deaths in 2022, of which non-small cell lung cancer (NSCLC) accounts for approximately 85–90% of all malignancies [1, 2]. Epidermal growth factor receptor (EGFR), a tyrosine kinase receptor, is the most common driver of NSCLC [3]. It plays a key role in the development of tumor proliferation. EGFR-tyrosine kinase inhibitors (TKIs) have become the standard first-line therapy for NSCLC patients. However, most patients typically develop disease progression following EGFR-TKI treatment for 9–14 months [4]. Osimertinib is a third-generation EGFR-TKI. Recent clinical trials, such as FLAURA2, demonstrate that combining Osimertinib with chemotherapy significantly prolongs progression-free survival (PFS) by nearly 9 months [5]. Another study conducted a phase III double-blind placebo-controlled trial involving 216 NSCLC patients who had received chemotherapy, showing a median progression-free survival of 39.1 months for Osimertinib, whereas the placebo group had a median of only 5.6 months [6]. Currently, drug resistance remains one of the most difficult problems in NSCLC treatment. Thus, it's urgent to investigate new therapeutic approaches and anti-cancer drugs.

Disruption of endoplasmic reticulum (ER) homeostasis interferes with normal ER function, accumulating unfolded and misfolded proteins, a process called ER stress (ERS) [7]. To overcome ERS, cells develop an unfolded protein response

(UPR) to resume protein folding capacity and re-establish cellular homeostasis [8]. However, a powerful and continuous ERS response can inhibit the protective UPR response of the ER [9], ultimately activating the ERS-mediated apoptosis pathway and enhancing the efficacy of drug therapy.

Traditional Chinese medicine (TCM) has shown promise as an antitumor agent. A variety of studies have explored the combination of TCM formulas with EGFR-TKIs to boost therapeutic effectiveness [10, 11]. For example, research has reported that tanshinone IIA can alleviate gefitinib resistance in human NSCLC cells [12]. Another study reported that costunolide, which was first isolated from the costus root, acts as a Mitogen-Activated Protein Kinase Kinase 1 (MEK1) or Protein Kinase B (AKT) 1/2 inhibitor to improve Osimertinib sensitivity [13]. XueFu ZhuYu (XFZY) decoction is a traditional Chinese prescription that compounds Taoren, Danggui, Chuanxiong, Honghua, Chishao, Dihuang, Zhiqiao, Chaihu, Jiegeng, Niuxi, and Gancao. The XFZY decoction is composed of various Chinese herbs that exhibit antitumor activities, block angiogenesis, and strengthen immunity [14]. Nevertheless, details concerning the synergistic effects of the XFZY decoction with Osimertinib remain unknown.

This study aimed to investigate whether XFZY decoction combined with Osimertinib can enhance the apoptosis of NSCLC cell lines and explore its underlying molecular mechanism. This study will provide a theoretical reference for NSCLC treatment using TCM.

2. Materials and methods

2.1 XFZY serum preparation

A total of 8 male (n = 4) and female (n = 4) Sprague-Dawley (SD) rats (200 ± 20 g, 6–8 weeks) were used in this study. All the rats were obtained from Dashuo Biological Technology Company (Chengdu, China). The XFZY decoction was extracted via decoction, filtration, and water bath evaporation at a concentration of 2.29 g of raw medicine/mL. The rats were randomly divided into two groups: based on the equivalent dose ratio table for human and animal surface area conversion [15], double equivalent dose (18.36 g/kg) XFZY decoction gavage for XFZY serum preparation (n = 4) and equal saline for the blank serum preparation (n = 4), twice a day, for 4 days. One hour after the last gavage, all the rats were subjected to blood collection via the abdominal aorta. All rats were anesthetized by intraperitoneal injection of 1% pentobarbital sodium before blood collection. Subsequently, they were sacrificed by cervical dislocation. This study was approved by the Animal Ethics Committee of West China Hospital of Sichuan University (No: 20230423001). The blood was centrifuged at 2000 r/min for 5 min, and the supernatant was inactivated in a water bath at 56 °C for 30 min, filtered through a 0.22 μm microporous membrane, and stored at −20 °C.

2.2 Cell culture and Cell Counting Kit-8 assay

Human lung adenocarcinoma cells (H1975: EGFR L858R/T790M, CL-0298) and (NCI-H1299: EGFR wild-type, CL-0165) were obtained from Procell (Wuhan, China). Authentication of H1975 and NCI-H1299 cell lines was performed using Short Tandem Repeat (STR) genotyping, and they were regularly examined for mycoplasma contamination. Roswell Park Memorial Institute 1640 medium (RPMI-1640) + 10% Fetal Bovine Serum (FBS) + 1% P/S medium was used to culture cells. After cells grew to the log phase, they were collected and seeded in 96-well plates (2×10^3 cells/well). The experiments are as follows. In Experiment 1, 0%, 5%, 10%, 15%, or 20% XFZY serum or 20% blank serum was added to the culture medium. In Experiment 2, the following mixture was added to the culture medium: control, XFZY serum (10%), Osimertinib (22 nmol/L), or XFZY serum + Osimertinib. Experiment 3: control, NAC (5 mmol/L, *N*-acetylcysteine), XFZY serum + Osimertinib, XFZY serum + Osimertinib + NAC. Next, the culture medium was incubated with 5% CO_2 at 37 °C. To each well, 10 μL of CCK8 solution (BS350A, Biosharp, Hefei, Anhui, China) was added, and the mixture was incubated for 2 h at 37 °C according to the manufacturer's protocol. Finally, a microplate reader (SpectraMax PLUS 384, Molecular Devices, San Jose, CA, USA) was used to measure the optical density of each well at 450 nm. Each experiment was conducted in four replicates.

2.3 Flow cytometry

Flow cytometry was performed to analyze cell apoptosis and ROS generation. In Experiments 2 and 3, H1975 and NCI-H1299 cells were collected after 24 h of treatment. For apoptosis analysis, the cells were stained with the Annexin V/Propidium Iodide (PI) Cell Apoptosis Detection Kit (KGA1030-100T, KeyGen Biotech, Nanjing, Jiangsu, China). For ROS generation, after drug treatment, the cells were fixed and incubated with H2DCFDA (no. D6883, Sigma-Aldrich, St. Louis, MO, USA) for 20 min in the dark. A Fluorescence Activated Cell Sorting Calibur (FACSCalibur) (no. 342945, Becton-Dickinson, Heidelberg, BE, Germany) flow cytometer was used to analyze the results via Cell Quest software (version 4.0.2, Becton-Dickinson, Heidelberg, BW, Germany). Each experiment was conducted in three replicates.

2.4 Transwell assay

For the invasion assay, the upper chambers were precoated with Matrigel (no. 356234, BD Biosciences, Franklin Lakes, NJ, USA) for 1 h. Next, the upper chambers were seeded with H1975 or NCI-H1299 cells (1×10^5 cells/well). The upper chambers were supplemented with culture medium without serum, and the lower chambers were supplemented with culture medium for drug treatment. Following culture, the mixture was incubated for 24 h at 37 °C. Finally, the invading NSCLC cells were fixed with 4% methanol, stained with 0.1% crystal violet, and counted under a light microscope (IX71, Olympus Corporation, Tokyo, Japan). Each experiment was conducted in three replicates.

2.5 Scratch assay

A scratch assay was performed to assess the migration of H1975 and NCI-H1299 cells. A 6-well plate was seeded with cells at a density of 5×10^5 cells/well and incubated overnight.

A sterile pipette tip was then used to create a single scrape in the confluent monolayer. After the monolayers were washed with PBS, different drugs were added. A Leica TCS 4D microscope (Leica Camera AG, Wetzlar, HE, Germany) was used to capture the same scraped section at 0 and 24 h. Each experiment was conducted in three replicates.

2.6 Transmission electron microscopy (TEM)

A transmission electron microscope (JEM-1400FLASH, JEOL, Akishima, Japan) was used to observe the ultrastructure of the endoplasmic reticulum. After drug treatment, H1975 cells were treated with XFZY serum, Osimertinib, or XFZY serum + Osimertinib. NCI-H1299 cells were treated with NAC, XFZY serum + Osimertinib, or XFZY serum + Osimertinib + NAC, and the cells were collected, fixed, and dehydrated. After that, each sample was embedded in epoxy resin and polymerized at 60 ℃ for 48 h. Ultrathin 70–80 nm sections were prepared and then stained with uranyl acetate and lead citrate for observation. Each experiment was conducted in three replicates.

2.7 Western blotting

H1975 and NCI-H1299 cells were collected after drug treatment. The cells were lysed at low temperatures with radioimmunoprecipitation assay buffer (RIPA) cell lysis buffer, and the supernatants were collected for protein concentration assessment and electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate the proteins, which were subsequently transferred onto polyvinylidene difluoride (PVDF) membranes. After the membrane was blocked, it was incubated with primary antibodies overnight at 4 ℃. The primary antibodies used included antibodies against ATF4 (1:1000, no. A0201, ABclonal, Woburn, MA, USA), CHOP (1:1000, no. A20987, ABclonal, Woburn, MA, USA), PERK (1:1000, no. A21255, ABclonal, Woburn, MA, USA), p-PERK (1:1000, no. AP0886, ABclonal, Woburn, MA, USA), and β-actin (1:50,000, no. AC026, ABclonal, Woburn, MA, USA). Then, the membrane was incubated with secondary antibodies for 2 h and detected with enhanced chemiluminescence (ECL). Scion Image 4.0 software (Scion Corporation, Frederick, MD, USA) was used to quantify the band sizes. Each experiment was conducted in three replicates.

2.8 Statistical analysis

All experiments were independently repeated at least three times. Data are expressed as the mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism software (version 9.0.3; GraphPad Software, San Diego, CA, USA). For comparisons between two groups, unpaired two-tailed Student's *t* tests were applied. For comparisons among three or more groups, one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test was performed. A p value < 0.05 was considered statistically significant.

3. Results

3.1 The combination of XFZY serum and osimertinib inhibited proliferation and promoted apoptosis in NSCLC cell lines

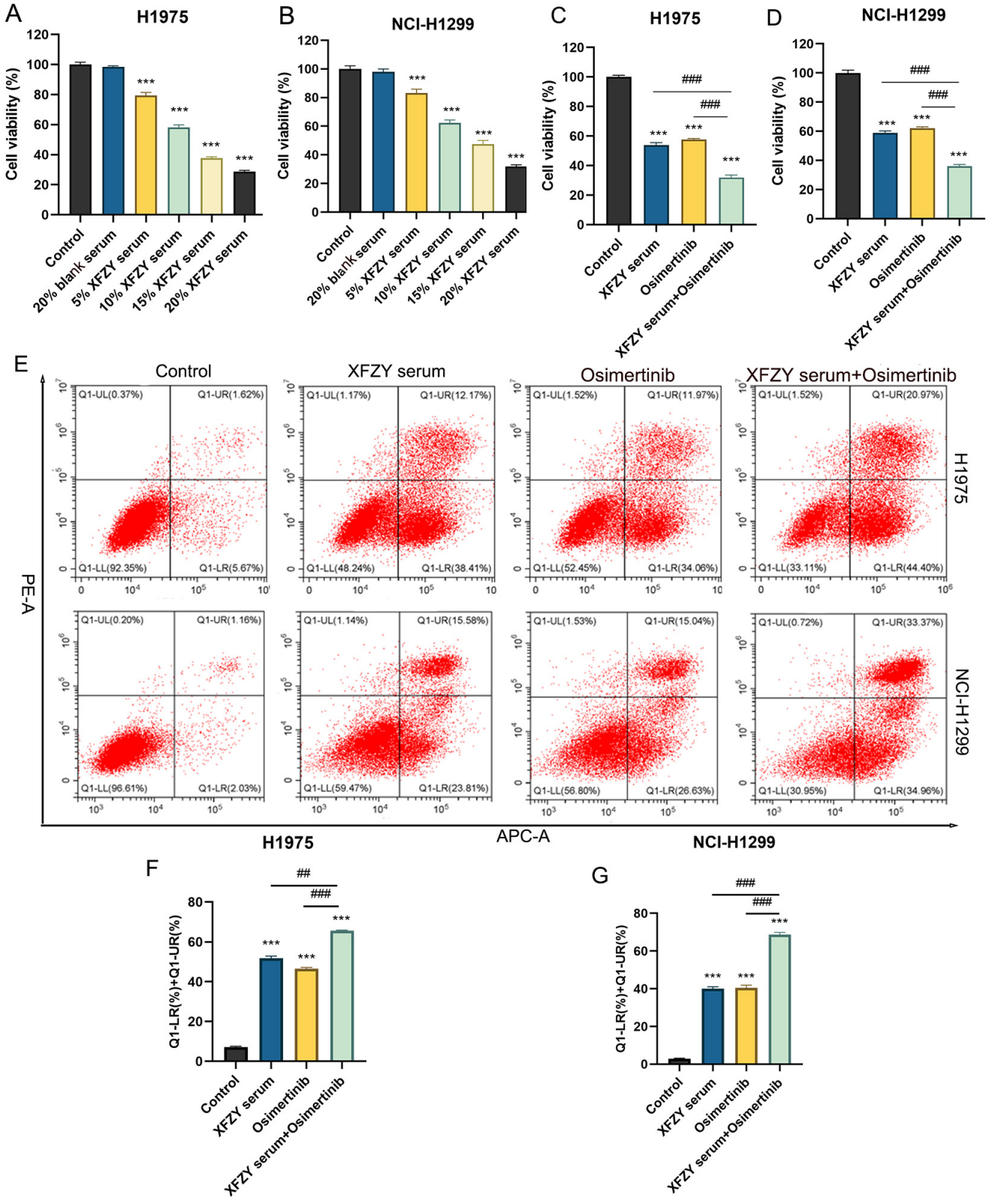
To determine the proliferation of H1975 and NCI-H1299 cells in different XFZY serum concentrations (5%, 10%, 15%, and 20%), we performed a CCK8 assay. The results revealed that 5% XFZY serum significantly inhibited H1975 (Fig. 1A) and NCI-H1299 (Fig. 1B) cell proliferation, while the XFZY serum IC50 was 12.91% for H1975 cells and 14.37% for NCI-H1299 cells. As shown in Fig. 1C,D, XFZY serum and Osimertinib treatment significantly inhibited H1975 and NCI-H1299 cell proliferation. Similarly, flow cytometry was performed to detect the effects of XFZY serum and Osimertinib treatment on the apoptosis of H1975 and NCI-H1299 cells (Fig. 1E). The results revealed that XFZY serum and Osimertinib treatment caused cell apoptosis (Fig. 1F,G). Interestingly, the drug combination showed the best effects, not only in terms of proliferation inhibition but also in terms of apoptosis promotion.

3.2 The combination of XFZY serum and Osimertinib inhibited the migration and invasion of NSCLC cell lines

As shown in Fig. 2A,B, compared with the control group, the XFZY serum + Osimertinib group presented the greatest inhibition of migration among all drug treatment groups. Furthermore, the Transwell assay results revealed that the invasion of H1975 cells (Fig. 2C) and NCI-H1299 cells (Fig. 2D) was inhibited by XFZY serum or Osimertinib treatment. However, the combination of XFZY serum and Osimertinib resulted in the greatest inhibition of cell invasion among the three-drug treatment groups.

3.3 The combination of XFZY serum and Osimertinib promoted ERS in NSCLC cell lines

The effects of the combination of XFZY serum and Osimertinib on ERS in H1975 and NCI-H1299 cells were investigated. Flow cytometry was used to quantify ROS production, and TEM was used to observe ER morphology. As shown in Fig. 3A, ROS production in H1975 and NCI-H1299 cells was promoted by XFZY serum and Osimertinib treatment. Fig. 3B shows that XFZY serum or Osimertinib induced rough ER expansion (green arrows) and mitochondrial swelling (blue arrows), and the combination of XFZY serum and Osimertinib induced severe rough ER expansion and mitophagy. ERS pathway proteins, including ATF4, CHOP, PERK, and p-PERK, were quantified via WB (Fig. 3C). The results indicated that ATF4 (Fig. 3D) and CHOP (Fig. 3E) expression were upregulated by XFZY serum and Osimertinib treatment in both H1975 and NCI-H1299 cells. In addition, the p-PERK/PERK ratio showed a similar trend (Fig. 3F). Importantly, the drug combination activated ERS to a maximum degree.



F I G U R E 1. The effects of the combination of XFZY serum and Osimertinib on the proliferation and apoptosis of NSCLC cell lines. A CCK8 assay was performed to detect cell viability, (A) H1975 and (B) NCI-H1299 cell viability under 5%, 10%, 15%, and 20% XFZY serum treatment; and (C) H1975 and (D) NCI-H1299 cell viability under XFZY serum and Osimertinib treatment each or in combination. (E) Flow cytometry was performed to detect cell apoptosis, and a bar chart was used to analyze the apoptosis of the (F) H1975 and (G) NCI-H1299 cell lines. Compared with the control group, $^{***}p < 0.001$; compared with the XFZY serum + Osimertinib group, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$. XFZY: XueFu ZhuYu; NCI: National Cancer Institute; PE-A: Phycoerythrin-Area; APC-A: Allophycocyanin-Area.

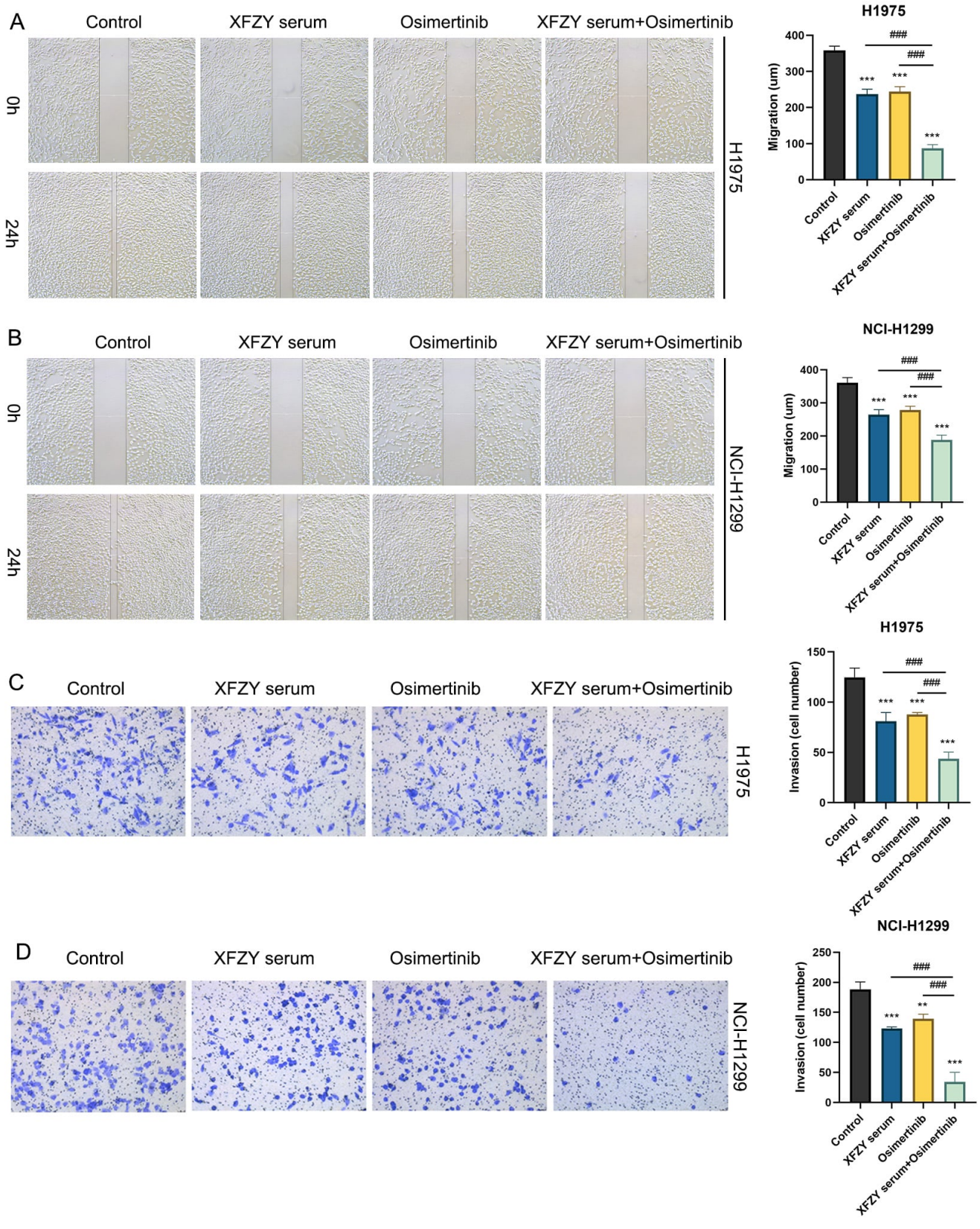


FIGURE 2. The effect of the combination of XFZY serum and Osimertinib on the migration and invasion of NSCLC cell lines. XFZY serum-treated H1975 and NCI-H1299 cell lines with or without Osimertinib. A scratch assay was performed to detect the migration of (A) H1975 and (B) NCI-H1299 cells. Transwell assays were performed to detect the invasion of (C) H1975 and (D) NCI-H1299 cells. Compared with the control group, $^{**}p < 0.01$, $^{***}p < 0.001$; compared with the XFZY serum + Osimertinib group, $^{\#\#\#}p < 0.001$. XFZY: XueFu ZhuYu; NCI: National Cancer Institute.

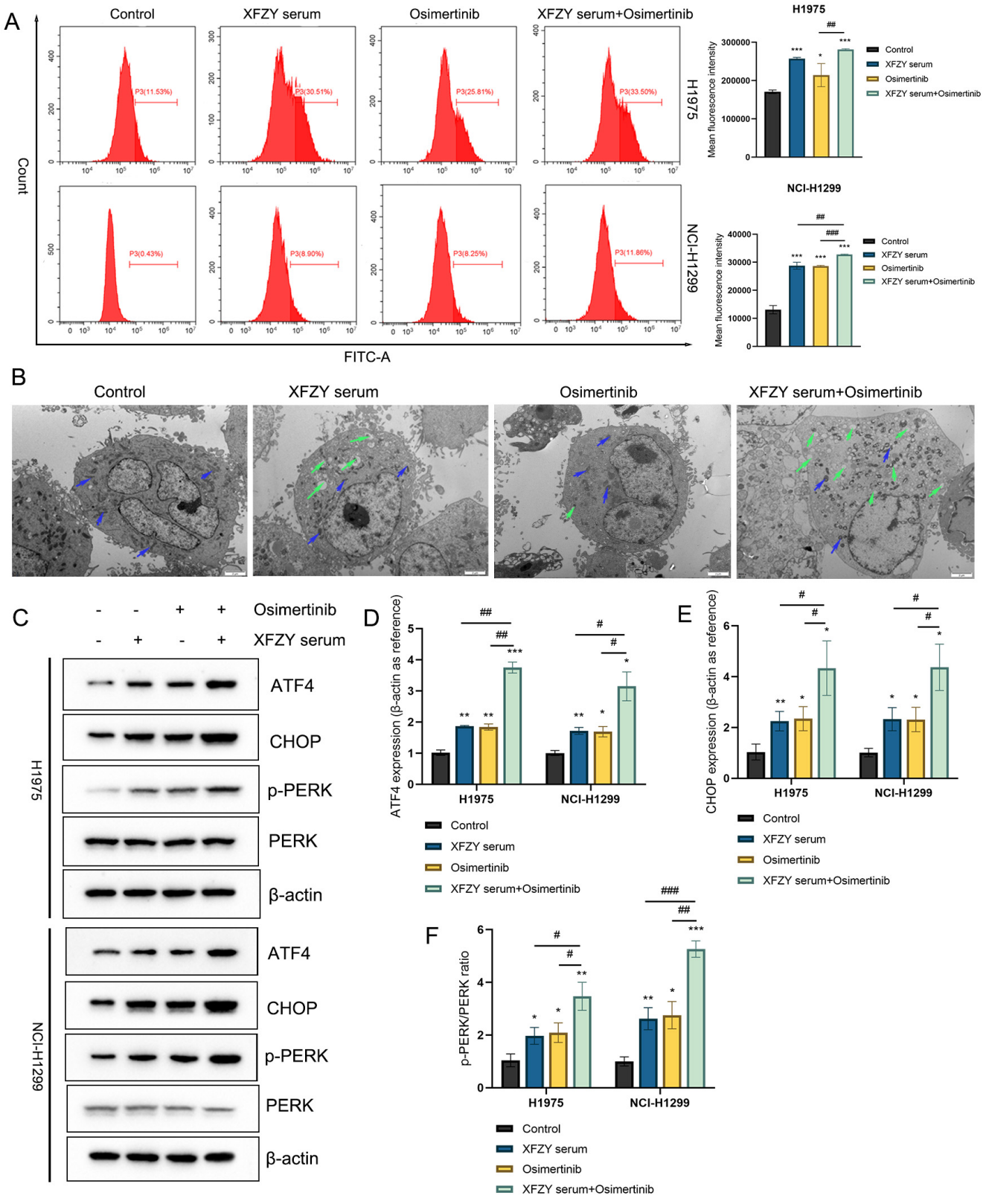


FIGURE 3. The effect of the combination of XFZY serum and Osimertinib on ERS in NSCLC cell lines. XFZY serum-treated H1975 and NCI-H1299 cell lines with or without Osimertinib. (A) ROS production was quantified by flow cytometry. (B) TEM was used to observe the morphology of the ER (green arrows) and Mitochondria (blue arrows); scale bar = 2 μm; (C) WB was performed to detect the expression of proteins, including (D) ATF4 and (E) CHOP, and (F) the p-PERK/PERK ratio. Compared with the control group, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; compared with the XFZY serum + Osimertinib group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$. XFZY: XueFu ZhuYu; NCI: National Cancer Institute; FITC-A: Fluorescein Isothiocyanate-Area; ATF4: Activating Transcription Factor 4; CHOP: C/EBP Homologous Protein; p-PERK: phosphorylated PKR-like ER kinase.

3.4 The combination of XFZY serum and Osimertinib inhibited the proliferation and enhanced the apoptosis of NSCLC cell lines by promoting ROS production

During the development of apoptosis, ROS production is a necessary step. A ROS scavenger (NAC) was used in the following experiments. As shown in Fig. 4A,B, compared with the XFZY serum + Osimertinib group, the XFZY serum + Osimertinib + NAC group showed higher cell viability in H1975 and NCI-H1299 cells. The apoptosis degree of H1975 and NCI-H1299 cells was analyzed via flow cytometry (Fig. 4C). In H1975 cells (Fig. 4D) and NCI-H1299 cells (Fig. 4E), NAC treatment significantly decreased apoptosis induced by XFZY serum + Osimertinib. Notably, NAC did not affect cell proliferation or apoptosis.

3.5 The combination of XFZY serum and Osimertinib inhibited the migration and invasion of NSCLC cell lines by promoting ROS production

Next, scratch and Transwell assays were performed to detect cell migration and invasion under treatment with XFZY serum + Osimertinib and NAC. The results revealed that, compared with the control group, the NAC group presented no obvious change. The XFZY serum + Osimertinib treatment significantly decreased H1975 cells (Fig. 5A) and NCI-H1299 cell migration (Fig. 5B). Importantly, compared with the XFZY serum + Osimertinib group, NAC addition significantly increases cell migration ability. As shown in Fig. 5C,D, the invasion of H1975 cells and NCI-H1299 cells tended to be similar to their migration.

3.6 The combination of XFZY serum and Osimertinib promoted ERS in NSCLC cell lines by promoting ROS production

To further investigate whether the combination of XFZY serum and Osimertinib promotes ERS in NSCLC cell lines by promoting ROS production. The experiments, including flow cytometry, TEM, and WB, were performed again. As shown in Fig. 6A, NAC decreased the level of ROS production in H1975 and NCI-H1299 cells after XFZY serum + Osimertinib treatment. Fig. 6B shows that XFZY serum + Osimertinib induced severe rough ER expansion and mitophagy, and NAC alleviated these effects. ERS pathway protein bands, including those of ATF4, CHOP, PERK, and p-PERK, are displayed in Fig. 6C. The results indicated that ATF4 (Fig. 6D) and CHOP (Fig. 6E) expression were upregulated by XFZY serum + Osimertinib in both H1975 and NCI-H1299 cells. Compared with XFZY serum + Osimertinib, XFZY serum + Osimertinib + NAC treatment downregulated ATF4 and CHOP expression. In addition, the p-PERK/PERK ratio showed a similar trend (Fig. 6F).

4. Discussion

Osimertinib, a third-generation EGFR-TKI, acquired resistance after its application in 2015, which was an obstacle to the treatment of NSCLC [16]. XFZY decoction, a key formula in Chinese medicine, is regularly utilized as a supplementary therapy for tumors. Research indicates that XFZY plays a significant role in hindering glioma growth through the regulation of the tumor microenvironment [14]. Furthermore, network pharmacology analysis revealed that there are 128 active ingredients and 80 key targets in the XFZY decoction for tumor treatment [17]. However, it is not clear whether XFZY decoction can improve Osimertinib sensitivity in NSCLC treatment. In this study, two NSCLC cell lines (H1975 and NCI-H1299) were used for drug experiments. Results indicated that XFZY serum and Osimertinib treatment can inhibit the proliferation, migration, and invasion abilities of NSCLC cells, but apoptosis was enhanced. Interestingly, the combination of XFZY serum and Osimertinib treatment shows the best effects. These findings demonstrated that XFZY serum has a synergistic effect with Osimertinib.

Evidence demonstrates that excessive ERS contributes to cancer cell death. For example, Mei Jing Piao *et al.* [18] reported that shikonin can reinforce the 5-fluorouracil-induced death of colorectal cancer cells and ovarian cells, via upregulating ERS. Yongliang Cui *et al.* [19] reported that Chalcomoracin increases ERS level by regulating the mitogen-activated protein kinase (MAPK) pathway, leading to the inhibition of hepatocellular carcinoma cell growth. The present study revealed that the combination of XFZY serum and Osimertinib significantly overloads ROS and ERS in NSCLC cell lines. However, the underlying molecular mechanism needs to be further explored.

ERS-responsive apoptosis is a novel apoptotic pathway. In this process, the phosphorylation of R-like endoplasmic reticulum kinase (PERK) is activated, and it further activates the phosphorylation of eIF2α, meanwhile, inducing the transcription of the activating transcription factor 4 (ATF4) [20, 21]. However, the continuous and overloading ERS can lead to an increase in the CHOP level. As reported, CHOP plays a key role in the process of ERS-responsive apoptosis by down-regulating the anti-apoptotic gene *Bcl-2* and increasing ROS generation [22, 23]. Our study revealed that the combination of XFZY serum and Osimertinib significantly upregulated protein expression of p-PERK, ATF4, and CHOP in H1975 and NCI-H1299 cells. Importantly, the combination of XFZY serum and Osimertinib induced severe rough ER expansion and mitophagy. The PERK-eIF2α-ATF4 pathway is deeply coupled with mitophagy at the endoplasmic reticulum-mitochondrial contact sites (MAM/MERCS) [24, 25]. They synergistically eliminate damaged mitochondria to aid cellular survival. However, when this process becomes sustained or excessive, it triggers cell death by accelerating ROS accumulation [20, 25]. Therefore, the results suggested that XFZY serum-induced cell apoptosis may involve regulating the PERK-eIF2α-ATF4 pathway and accumulating ROS.

Considering the role of ROS in the process of ERS and cell apoptosis, we inferred that ROS generation might be a potential target of XFZY serum to cause NSCLC cell death. Therefore, a ROS scavenger (*N*-acetylcysteine, NAC) was applied to treat H1975 and NCI-H1299 cells with or without XFZY serum + Osimertinib. These results are consistent with our assumption that the combination of XFZY serum

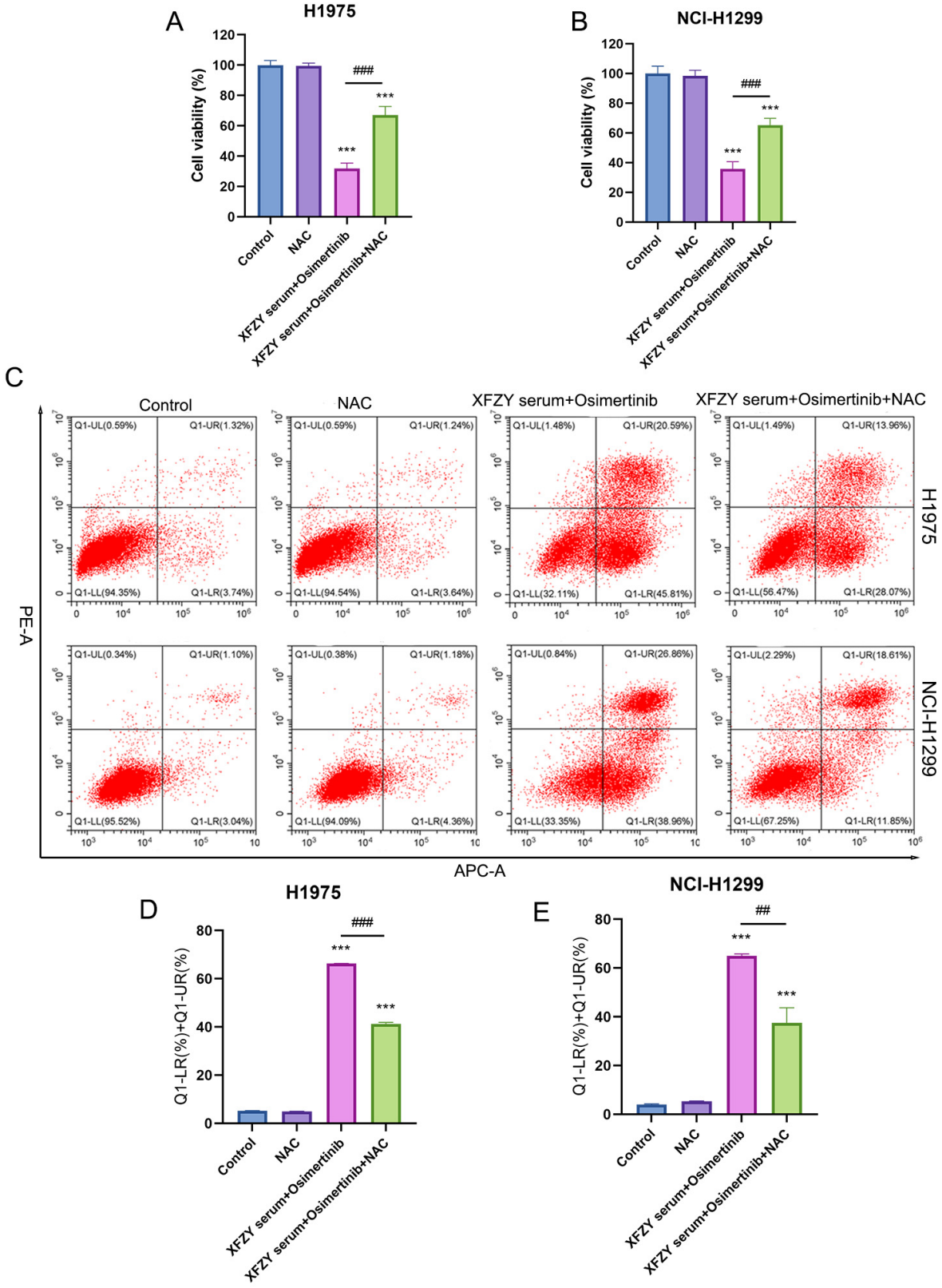


FIGURE 4. The combination of XFZY serum and Osimertinib inhibited the proliferation and apoptosis of NSCLC cell lines by promoting ROS production. The combination of XFZY serum and Osimertinib was used to treat H1975 and NCI-H1299 cell lines with or without NAC. A CCK8 assay was performed to detect the proliferation of (A) H1975 and (B) NCI-H1299 cells. (C) Flow cytometry was performed to detect cell apoptosis, and a bar chart was used to analyze the apoptosis of the (D) H1975 and (E) NCI-H1299 cell lines. Compared with the control group, ***$p < 0.001$; compared with the XFZY serum + Osimertinib group, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$. XFZY: XueFu ZhuYu; NAC: *N*-acetylcysteine; NCI: National Cancer Institute; PE-A: Phycoerythrin-Area; APC-A: Allophycocyanin-Area.

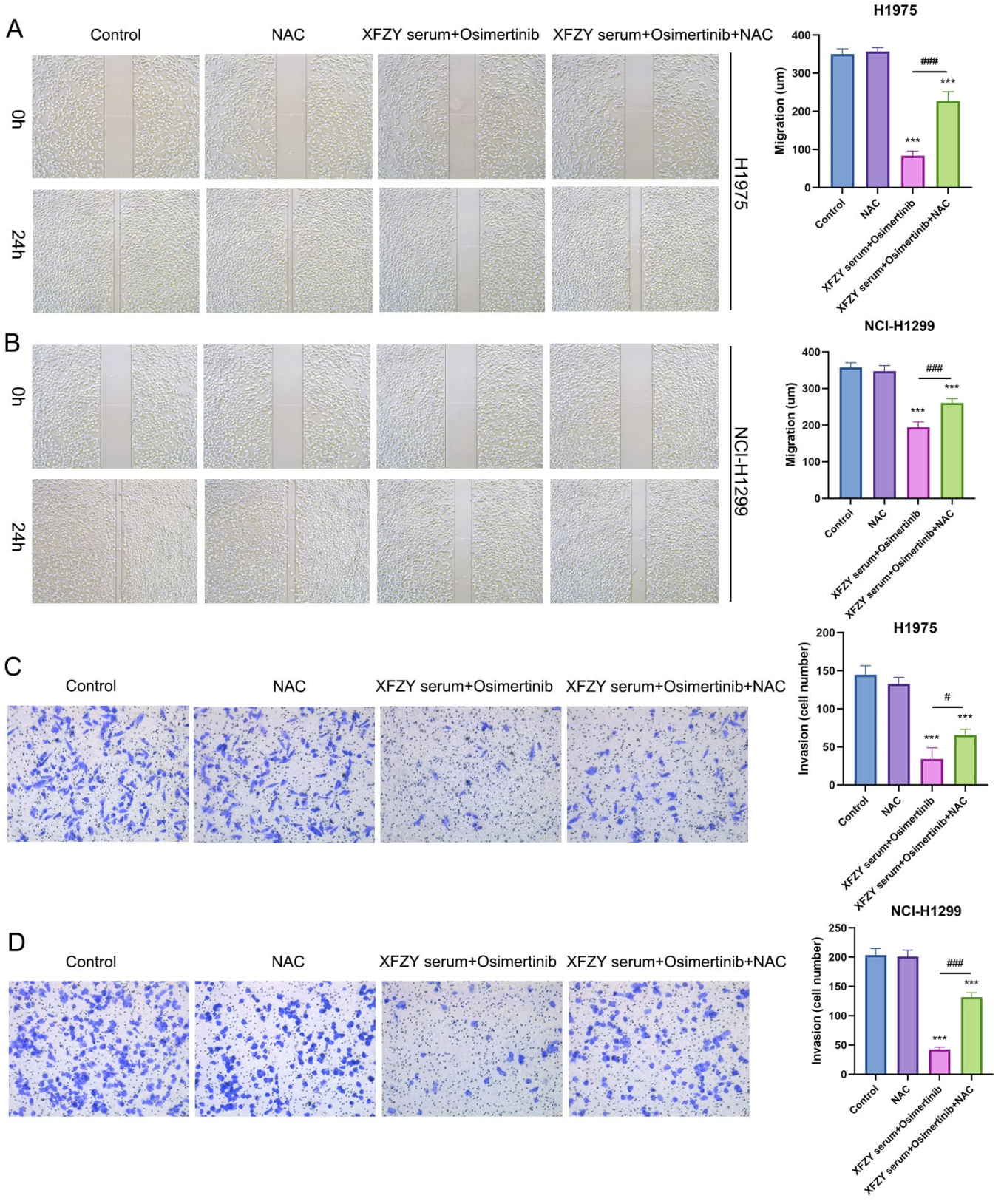


FIGURE 5. The combination of XFZY serum and Osimertinib inhibited the migration and invasion of NSCLC cell lines by promoting ROS production. The combination of XFZY serum and Osimertinib was used to treat H1975 and NCI-H1299 cell lines with or without NAC. A scratch assay was performed to detect the migration of (A) H1975 and (B) NCI-H1299 cells. Transwell assays were performed to detect the invasion of (C) H1975 and (D) NCI-H1299 cells. Compared with the control group, ***$p < 0.001$; compared with the XFZY serum + Osimertinib + NAC group, $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$. XFZY: XueFu ZhuYu; NAC: *N*-acetylcysteine; NCI: National Cancer Institute.

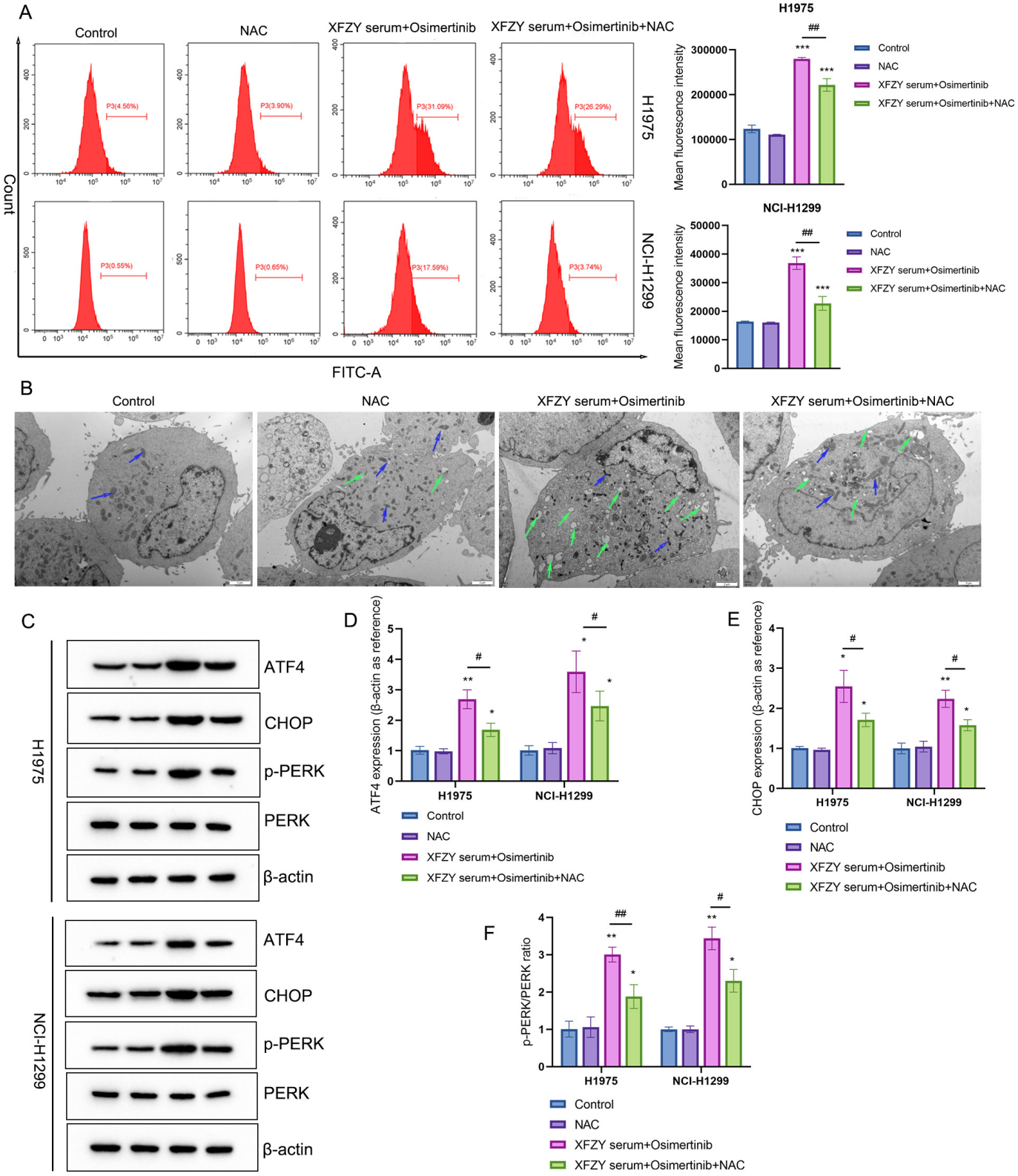


FIGURE 6. The combination of XFZY serum and Osimertinib promoted ERS in NSCLC cell lines by promoting ROS production. The combination of XFZY serum and Osimertinib was used to treat H1975 and NCI-H1299 cell lines with or without NAC. (A) ROS production was quantified by flow cytometry. (B) TEM was used to observe the morphology of the ER (green arrows) and Mitochondria (blue arrows); scale bar = 2 μm. (C) WB was performed to detect the expression of proteins, including (D) ATF4 and (E) CHOP, and (F) the p-PERK/PERK ratio. Compared with the control group, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; compared with the XFZY serum + Osimertinib group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$. XFZY: XueFu ZhuYu; NAC: *N*-acetylcysteine; NCI: National Cancer Institute; FITC-A: Fluorescein Isothiocyanate-Area; ATF4: Activating Transcription Factor 4; CHOP: C/EBP Homologous Protein; p-PERK: phosphorylated PKR-like ER kinase.

and Osimertinib significantly increases ROS generation, which NAC could counteract to a certain degree. In addition, the application of NAC alleviated apoptosis and ERS and increased the migration and invasion of cancer cells treated with XFZY serum + Osimertinib.

5. Conclusions

In brief, this study demonstrated that the combination of XFZY serum and Osimertinib could significantly inhibit cell proliferation, migration, and invasion and promote the apoptosis and ERS of H1975 and NCI-H1299 cells. However, ROS scavengers could offset the effects of XFZY serum and Osimertinib treatment. These findings suggested that XFZY decoction could increase the Osimertinib sensitivity of NSCLC cell lines by enhancing the ERS response and activating the PERK-ATF4-CHOP signaling pathway. This study is limited by its reliance on *in vitro* cell models, without validation in clinical samples or *in vivo* systems. Consequently, the translational relevance remains uncertain. Future work will incorporate clinical specimens and animal models to confirm and expand our findings.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

MLS—conceptualization; methodology; writing–original draft preparation; funding acquisition. YL—methodology; formal analysis and investigation. QZ—methodology. ZLL—formal analysis and investigation. YCH—visualization. DDZ—conceptualization; writing–review and editing.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Animal Ethics Committee of West China Hospital of Sichuan University (No: 20230423001).

ACKNOWLEDGMENT

Not applicable.

FUNDING

This study was supported by the General Program of Chongqing Natural Science Foundation (No. cstc2021jcyj-msxmX0860).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Sharma R, Khubchandani J. Global, regional, and national burden of tracheal, bronchus, and lung cancer in 2022: evidence from the GLOBOCAN study. Epidemiologia. 2024; 5: 785–795.

[2] Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. CA: A Cancer Journal for Clinicians. 2024; 74: 12–49.

[3] Chatterjee K, Ray A, Chattopadhyay B. Incidence and characteristics of Epidermal Growth Factor Receptor (EGFR) mutation in non-small-cell lung cancer (Adenocarcinoma histology): a report of 106 patients from Kolkata. Indian Journal of Cancer. 2017; 54: 305–307.

[4] Oxnard GR, Arcila ME, Sima CS, Riely GJ, Chmielecki J, Kris MG, *et al*. Acquired resistance to EGFR tyrosine kinase inhibitors in EGFR-mutant lung cancer: distinct natural history of patients with tumors harboring the T790M mutation. Clinical Cancer Research. 2011; 17: 1616–1622.

[5] Planchard D, Jänne PA, Cheng Y, Yang JC, Yanagitani N, Kim SW, *et al*. A plain language summary of results from the FLAURA2 study: Initial (first-line) osimertinib treatment with or without chemotherapy in patients with untreated EGFR-mutated advanced NSCLC. Future Oncology. 2025; 21: 903–915.

[6] Lu S, Kato T, Dong X, Ahn MJ, Quang LV, Soparattanapaisarn N, *et al*. Osimertinib after chemoradiotherapy in stage III EGFR-mutated NSCLC. The New England Journal of Medicine. 2024; 391: 585–597.

[7] Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nature Reviews Molecular Cell Biology. 2007; 8: 519–529.

[8] Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. Nature Reviews Molecular Cell Biology. 2012; 13: 89–102.

[9] Chen BL, Sheu ML, Tsai KS, Lan KC, Guan SS, Wu CT, *et al*. CCAAT-enhancer-binding protein homologous protein deficiency attenuates oxidative stress and renal ischemia-reperfusion injury. Antioxidants & Redox Signaling. 2015; 23: 1233–1245.

[10] Kwag E, Kim SD, Shin SH, Oak C, Park SJ, Choi JY, *et al*. A randomized, multi-center, open label study to compare the safety and efficacy between afatinib monotherapy and combination therapy with HAD-B1 for the locally advanced or metastatic NSCLC patients with EGFR mutations. Integrative Cancer Therapies. 2024; 23: 15347354241268231.

[11] Xi Z, Dai R, Ze Y, Jiang X, Liu M, Xu H. Traditional Chinese medicine in lung cancer treatment. Molecular Cancer. 2025; 24: 57.

[12] Wang R, Luo Z, Zhang H, Wang T. Tanshinone IIA reverses gefitinib-resistance in human non-small-cell lung cancer via regulation of VEGFR/Akt pathway. OncoTargets and Therapy. 2019; 12: 9355–9365.

[13] Tian X, Wang R, Gu T, Ma F, Laster KV, Li X, *et al*. Costunolide is a dual inhibitor of MEK1 and AKT1/2 that overcomes osimertinib resistance in lung cancer. Molecular Cancer. 2022; 21: 193.

[14] Liu J, Zhang J, Huang L, Zhu X, Chen W, Hu P. XuefuZhuyu Tang exerts antitumor effects by inhibiting glioma cell metastasis and invasion via regulating tumor microenvironment. OncoTargets and Therapy. 2016; 9: 3603–3612.

[15] Meng L, Zhang X, Wang H, Dong H, Gu X, Yu X, *et al*. Yangyin Yiqi mixture ameliorates bleomycin-induced pulmonary fibrosis in rats through inhibiting TGF-β1/Smad pathway and epithelial to mesenchymal transition. Evidence-Based Complementary and Alternative Medicine. 2019; 2019: 2710509.

[16] Thress KS, Paweletz CP, Felip E, Cho BC, Stetson D, Dougherty B, *et al*. Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M. Nature Medicine. 2015; 21: 560–562.

[17] Wang S, Wu C, Li Y, Ye B, Wang S, Li G, *et al*. Analysis of the anti-tumour effect of XueFu Zhuyu decoction based on network pharmacology and experimental verification in drosophila. Frontiers in Pharmacology. 2022; 13: 922457.

[18] Piao MJ, Han X, Kang KA, Fernando PDSM, Herath HMUL, Hyun JW. The endoplasmic reticulum stress response mediates shikonin-induced apoptosis of 5-fluorouracil-resistant colorectal cancer cells. Biomolecules & Therapeutics. 2022; 30: 265–273.

[19] Cui Y, Lan L, Lv J, Zhao B, Kong J, Lai Y. Chalcomoracin promotes

apoptosis and endoplasmic reticulum stress in hepatocellular carcinoma cells. The Journal of Antibiotics. 2024; 77: 428–435.

[20] Chen X, Shi C, He M, Xiong S, Xia X. Endoplasmic reticulum stress: molecular mechanism and therapeutic targets. Signal Transduction and Targeted Therapy. 2023; 8: 352.

[21] Teske BF, Wek SA, Bunpo P, Cundiff JK, McClintick JN, Anthony TG, *et al*. The eIF2 kinase PERK and the integrated stress response facilitate activation of ATF6 during endoplasmic reticulum stress. Molecular Biology of the Cell. 2011; 22: 4390–4405.

[22] Chan CP, Kok KH, Jin DY. CREB3 subfamily transcription factors are not created equal: recent insights from global analyses and animal models. Cell & Bioscience. 2011; 1: 6.

[23] Huang SM, Cheung CW, Chang CS, Tang CH, Liu JF, Lin YH, *et al*. Phloroglucinol derivative MCPP induces cell apoptosis in human colon cancer. Journal of Cellular Biochemistry. 2011; 112: 643–652.

[24] Rühmkorf A, Harbauer AB. Role of mitochondria-ER contact sites in mitophagy. Biomolecules. 2023; 13: 1198.

[25] Kwon J, Kim J, Kim KI. Crosstalk between endoplasmic reticulum stress response and autophagy in human diseases. Animal Cells and Systems. 2023; 27: 29–37.

How to cite this article: Mingling Sun, Yong Liu, Qiong Zhang, Zonglang Lai, Yancheng He, Dandan Zheng. The combination of Xuefu Zhuyu decoction and osimertinib inhibits the proliferation of non-small cell lung cancer cells by activating endoplasmic reticulum stress. Journal of Men's Health. 2025; 21(10): 79-90. doi: 10.22514/jomh.2025.128.