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TROP2 regulates cisplatin sensitivity of triple-negative breast cancer cells by regulating endoplasmic reticulum stress

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Summary. Triple-negative breast cancer (TNBC) is a kind of breast cancer with a high metastasis rate and poor prognosis. As a transmembrane glycoprotein, tumor-associated calcium signal transducer 2 (TROP2) plays a certain role in the cancers. This study aimed to explore the potential mechanism of TROP2 affecting cisplatin (CDDP) resistance in TNBC from endoplasmic reticulum stress (ERS). MDA-MB-231 and CDDP-resistant cell lines MDA-MB-231/CDDP were used in this study, and the expression of TROP2 was detected by western blotting. After transfecting with the interference sequence of siRNA targeting TROP2, cell proliferation and apoptosis were detected by the cell counting kit-8, colony formation, and flow cytometry, and the expression of ERS-marker proteins was detected by western blotting. Furthermore, the effects of ERS in TROP2 on drug resistance of TNBC cells were explored by using ERS inhibitor 4-phenylbutyric acid (4-PBA). Results found that TROP2 expression in MDA-MB-231/CDDP was significantly upregulated compared with MDA-MB-231. The expression of TROP2 in MDA-MB-231/CDDP was significantly decreased after transfection with siRNA-TROP2, and the proliferation of MDA-MB-231 and MDA-MB-231/CDDP cells was significantly decreased after further induction with CDDP. TROP2 significantly affected TNBC cell cloning, apoptosis, and the expression of ERS-related marker proteins, while 4-PBA reversed the promoting effects of siRNA-TROP2 on apoptosis and ERS, as well as the inhibitory effects on cell proliferation, suggesting that TROP2 affected the resistance of TNBC cells to CDDP through ERS. In conclusion, TROP2 inhibited apoptosis of TNBC cells, improved the cell cloning ability, and regulated the sensitivity of TNBC cells to CDDP through ERS.

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Introduction

Breast cancer is the most common malignant tumor in women. According to the World Health Organization, 2.3 million women were diagnosed with breast cancer and 670,000 died in 2022 (WHO, 2022). Triple-negative breast cancer (TNBC) refers to breast cancer without the expression of three receptors, including the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), and its incidence accounts for about 15% of all breast cancers (Yin et al., 2020). Compared with other breast cancer subtypes, TNBC is characterized by high invasiveness, early metastasis, high recurrence rate, and poor prognosis, and most deaths occur within the first five years after treatment (Howard and Olopade, 2021). Due to the lack of hormone receptors and HER2, TNBC is not sensitive to endocrine or biological therapies, and chemotherapy is still the standard treatment (Derakhshan and Reis-Filho, 2022). Several clinical trials have confirmed that platinum-based systematic therapy has a good effect on TNBC, especially in patients with a BRCA1 mutation who are more sensitive to cisplatin (CDDP) (Zhu et al., 2022). CDDP is the first platinum-based anti-tumor drug to be marketed, and a study has shown that the total clinical effective rate of CDDP monotherapy for TNBC has reached 50%, and the pathological complete response rate was similar to that of multi-drug combination therapy (Silver et al., 2010). CDDP mainly produces anti-tumor effects by destroying the genomic DNA of cells (Achkar et al., 2018; Riddell and Lippard, 2018). Also, TNBC has certain DNA damage repair obstacles, so this type of breast cancer is highly sensitive to platinum drugs. The study found that the pathologic complete response (pCR) of breast cancer

patients receiving neoadjuvant and adjuvant chemotherapy with CDDP was significantly higher in TNBC than in non-TNBC patients (Poggio et al., 2018). Compared with platinum-free chemotherapy regimens, platinum-containing chemotherapy regimens can prolong patients' disease-free survival (Zhao et al., 2023). Platinum drugs are considered an effective choice for the treatment of postoperative recurrence and metastasis of TNBC (Li et al., 2022). However, similar to other chemotherapy drugs, drug resistance has become a serious and intractable problem in the treatment of TNBC with platinum-based drugs. If platinum resistance occurs, tumor cells will be more aggressive and metastatic, and patients will benefit less from treatment and have a poor prognosis (Ferrari et al., 2022). It is an urgent problem to explore the mechanism of CDDP resistance of TNBC.

Tumor-associated calcium signal transducer 2 (TROP2) is a 46 kDa type I transmembrane glycoprotein, which shares 49% homology and 67% similarity with EpCAM, and is expressed in skin, liver, breast, renal tubules, and other tissues (McDougall et al., 2015; Zaman et al., 2019). As a member of the TACSTD gene family, TROP2 is considered a cell surface marker of invasive trophoblast cells (McDougall et al., 2015). Studies have shown that TROP2 is related to cell proliferation, invasion, metastasis, and poor prognosis of various cancer types (Li et al., 2016; Akarken and Dere, 2021). TROP2 is a key therapeutic target, which is related to the malignancy of various solid tumors, including breast cancer (Goldenberg et al., 2018). However, the role of TROP2 in breast cancer remains unclear.

The endoplasmic reticulum (ER) is an intracellular organelle that plays an important role in maintaining intracellular balance and the delicate balance between health and disease (Wang and Kaufman, 2016). When a stressor is applied to cells, protein folding in the ER goes wrong, and the accumulation of unfolded proteins and imbalance of calcium levels lead to the activation of the ER stress (ERS) pathway (Ansari et al., 2018; Khaket et al., 2018). ERS is a key signal transduction event that induces apoptosis and subsequently enhances the cytotoxic effects of a variety of chemotherapy drugs (Mishra et al., 2018). ERS plays a role in the process of cell-tumor microenvironment interaction (Rodvold et al., 2016; Chen et al., 2018), and the activation of the ERS pathway can promote the antitumor effect of some drugs (Xu et al., 2020; Samanta et al., 2021). The key ERS sensor IRE1a signaling pathway directly or indirectly affects the malignant growth, angiogenesis, and progression of tumors through the microenvironment (Cubillos-Ruiz et al., 2017; Harnoss et al., 2020).

This study first analyzed the expression of TROP2 in CDDP-resistant TNBC cells and TNBC cells to reveal the sensitivity of CDDP drugs to TNBC cell proliferation and then analyzed the effects of TROP2 on TNBC cell apoptosis, cloning, and ERS. The manuscript explores the molecular mechanism of TROP2 affecting the sensitivity of TNBC cells to CDDP and provides a theoretical basis for the drug treatment of TNBC.

Materials and methods

Construction of the MDA-MB-231/CDDP cell line

MDA-MB-231/CDDP cell lines were constructed by repeatedly treating MDA-MB-231 cells (Procell, Inc.) with high concentrations of CDDP (Selleck Chem, Inc.) for a short time (Coley, 2004). MDA-MB-231 parent cells in the logarithmic growth phase had CDDP solution added at an initial concentration of 5 μm and placed in the cell incubator for 48h and then restored to a complete medium without CDDP. This was an action cycle of this concentration. When the cell morphology returned to normal and entered the logarithmic growth phase, the same concentration was repeated. For each concentration of CDDP, three cycles were completed to increase the concentration of CDDP, and the above steps were repeated until the final concentration reached 50 μm. The cells were incubated in a CDDP-free medium for at least six months, and the assay remained resistant to CDDP for subsequent experiments. The survival rates of MDA-MB-231 and MDA-MB-231/CDDP cells treated with different concentrations of CDDP (0, 1, 5, 10, 20, 40, 80 μM) were detected by the cell counting kit-8 (CCK8) assay, and the IC_{50} of the two cell lines and CDDP resistance fold (RF) of MDA-MB-231/CDDP cells were calculated to demonstrate CDDP resistance in MDA-MB231/CDDP cells.

CCK8 assay

The supernatant was discarded after the cells were treated differently, and then 10 μL of CCK8 dilution working solution (cat. no. BS350A; Biosharp, Inc.) diluted 1:10 in serum-free medium was added to each well. The culture plate was gently shaken several times and incubated at 37°C for 2h. The absorbance values of each well were measured with a microplate reader at 450 nm wavelength, and the inhibition rate of cell proliferation was calculated.

Cell culture and experimental grouping

MDA-MB-231 and MDA-MB-231/CDDP cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Procell, Inc.) supplemented with 10% fetal bovine serum (FBS; TRAN, Inc.). Both cell lines were maintained in a humidified atmosphere containing 5% CO_2 at 37°C. The cell experiment was divided into two parts. In the first part, the two types of cells were divided into siRNA-NC, siRNA-NC+CDDP (50 μM), siRNA-TROP2, and siRNA-TROP2+CDDP (50 μM) groups. The CDDP dose of the two cell lines was 35 μM for 24h. The second part was divided into siRNA-NC, siRNA-NC+4-PBA (0.5 μmol/L), siRNA-TROP2, siRNA-TROP2+4-PBA (0.5 μmol/L), siRNA-TROP2+CDDP

(IC_{50}, 12 μM for MDA-MB-231, 61 μM for MDA-MB-231/CDDP), and siRNA-TROP2+CDDP+4-PBA (0.5 μmol/L 4-PBA, IC_{50} CDDP) groups. According to the experimental group, after transfection with siRNA, the cells were pretreated with 0.5 μmol/L ERS inhibitor 4-phenylbutyric acid (4-PBA) for 2h, and IC_{50} CDDP was induced for 24h.

Cell transfection

Using the *TROP2* mRNA sequence published in NCBI (NM_002353.3), the siRNA design software (BLOCK-iT™ RNAi Designer) of the Invitrogen website was used to design interference primers. Cells were transfected with Lipofectamine 2000 (cat. no. 11668-019, Invitrogen) under their instructions. Lipofectamine 2000 and siRNA at the recommended dose were added into the medium, mixed with the solution, and allowed to stand for 30 min. The Lip2000-siRNA mixed solution was used to treat cells for 6h and then replaced with a maintenance medium for another 36h.

Western blot analysis

Total cellular protein was obtained by lysing TNBC cells in RIPA buffer (Pierce; Servicebio, Inc.). Protein concentrations were detected using a BCA Protein Assay Kit (Beyotime, Inc.). Samples containing ~30 μg of protein were separated by 10% SDS-PAGE, and the protein bands were transferred onto PVDF membranes (Invitrogen; Sigma Aldrich, Inc.). The membranes were then blocked with 5% skim milk and subsequently incubated overnight with primary antibodies against TROP2 (cat. no. A8129, dilution: 1:2000; ABclonal), Caspase-12 (cat. no. ab8118, dilution: 1:2000; Abcam), CHOP (cat. no. A20987, dilution: 1:2000, ABclonal), PARP (cat. no. A0010, dilution: 1:2000; ABclonal), GRP78 (cat. no. A4908, dilution: 1:2000; ABclonal), cleaved-PARP (cat. no. 5625, dilution: 1:1000; CST), and β-actin (cat. no. AC026, dilution: 1:50000; ABclonal) at 4°C. On the following day, the membranes were incubated with an HRP-conjugated secondary antibody (cat. no. ab6721, dilution: 1:5000; Abcam). The proteins were detected using an enhanced chemiluminescence kit (ZenBio, lnc.). Quantification of western blot bands was performed using Image-Pro Plus (Version 6; Media Cybernetics, Inc.).

Colony formation assay

A total of 800 MDA-MB-231 and MDA-MB-231/CDDP cell suspensions of different treatments were inoculated into a 6-well plate and incubated at 37°C and 5% CO_2 for about 14 days. The culture was terminated when visible clones appeared in the 6-well plate. After the supernatant was discarded, the cells were fixed with pure ethanol for 30 min, stained with 0.1% crystal violet for 15 min, and the images were collected after air drying.

Flow cytometry

Cell apoptosis was detected with an Annexin V-APC/PI Double Staining cell apoptosis detection Kit (cat. no. KGA1030; Keygen lnc.). MDA-MB-231 and MDA-MB-231/CDDP cells were harvested, washed twice in PBS, stained with Annexin V-APC/PI for 15 min, and analyzed with a flow cytometer (CytoFLEX, Beckman).

Statistical methods

All statistical analyses were performed using IBM SPSS Statistics 21.0 (IBM Corp.). Quantitative results are expressed as the mean ± SD of data obtained from at least three experiments. Differences between two groups were analyzed using Student's t-test and differences among groups were assessed by one-way analysis of variance (ANOVA). $P<0.05$ was considered statistically significant.

Results

TROP2 was highly expressed in CDDP-resistant TNBC cells

To prove the drug resistance of MDA-MB-231/CDDP cells, MDA-MB-231 and MDA-MB-231/CDDP cells were treated with different doses of CDDP. The cell proliferation activity was analyzed by CCK8, and the results are shown in Fig. 1A. The IC_{50} of MDA-MB-231 and MDA-MB-231/CDDP against CDDP was 16.43 and 84.92 μM, respectively, and the RF of MDA-MB-231/CDDP cells was 5.17, suggesting that the drug resistance of MDA-MB-231/CDDP cells met the requirements of drug-resistant strains. The expression level of TROP2 in TNBC cells was further detected by Western blot, and results showed that the expression of TROP2 in CDDP-resistant TNBC cells was significantly higher than that in TNBC cells ($P<0.05$, Fig. 1B). To explore the effect of TROP2 expression on the drug resistance of TNBC cells, siRNA interference sequences were constructed and transfected into two cell lines, and the expression level of TROP2 in the cells was detected by western blot. As shown in Fig.1C, after transfection of siRNA-TROP2, TROP2 expression in MDA-MB-231 and MDA-MB-231/CDDP cells was significantly downregulated ($P<0.01$). The effects of siRNA-TROP2 on the proliferation of MDA-MB-231/MDA-MB-231/CDDP cells induced by CDDP was further analyzed, as shown in Fig. 1D. After transfection of siRNA-TROP2, the cell proliferation of MDA-MB-231 and MDA-MB-231/CDDP cells decreased significantly ($P<0.05$), and the IC_{50} of cell proliferation in MDA-MB-231 cells decreased from

16.242 μM to 12.079 μm, and the IC_{50} in MDA-MB-231/CDDP cells decreased from 81.817 μm to 60.852 μm.

Silencing TROP2 and CDDP treatment promoted the apoptosis of TNBC cells and reduced cell cloning ability

To confirm the effects of TROP2 on drug resistance of TNBC cells *in vitro*, siRNA-TROP2 was transfected into MDA-MB-231 and MDA-MB-231/CDDP cell lines, and a series of functional experiments were performed. Firstly, the apoptosis of MDA-MB-231 and MDA-MB-231/CDDP cells transfected with siRNA-TROP2 was analyzed by flow cytometry with Annexin V-APC/PI double staining. As shown in Figure 2A, after transfection of siRNA-TROP2, the apoptosis rate of MDA-MB-231 cells (7.77±0.18%) was higher than that of the si-NC group (6.59±0.38%) (P>0.05), and the apoptosis rate of MDA-MB-231/CDDP cells (16.97±0.57%) was significantly higher than that of the si-NC group (2.48±0.17%) (P<0.01). The apoptosis rate of the two cells transfected with siRNA-TROP2 was significantly increased after CDDP induction (P<0.01). A colony formation assay was used to further test the effect of TROP2 on the cloning ability of TNBC cells. As shown in Figure 2B, after transfection of siRNA-TROP2, the number of MDA-MB-231 cell clones formed (117.33±4.51) was significantly lower than that of the si-NC group (251.67±15.82) (P<0.01), and the clone formation number of MDA-MB-231/CDDP cells (194.00±1.63) was also significantly lower than that of the si-NC group (230.00±12.36) (P<0.01). The number of clone formations of the two cells after CDDP induction was significantly lower (P<0.01).

Effect of silencing TROP2 and CDDP induction on ERS

As shown in Figure 3, compared with the siRNA-NC group, the protein levels of caspase-12, cleaved-PARP, PARP, GRP78, and CHOP in MDA-MB-231 and MDA-MB-231/CDDP cells were significantly upregulated in the siRNA-NC+CDDP and siRNA-TROP2 groups (P<0.05). Compared with the siRNA-NC+CDDP or siRNA-TROP2 group, the expression of these proteins in the siRNA-TROP2+CDDP group of the two cell lines was further increased (P<0.01).

The ERS inhibitor 4-PBA reversed the inhibitory effect of siRNA-TROP2 and CDDP co-treatment on TNBC cell proliferation

To further demonstrate the influence of ERS participation in TROP2 on TNBC cell resistance, a series of subsequent functional experiments were

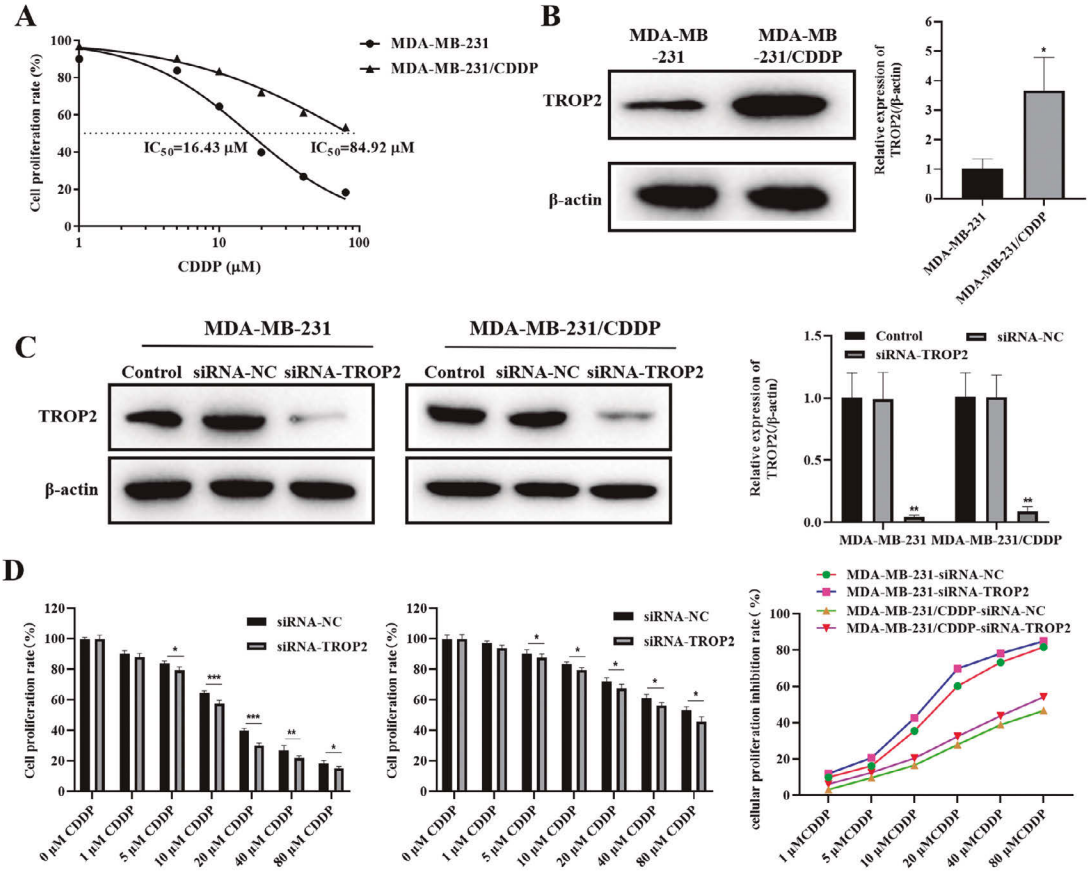

Fig. 1. The expression of TROP2 in TNBC cells and CDDP-resistant TNBC cells. **A.** The proliferation rate and IC_{50} of MDA-MB-231 and MDA-MB-231/CDDP cells were detected by CCK8. **B.** Western blot analysis of TROP2 expression in MDA-MB-231 and MDA-MB-231/CDDP cells. **C.** Western blot analysis of TROP2 expression in MDA-MB-231 and MDA-MB-231/CDDP cells after transfection of siRNA-TROP2. **D.** CCK8 was used to detect the cell proliferation rate induced by different concentrations of CDDP. Data were expressed as mean ± standard deviation; compared with the control group, **P<0.01. TNBC, triple-negative breast cancer; TROP2, tumor-associated calcium signal transduction factor 2; CDDP, cisplatin.

conducted after pretreatment with ERS inhibitor 4-PBA. Apoptosis of MDA-MB-231 and MDA-MB-231/CDDP cells was analyzed by flow cytometry with Annexin V-APC/PI double staining. As shown in Fig.4A and C, compared with the siRNA-NC group, the apoptosis rate of the siRNA-TROP2 group was significantly increased (P<0.01), and siRNA-NC+4-PBA had no significant effect on cell apoptosis (P>0.05). Compared with the siRNA-TROP2 group, siRNA-TROP2+CDDP treatment also significantly increased the cell apoptosis rate (P<0.01), siRNA-TROP2+4-PBA had no significant effect on cell apoptosis (P>0.05), indicating that siRNA-TROP2 or CDDP treatment could significantly promote cell apoptosis. The apoptosis rate of the co-treatment group was significantly higher than that of the single treatment group, 4-PBA had no significant effect on the apoptosis rate of siRNA-NC/siRNA-TROP2 transfected cells. In addition, compared with the siRNA-TROP2+CDDP group, the apoptosis rate of the siRNA-TROP2+CDDP+4-PBA group was significantly decreased (P<0.01), indicating that TROP2 affects apoptosis and drug resistance of TNBC by activating ERS. A colony formation assay was used to further test the effect of 4-PBA on the cloning ability of CDDP-resistant TNBC cells transfected with siRNA-TROP2. As shown in Figure 4B and D, the number of clone formations of MDA-MB-231 and MDA-MB-231/CDDP cells significantly decreased after siRNA-TROP2

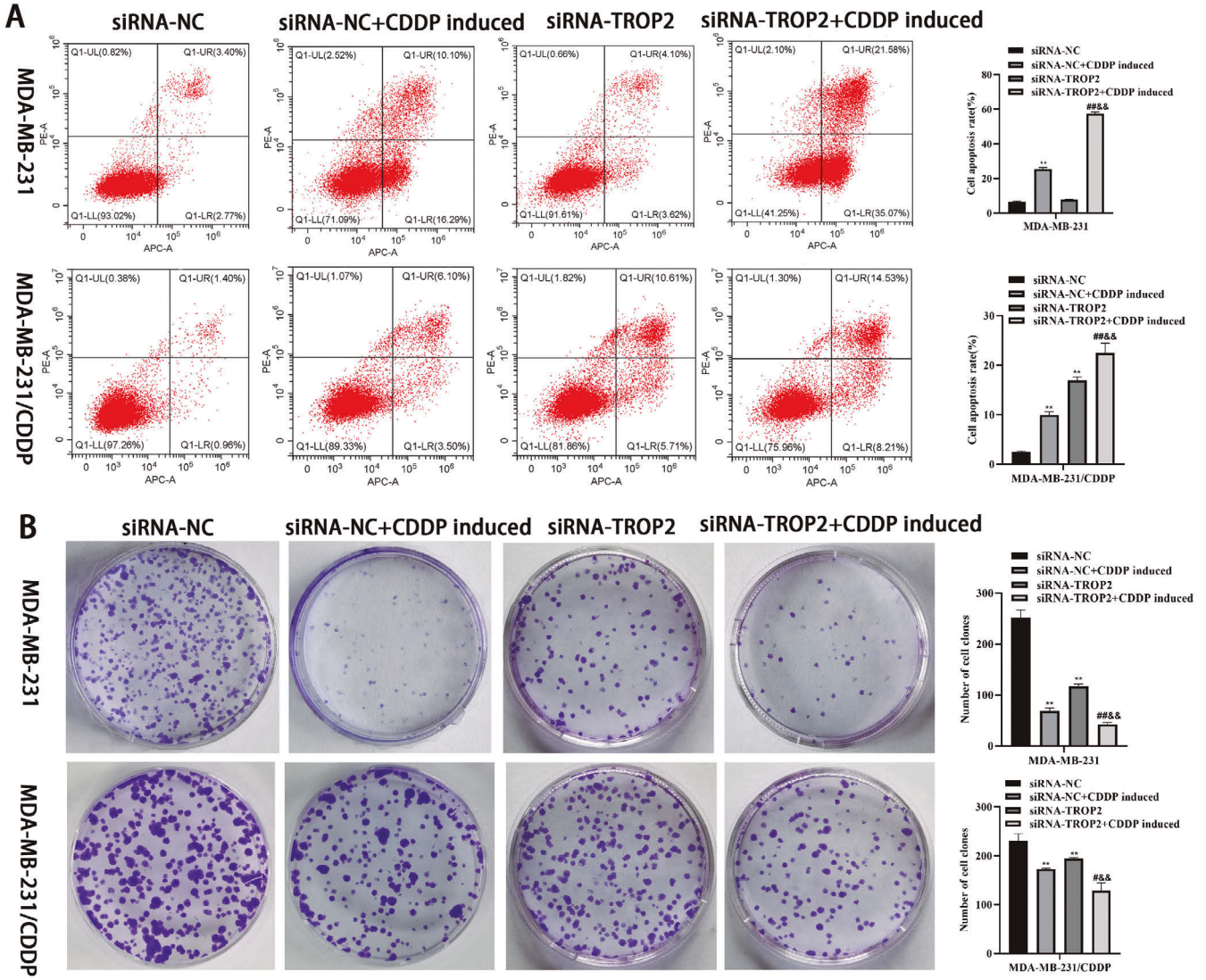

Fig. 2. Effect of siRNA-TROP2 and CDDP on the apoptosis and cloning ability of CDDP-resistant TNBC cells. **A.** Annexin V-APC/PI double labeling flow cytometry analysis of the apoptosis rate. **B.** The colony formation assay was used to detect cell cloning ability. Data were expressed as mean ± standard deviation; Compared with siRNA-NC group, $^{**}P$<0.01; Compared with the siRNA-NC+CDDP-induced group, $^{\#}P$<0.05, $^{\#\#}P$<0.01; Compared with siRNA-TROP2 group, $^{\&\&}P$<0.01. TNBC, triple-negative breast cancer; TROP2, tumor-associated calcium signal transduction factor 2; CDDP, cisplatin.

transfection and CDDP treatment (37.33±4.73, 56.67±13.05, P<0.01), and the number of clone formations was significantly increased after adding 4-PBA (64.00±4.58, 125.00±1.00, P<0.01).

ERS mediated by siRNA-TROP2 and CDDP co-treatment was reversed by 4-PBA

Western blot was used to detect the expression of ERS-related proteins in MDA-MB-231 and MDA-MB-231/CDDP cells. As shown in Figure 5, the protein levels of caspase-12, cleaved-PARP, PARP, GRP78, and CHOP in MDA-MB-231 and MDA-MB-231/CDDP cells were significantly up-regulated after siRNA-TROP2 transfection and CDDP co-treatment (P<0.01). The protein expression levels of caspase-12, cleaved-PARP, GRP78, and CHOP were significantly downregulated after adding 4-PBA (P<0.05).

Discussion

TNBC is the major subtype that causes death in breast cancer patients. At present, chemotherapy is still one of the main treatment methods for TNBC, but the resistance of tumor cells to chemotherapy drugs seriously affects the therapeutic effect, and chemotherapy resistance often leads to treatment failure. TROP2 is a new proto-oncogene, which is closely related to the occurrence and development of various tumors. Anti-TROP2 antibody drug conjugations Trodelvy™ (Sacituzumab govitecan) are approved for the treatment of metastatic TNBC (Liu et al., 2022). In this study, we found that TROP2 expression in CDDP-resistant TNBC cells was significantly higher than that in TNBC cells, and TROP2 expression and cell proliferation in TNBC cells were significantly reduced after the silencing of TROP2 expression; the inhibition rate of cell proliferation gradually increased with the increase of CDDP concentration. The results indicated that TROP2 affected the resistance of TNBC cells to CDDP. Membrane-localized TROP2 has been reported to be a prognostic target that cannot be ignored. Breast cancer patients with intracellular TROP2 retention have a lower tumor recurrence rate and a higher survival rate (Ambrogi et al., 2014), suggesting that TROP2 is a potential target for the treatment of TNBC. In addition, we found that silencing the expression of TROP2 significantly promoted the apoptosis of TNBC cells and the expression of ERS-labeled proteins.

In recent years, attention has been focused on how to treat cancers with ERS. Previous studies have reported that targeted ERS plays a tumor suppressor role in human hepatocellular carcinoma (Xu et al., 2022), non-small cell lung cancer (Ma et al., 2017), and ovarian cancer (Liu et al., 2017). The ER is an important organelle for protein synthesis, processing, and folding,

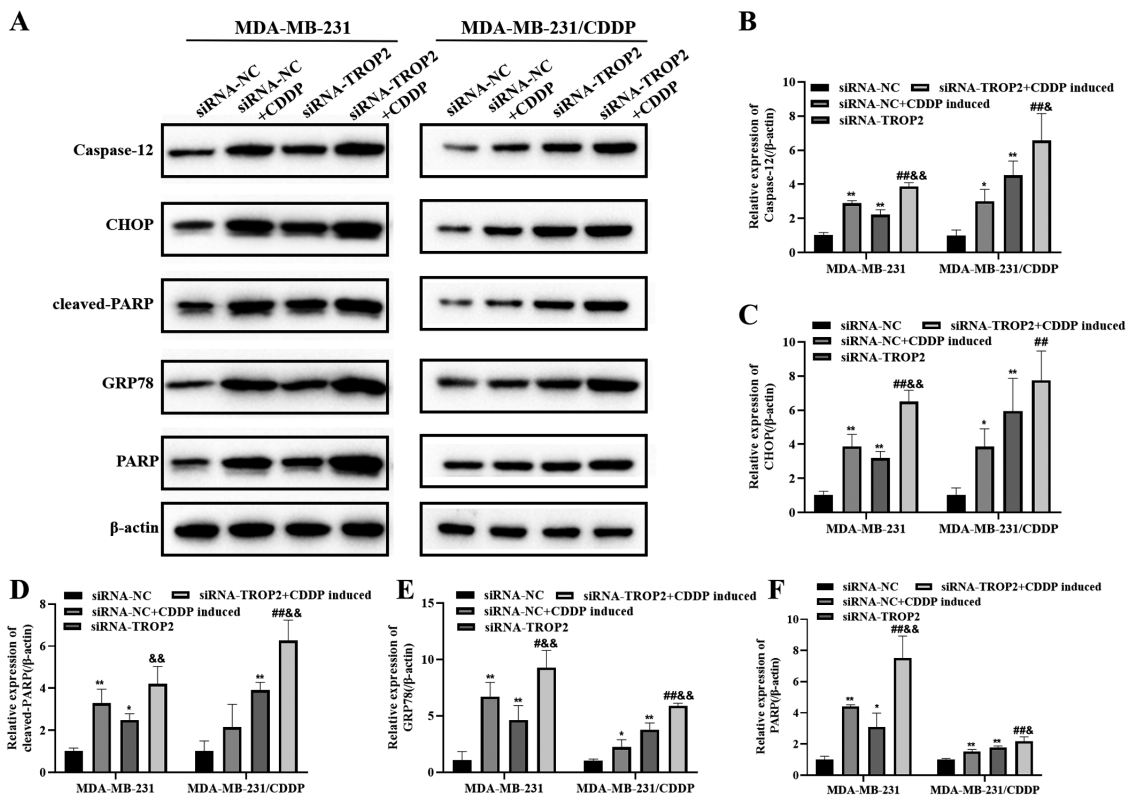

Fig. 3. The expression of ERS-related proteins induced by siRNA-TROP2 and CDDP. **A.** Bands of ERS-related proteins detected by Western blot. **B-F.** Statistical histogram of the relative expression of these proteins. Data were expressed as mean ± standard deviation; compared with the siRNA-NC group, *P<0.05, **P<0.01; Compared with the siRNA-NC+CDDP-induced group, $^{\#}P$<0.05, $^{\#\#}P$<0.01; compared with siRNA-TROP2 group, $^{\&}P$<0.05, $^{\&\&}P$<0.01. TNBC, triple-negative breast cancer; TROP2, tumor-associated calcium signal transduction factor 2; CDDP, cisplatin, ERS, endoplasmic reticulum stress.

and plays an important role in regulating Ca^{2+} homeostasis. Disturbance of ER homeostasis leads to the accumulation of unfolded or misfolded proteins, which leads to an increase in intracellular Ca^{2+} concentration, leading to ERS and ultimately to apoptosis (Oakes, 2020). Under normal physiological conditions, cells can alleviate cell damage caused by ERS through unfolded protein response (UPR). As an important compensatory mechanism for ERS, UPR is mainly mediated by the molecular chaperone GRP78 and ER transmembrane receptor proteins PERK, ATF6, and IRE1GRP78. In this study, we found that silencing TROP2 expression significantly promoted the expression of GRP78 in TNBC cells, suggesting that silencing TROP2 could promote UPR-mediated ERS in TNBC cells. In addition, when ERS occurs, transmembrane proteins dissociate from GRP78 and bind to accumulated unfolded proteins, thereby activating protein responses in downstream pathways, activating ERS-related signal transduction pathways, and repairing less damaged cells. This stress response in the ER is thought to be a protective mechanism of cells against external stimuli. However, when the duration of ERS is too long or the intensity is too high, ERS will initiate the expression of the stress protein regulatory gene CHOP and cause cell apoptosis. This study also found that silenced TROP2 promoted the expression of CHOP, Caspase-12, cleaved PARP, and PARP in TNBC cells. The ERS apoptosis pathway is a

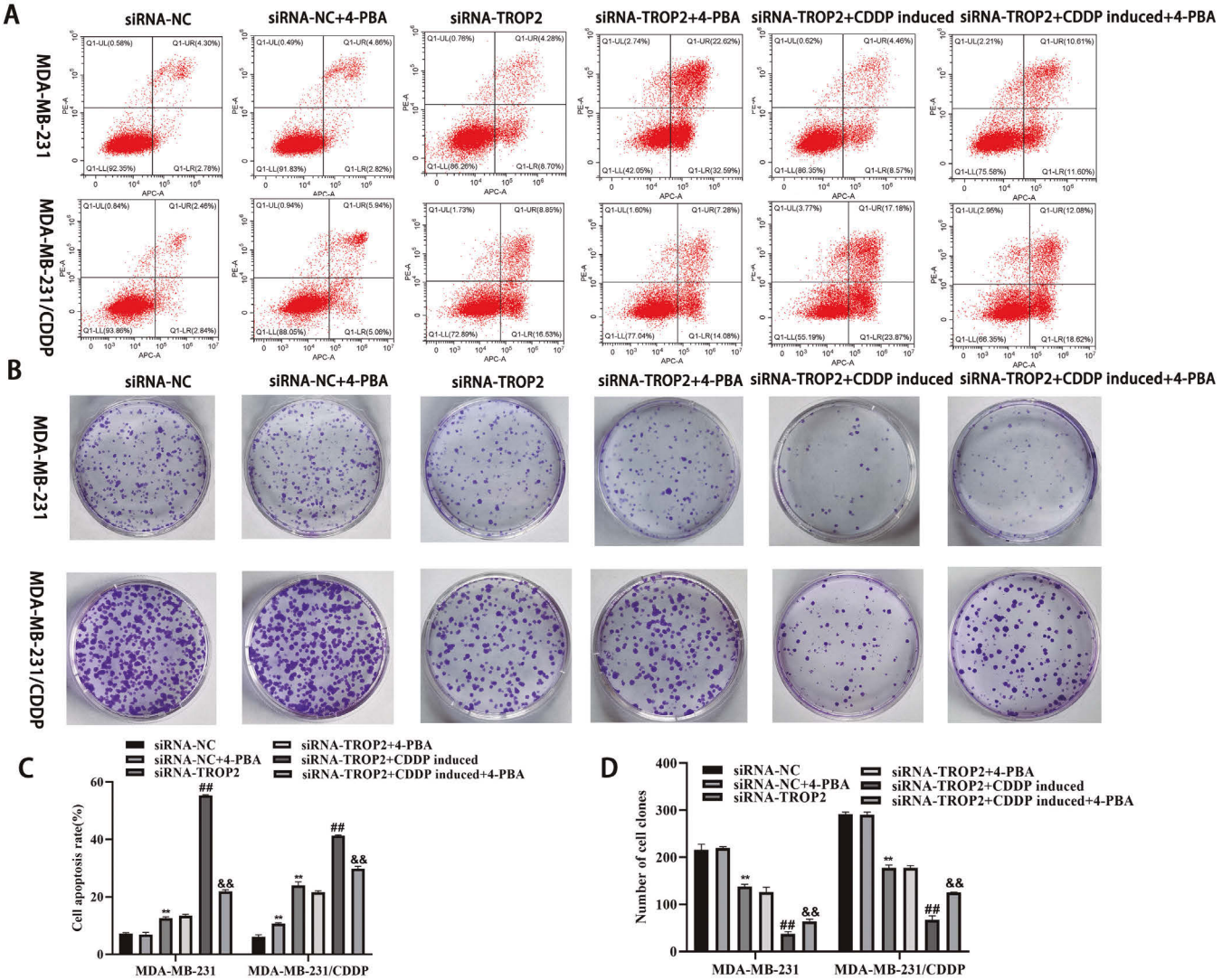

Fig. 4. TROP2 mediates the effect of ERS on the apoptosis and cloning ability of CDDP-resistant TNBC cells. **A, C.** Annexin V-APC/PI double labeling flow cytometry analysis of apoptosis rate. **B, D.** The colony formation assay was used to detect cell cloning ability. Data were expressed as mean ± standard deviation; compared with the siRNA-NC group, $^{**}P<0.01$; compared with the siRNA-TROP2 group, $^{\#\#}P<0.01$; compared with the siRNA-TROP2+CDDP group, $^{\&\&}P<0.01$. TNBC, triple-negative breast cancer; TROP2, tumor-associated calcium signal transduction factor 2; CDDP, cisplatin, ERS, endoplasmic reticulum stress.

new apoptotic regulatory pathway after the receptor pathway and mitochondrial pathway. CHOP is considered one of the important transcription factors regulating apoptosis signals by ERS, which can upregulate Bax and downregulate Bcl-2 to induce apoptosis by activating related pathways (Wang et al., 2018). Caspase-12 is a member of the aspartate cysteine-specific protease family and is usually inactive. When ERS occurs, Caspase-12 is activated, resulting in the separation of pro-caspase-12 from TRAF2 to form a dimer, and activation of caspase-3 to induce cell apoptosis (Dubois et al., 2013). Caspase-3 and cleaved-PARP are the most studied apoptosis-related factors. Studies showed that cleaved-caspase-3, PARP, and cleaved-PARP expression are upregulated in methamphetamine-induced neuronal apoptosis (Cai et al., 2016). Therefore, our study suggested that silencing TROP2 may play an anticancer role in promoting TNBC cell apoptosis by inducing the expression of related proteins during ERS. In addition, we further confirmed, using the ERS inhibitor 4-PBA, that the promoting effect of silted TROP2 on ERS and apoptosis was reversed after CDDP and 4-PBA treatment. As a small molecule chemical chaperone, 4-PBA can stabilize the structure of peptide bonds and improve the folding and transport capacity of proteins in the ER lumen, thereby reducing ER load and improving the adaptive capacity of ER (Ayala et al., 2012). Under ERS, 4-PBA can inhibit ERS and cell apoptosis by downregulating the expression levels of GRP78, IRE1, and CHOP and upregulating the expression of anti-apoptotic factor Bcl-2 (Kim et al., 2012). Therefore, the use of 4-PBA further suggested that the involvement of TROP2 in TNBC resistance to CDDP was affected by regulating ERS.

Conclusion

In conclusion, downregulation of TROP2 expression was found to inhibit TNBC cell cloning and promote cell apoptosis. In addition, TROP2 affected the sensitivity of TNBC cells to CDDP drugs, and its mechanism was closely related to the activation of the ERS response, revealing that TROP2 could be used as a potential target for TNBC treatment.

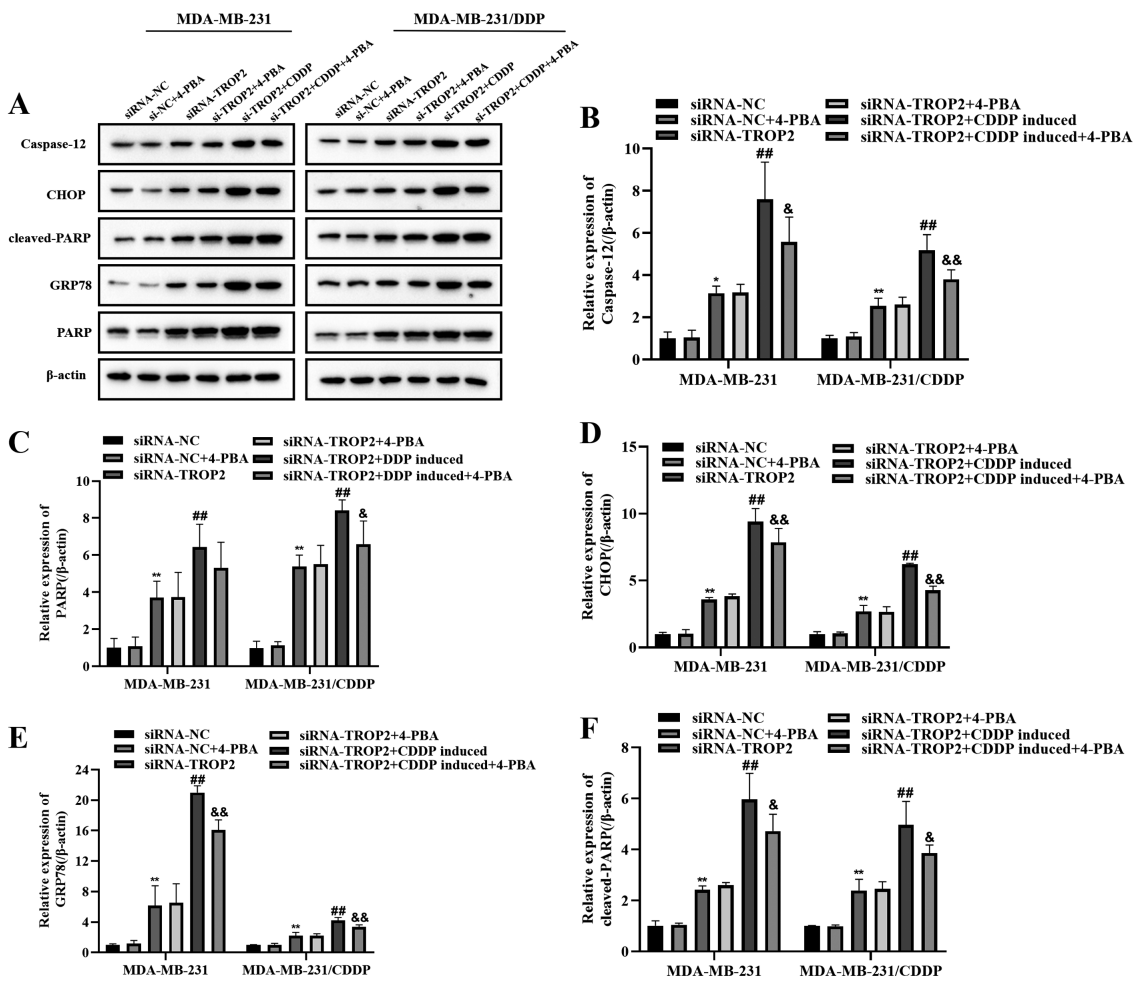

Fig. 5. The expression of ERS-related proteins was detected by Western blot. **A.** Bands of ERS-related proteins. **B-F.** Statistical histogram of the relative expression of these proteins. Data were expressed as mean ± standard deviation; compared with the siRNA-NC group, $^{*}P<0.05$, $^{**}P<0.01$; compared with the siRNA-TROP2 group, $^{\#\#}P<0.01$; compared with the siRNA-TROP2+CDDP group, $^{\&}P<0.05$, $^{\&\&}P<0.01$. TNBC, triple-negative breast cancer; TROP2, tumor-associated calcium signal transduction factor 2; CDDP, cisplatin, ERS, endoplasmic reticulum stress.

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Authors' Contributions. (I) conceived and designed the experiments: Mingqi Zhang, Jianzhong Xu, and Ning Li; (II) performed the experiments: Mingqi Zhang, Jianzhong Xu, Qing Liu, Xi Yan, and Ning Li; (III) analyzed and interpreted the data: Mingqi Zhang, Jianzhong Xu, and Ning Li; (IV) wrote the paper: Mingqi Zhang and Jianzhong Xu.
Data availability statement. The data used to support the findings of this study are available from the corresponding author.

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