

RESEARCH ARTICLE



Tabersonine enhances cisplatin sensitivity by modulating Aurora kinase A and suppressing epithelial–mesenchymal transition in triple-negative breast cancer

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ABSTRACT

Context: Tabersonine has been investigated for its role in modulating inflammation-associated pathways in various diseases. However, its regulatory effects on triple-negative breast cancer (TNBC) have not yet been fully elucidated.

Objective: This study uncovers the anticancer properties of tabersonine in TNBC cells, elucidating its role in enhancing chemosensitivity to cisplatin (CDDP).

Materials and methods: After tabersonine (10 μ M) and/or CDDP (10 μ M) treatment for 48 h in BT549 and MDA-MB-231 cells, cell proliferation was evaluated using the cell counting kit-8 and colony formation assays. Quantitative proteomics, online prediction tools and molecular docking analyses were used to identify potential downstream targets of tabersonine. Transwell and wound-healing assays and Western blot analysis were used to assess epithelial–mesenchymal transition (EMT) phenotypes.

Results: Tabersonine demonstrated inhibitory effects on TNBC cells, with IC₅₀ values at 48 h being 18.1 μ M for BT549 and 27.0 μ M for MDA-MB-231. The combined treatment of CDDP and tabersonine synergistically suppressed cell proliferation in BT549 and MDA-MB-231 cells. Enrichment analysis revealed that the proteins differentially regulated by tabersonine were involved in EMT-related signalling pathways. This combination treatment also effectively restricted EMT-related phenotypes. Through the integration of online target prediction and proteomic analysis, Aurora kinase A (AURKA) was identified as a potential downstream target of tabersonine. AURKA expression was reduced in TNBC cells post-treatment with tabersonine.

Discussion and conclusions: Tabersonine significantly enhances the chemosensitivity of CDDP in TNBC cells, underscoring its potential as a promising therapeutic agent for TNBC treatment.

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Introduction

Breast cancer (BC), the most common cancer among women, is considered a crucial global public health concern (Nedeljkovic and Damjanovic 2019). Triple-negative BC (TNBC), an aggressive subtype of BC, accounts for approximately 15–20% of all BC cases. TNBC is characterized by the absence of oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) expression, which limits the effectiveness of targeted therapies (Yin et al. 2020). Although cytotoxic chemotherapy is the main treatment for TNBC, patients with this subtype experience a higher mortality rate of 40% (Bou Zerdan et al. 2022). The considerable heterogeneity of TNBC cells often leads to inherent resistance to chemotherapeutic agents, posing a significant obstacle to managing this cancer (Deepak et al. 2020). Consequently, it is imperative to identify innovative approaches to improve TNBC treatment outcomes.

Cisplatin (CDDP), one of the first metal-based chemotherapeutic drugs, is widely applied in treating multiple cancers (Ghosh 2019). CDDP exerts its cytotoxic effects on tumour cells by inducing the DNA damage response and subsequently triggering apoptosis (Chen and Chang 2019). In TNBC, CDDP has

been reported to induce pyroptosis by activating the maternally expressed gene 3 (MEG3)/NOD-like receptor family pyrin domain-containing 3 (NLRP3)/caspase-1/gasdermin D (GSDMD) pathway, thus exerting antitumor effects (Yan et al. 2021). CDDP-based combination chemotherapy can also improve therapeutic efficacy in TNBC patients (Lin et al. 2022). A randomized phase 3 trial has shown that CDDP combined with nab-paclitaxel significantly prolongs progression-free survival of TNBC patients (Wang B et al. 2022). However, the emergence of drug resistance and undesirable side effects has been observed in TNBC patients undergoing CDDP treatment (Yu et al. 2020). Consequently, there is an urgent need to identify innovative strategies that improve CDDP sensitivity in TNBC.

Recently, researchers have increasingly focused on investigating the potential of natural products in treating BC. Tabersonine, a terpene indole alkaloid derived from the medicinal plant *Catharanthus roseus* (Linn.) G. Don (Apocynaceae) has received growing interest due to its bioactive properties in regulating inflammatory diseases. For example, tabersonine has been found to inhibit the activation of the nuclear factor- κ B (NF- κ B) signalling pathway, resulting in the amelioration of obesity-induced renal injury (Qian et al. 2023). Tabersonine has shown efficacy

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in mitigating inflammation-associated cardiomyopathy by binding directly to transforming growth factor beta-activated kinase 1 (TAK1) and inhibiting phosphorylation (Chen, Lin, Y et al. 2023). Furthermore, tabersonine has been reported to serve as an intermediate in the synthesis of vindoline (Liu T et al. 2021), indicating its potential to generate antitumor activity. However, the specific effects of tabersonine on TNBC progression need to be elucidated.

This study identified tabersonine as a promising antitumor compound that enhanced the sensitivity of TNBC cells to CDDP. Using quantitative proteomics and bioinformatic analysis, it was discovered that a combination of tabersonine and CDDP effectively inhibited cell invasion and migration by inhibiting the epithelial–mesenchymal transition (EMT) process. Furthermore, our findings indicate that tabersonine exerts its inhibitory effects by down-regulating the expression of Aurora kinase A (AURKA) in TNBC cells. These findings suggest that tabersonine, a potential antitumor agent, has the potential to overcome CDDP resistance in TNBC therapy.

Materials and methods

Bioinformatics analyses

To identify potential tabersonine targets, the open-access PharmMapper Server (<http://lilab.ecust.edu.cn/pharmmapper/>) was used to predict binding potentials (Liu X et al. 2010). Subsequently, the online UALCAN database tool (<https://ualcan.path.uab.edu/>) (Chandrashekar et al. 2022) was used to validate AURKA expression in normal and BC samples, as well as in various BC subtypes. The association between AURKA expression and platinum sensitivity was investigated using data sets from GSE21653, GSE22219, GSE97342 and TCGA_BRCA, accessed through the Biomarker Exploration of Solid Tumours (BEST) database (https://rookietopia.com/app_direct/BEST/) (Chen, Yan, X et al. 2023). To further assess the impact of AURKA expression on prognosis post-chemotherapy, the Kaplan–Meier plotter (<https://kmplot.com/analysis/>) was used to analyse distant-metastasis-free survival (DMFS) and relapse-free survival (RFS) in TNBC patients (Gyorffy 2021). The STRING database (<https://string-db.org/>) (Gyorffy 2021) was used to construct protein association networks of AURKA with EMT-related genes.

Cells and reagents

The human TNBC cell lines BT549 (CVCL_1092, CX0068) and MDA-MB-231 (CVCL_0062, CL-0150B) were obtained from the Center for Molecular Medicine of the Xiangya Hospital. The BT549 cell line was cultured in 1640 medium (Gibco, C11875500BT, Carlsbad, CA), while the MDA-MB-231 cell line was maintained in Dulbecco's modified eagle medium (DMEM, Corning, 10-013-CV, NY). Culture medium was supplemented with 10% foetal bovine serum (Gibco, 10091-148) and 1% penicillin-streptomycin (Gibco, 15140-122). Tabersonine (Selleckchem, S9427, Houston, TX) and CDDP (APEX BIO, A8321, Houston, TX) were respectively dissolved in dimethylsulfoxide (DMSO) to prepare 10 mM stock solutions.

Cell counting kit 8 assay

For cytotoxic analysis, the BT549 and MDA-MB-231 cell lines were seeded in 96-well plates at a density of 2×10^3 cells per

well, using complete medium. Various concentrations of tabersonine (0, 1, 5, 10, 20, 40 and 80 μ M) and CDDP (0, 1, 5, 10, 20, 40 and 80 μ M) were added to the wells and incubated for 48 h. To examine the effects of these agents on cell proliferation, cells were treated with 10 μ M of CDDP, 10 μ M of tabersonine, or a combination of both and incubated for 48 h. For cell viability assays, CCK-8 reagent (Bimake, B34304, Houston, TX) was added to each well (10 μ L/well) and incubated for 2 h. The optical density (OD) at 450 nm was measured using a VICTOR X2 microplate reader (PerkinElmer, Waltham, MA). The half-maximal inhibitory concentration (IC_{50}) values were calculated using GraphPad Prism version 8.0 software (La Jolla, CA), employing a variable slope analysis.

Colony formation assay

BT549 and MDA-MB-231 cells were seeded in 6-well plates at a density of 1×10^3 cells per well and treated with the indicated drugs (tabersonine and/or CDDP) at concentrations of 10 μ M for 48 h. Subsequently, the medium containing the drugs was replaced with a fresh complete medium, and the cells were further incubated at 37°C for approximately 14 d. After incubation, colonies were fixed with methanol and stained with 0.3% w/v crystal violet at room temperature for 25 min. Colonies consisting of a cluster of 50 or more cells were counted.

Quantitative proteomics analysis

Label-free 4D quantitative proteomics was conducted by Majorbio (Shanghai, China) to determine alterations in protein expression in TNBC cells after tabersonine treatment. For quantitative proteomic analysis, BT549 and MDA-MB-231 cells (5×10^6 cells) were pretreated with 10 μ M tabersonine for 24 h. Subsequently, cells were collected, immediately snap-frozen in liquid nitrogen, and stored at -80°C . Total protein extraction, peptide desalination, and quantification were performed using the technology platform provided by Majorbio. The bioinformatic analysis of the proteomic data was then conducted using the Xiantao database (<https://www.xiantaozi.com/>).

Western-blot analysis

Proteins were extracted from cells using radioimmunoprecipitation assay (RIPA, Sidbio, SDP0001, China) lysis buffer containing a 1% fresh protease inhibitor cocktail (Selleckchem, B14001). Equal amounts of protein extracts (50 μ g per well) from each sample were separated by SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA). The membranes were blocked with 5% skim milk for 1 h at room temperature. Following blocking, membranes were incubated overnight at 4°C with primary antibodies: anti-AURKA (human, 1:1,000 dilution, RRID_AB_2882103, 66757-1-Ig, Proteintech, Rosemont, IL), anti-E-cadherin (human, 1:1,000 dilution, RRID_AB_10697811, 20874-1-AP, Proteintech), anti-N-cadherin (human, 1:1,000 dilution, RRID_AB_2813891, 22018-1-AP, Proteintech), anti-Vimentin (human, 1:3,000 dilution, RRID_AB_2273020, 10366-1-AP, Proteintech) and anti- β -actin (human, 1:20,000 dilution, RRID_AB_2687938, 66009-1-Ig, Proteintech). The membranes were then washed with 1× Tris-buffered saline containing 1% Tween 20 and incubated with horseradish peroxidase-conjugated secondary antibodies (anti-mouse: human, 1:3,000 dilution, RRID_AB_2722565, SA00001-1,

Proteintech; anti-rabbit: human, 1:3,000 dilution, RRID_AB_2722564, SA00001-2, Proteintech) for 1 h at room temperature. Finally, immunoblots were visualized using an enhanced chemiluminescence detection kit (34580, Thermo Scientific, Waltham, MA). β -actin was used as a loading control to normalize protein levels.

Transwell assay

To assess the invasive capacity of BT549 and MDA-MB-231 cells, they were first pretreated with 10 μ M CDDP and/or 10 μ M tabersonine for 48 h. The upper chamber of the Transwell apparatus (8.0 μ m pore size; Corning, 3422) was precoated with Matrigel (Corning, 354234). Cells (1×10^5) were then seeded in the upper chamber in 200 μ L of serum-free medium, while 600 μ L of DMEM/1640 supplemented with 10% FBS was added to the bottom reservoir. Following a 24 h incubation at 37°C, cells that invaded the membrane to the lower surface were fixed with 4% paraformaldehyde for 30 min and stained with 0.1% crystal violet for 5 min. Invading cells were observed and captured under a microscope in randomly selected visual fields.

Wounding-healing assay

BT549 and MDA-MB-231 cells were cultured in 6-well plates and treated with CDDP and/or tabersonine for 48 h. The wounds were introduced into the cell monolayers using a 200 μ L sterile pipette tip. Cells were washed twice with PBS to remove detached cells and debris. Cell migration was monitored, and the migration distance was investigated using a microscope. The area of cell migration, indicative of wound closure, was quantified as a percentage using ImageJ software.

Molecular docking analysis

SwissDock tool (<http://www.swissdock.ch/>) Molecular Modelling Group, Switzerland), MOE 2019 software (Chemical Computing Group, Canada) and UCSF Chimera version 1.16 software (UCSF Resource, USA) were used to predict interactions between

tabersonine and AURKA or TAK1. The molecular structure of tabersonine was initially retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). The crystal structures of the AURKA protein (PDB IDs: 2X6D, 2J50, 2X6E) and the tabersonine binding protein (PDB ID: 4GS6) were obtained from the RCSB Protein Data Bank (<http://www.rcsb.org/>) and subsequently pre-processed using MOE software. To assess binding affinity, we individually uploaded the tabersonine ligands and protein files to SwissDock for molecular docking. The optimal interaction model was selected based on the lowest Delta G value. These models were visualized using the UCSF Chimera version 1.16 software. Two-dimensional (2D) interaction predictions were performed using MOE 2019 software (Molecular Operating Environment, version 2019).

Statistical analysis

All statistical analyses were performed with GraphPad Prism version 8 and SPSS version 23.0 software (SPSS, Chicago, IL). Group comparisons were conducted using one-way ANOVA or Student's *t*-test, as appropriate. Continuous data are presented as means \pm standard deviation (SD). Statistical significance was established at the following levels: **p* < 0.05; ***p* < 0.01.

Results

Tabersonine increases the chemosensitivity of CDDP in TNBC cells

To investigate the effects of tabersonine on cell growth, TNBC cells were treated with various concentrations of tabersonine for 48 h. Figure 1(A,B) shows that CDDP IC₅₀ values were 29.4 and 39.5 μ M in BT549 and MDA-MB-231 cells, respectively. Furthermore, the tabersonine IC₅₀ values in the BT549 and MDA-MB-231 cells were 18.1 and 27.0 μ M, respectively (Figure 1(C,D)). Subsequent experiments used 10 μ M of CDDP and 10 μ M of tabersonine, based on these IC₅₀ values, as these concentrations only inhibited cell growth slightly. The proliferation of TNBC cells was further assessed under combined treatment with these two agents. Combining CDDP and tabersonine significantly inhibited BT549 and MDA-MB-231 cell proliferation

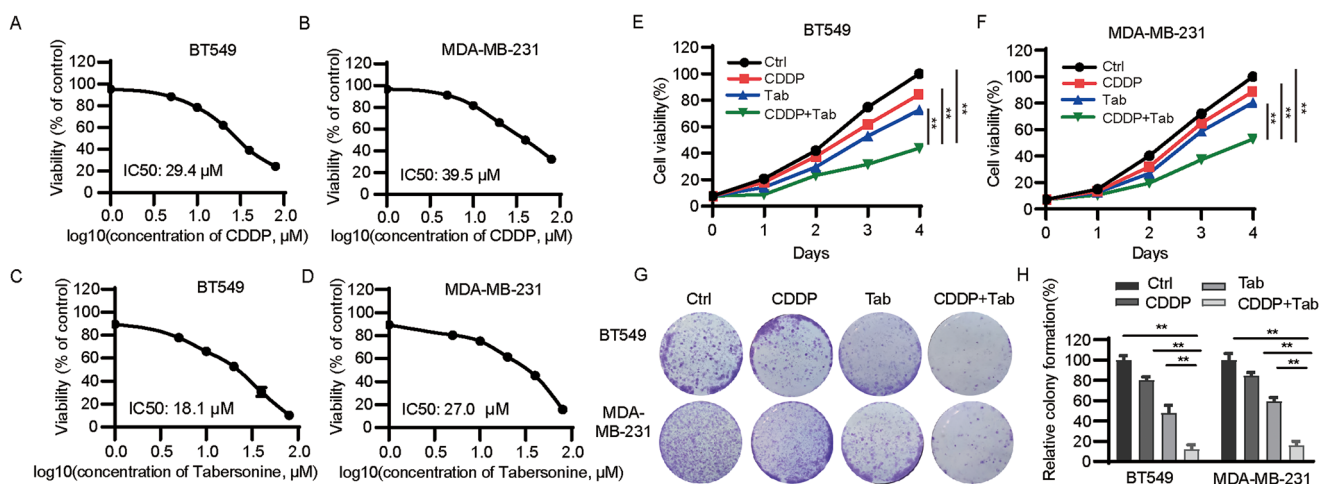


Figure 1. Tabersonine increases CDDP sensitivity in TNBC cells. (A,B) CCK-8 assays were used to detect IC₅₀ values in BT549 and MDA-MB-231 cells after incubation with CDDP for 48 h. (C,D) IC₅₀ values of tabersonine in BT549 and MDA-MB-231 cells, after treatment for 48 h. (E,F) BT549 and MDA-MB-231 cells were treated with 10 μ M CDDP, 10 μ M tabersonine or (10 μ M CDDP + 10 μ M tabersonine), followed by the measurement of cell viability using CCK-8 assays. (G,H) The colony formation potential was determined in BT549 and MDA-MB-231 cells after treatment with 10 μ M CDDP, 10 μ M tabersonine or (10 μ M CDDP + 10 μ M tabersonine). ***p* < 0.01. Tab: tabersonine; CDDP: cisplatin.

compared to treatment with CDDP or tabersonine alone (Figure 1(E,F)). Additionally, colony formation assays were conducted to corroborate the combined effects on cell growth. The results revealed that the combined treatment of CDDP and tabersonine synergistically suppressed TNBC cell growth (Figure 1(G,H)). These results suggest that tabersonine enhances CDDP-mediated inhibition of cell viability in TNBC cells.

Quantitative proteomic analysis based on tabersonine-treated TNBC cells

To investigate significant protein alterations after tabersonine treatment, the TNBC cell lines BT549 and MDA-MB-231 were used for proteome profiling. Differentially expressed proteins

(DEPs) were identified using volcano plots, applying a 1.2-fold change (FC) threshold and a p value < 0.05 . In total, 1021 and 1046 DEPs were identified in the tabersonine-untreated and -treated groups, respectively, for BT549 and MDA-MB-231 cells (Figure 2(A,B)). Among these, 502 proteins were up-regulated and 519 were down-regulated in BT549 cells. In MDA-MB-231 cells, 596 proteins were up-regulated and 450 were down-regulated.

To elucidate the biological functions of these DEPs, enrichment analysis of the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways was performed using the Xiantao database. Figure 2(C,D) illustrates the top 10 enriched pathways, revealing that DEPs were predominantly associated with epithelial EMT-related pathways, such as cadherin binding, cell-substrate junction and focal adhesion. Previous studies have

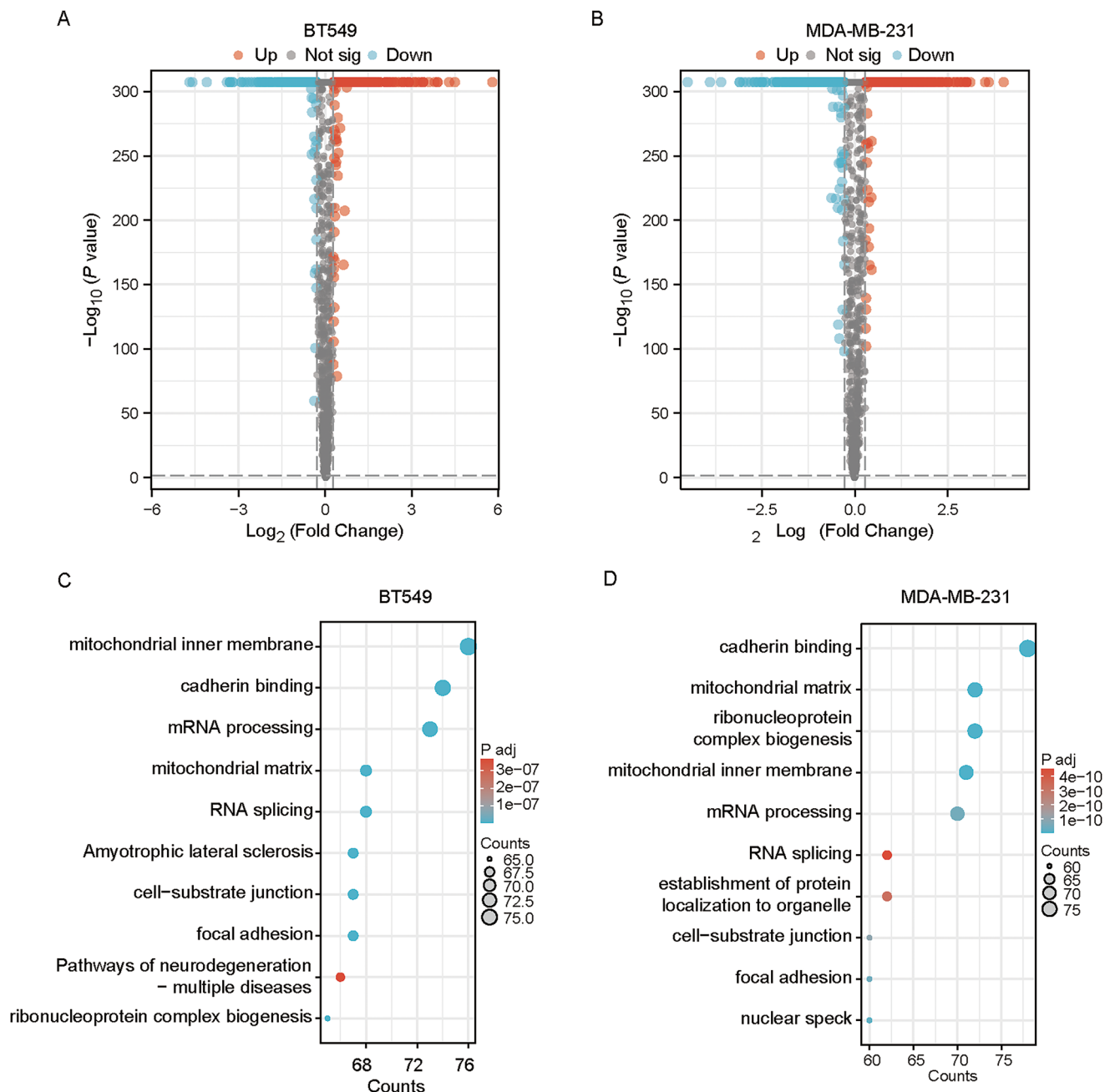


Figure 2. Quantitative proteomic analysis demonstrates differentially expressed proteins (DEPs) and enrichment analysis in tabersonine-treated TNBC cells. (A,B) The volcano plots illustrated DEPs between tabersonine-untreated and treated (10 μ M) TNBC cells (BT549 and MDA-MB-231). (C,D) GO and KEGG enrichment analysis of 10 main pathways in BT549 and MDA-MB-231 cells.

reported that EMT contributes to CDDP chemoresistance by transforming stationary epithelial cells into migratory mesenchymal cells, thus modulating adhesive functions (Ashrafizadeh et al. 2020). These proteomic findings suggest that tabersonine may exert its inhibitory effects by affecting EMT in TNBC cells.

Combined treatment of tabersonine and CDDP inhibits EMT in TNBC cells

To further validate whether tabersonine exerts antitumor effects and enhances the chemosensitivity of CDDP by modulating EMT, we conducted Transwell assays. These assays demonstrated that tabersonine significantly reduced cell invasion in BT549 and MDA-MB-231 cells (Figure 3(A,B)). The combination treatment with CDDP and tabersonine further strengthened this inhibitory effect, resulting in more than 80% reduction in cell invasiveness. We also evaluated the impact of these agents on cell migration using wound-healing assays. Although tabersonine alone effectively reduced migration in TNBC cells, combined treatment with CDDP and tabersonine resulted in a more substantial suppression of migration capacity than either agent alone in both cell lines, as demonstrated in Figure 3(C–E).

Several expressional changes in epithelial and mesenchymal markers commonly occur when tumour cells undergo EMT (Serrano-Gomez et al. 2016). Therefore, we examined the

expression of EMT-related proteins in BT549 and MDA-MB-231 cells using Western blotting. The results revealed an up-regulation of the epithelial marker E-cadherin and a decrease in the mesenchymal markers N-cadherin and Vimentin (Figure 3(F)). These findings suggest that tabersonine may enhance the sensitivity of TNBC cells to CDDP by suppressing EMT phenotypes.

AURKA is identified as a potential target for tabersonine and is associated with chemotherapeutic sensitivity in TNBC

To identify potential targets of tabersonine, an integrated analysis was performed using the PharmMapper Server database and quantitative proteomics. This analysis identified 84 potential tabersonine targets (Norm Fit > 0.5). These predicted targets were then cross-referenced with tabersonine-regulated DEPs from quantitative proteomic analysis. As shown in Figure 4(A–C), five co-DEPs were identified: 3 up-regulated (MAPK14, GSK3B and AKR1C3) and 2 down-regulated (AURKA and NQO1). MAPK14 and AURKA were selected for further investigation due to their more pronounced expression differences. However, the existing literature suggests that MAPK14 may promote BC progression (Wu X et al. 2014; Canovas et al. 2018), a finding contrary to our results. Consequently, we focused on AURKA for subsequent analyses.

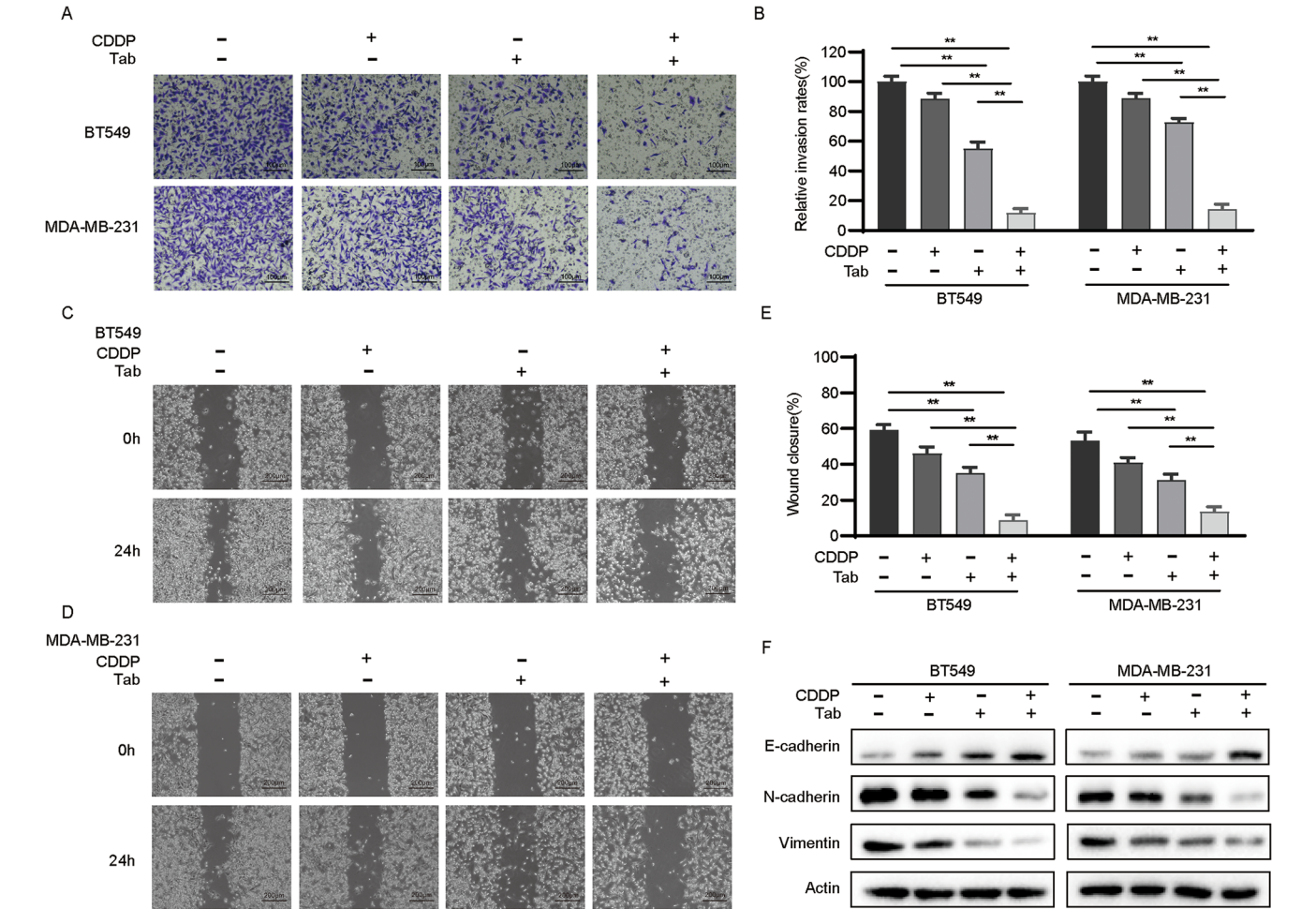


Figure 3. Tabersonine increases CDDP sensitivity by inhibiting EMT in TNBC cells. (A,B) The Transwell assay demonstrates cell invasive ability in BT549 and MDA-MB-231 cells after treatment of 10 μM CDDP, 10 μM tabersonine or (10 μM CDDP + 10 μM tabersonine). (C–E) Wound healing assays of BT549 and MDA-MB-231 cells that were treated with 10 μM CDDP, 10 μM tabersonine or (10 μM CDDP + 10 μM tabersonine). (F) Western blot examined the expression of EMT-related proteins, including E-cadherin, N-cadherin and Vimentin, in BT549 and MDA-MB-231, after treatment of 10 μM CDDP, 10 μM tabersonine or (10 μM CDDP + 10 μM tabersonine). ***p* < 0.01. Tab: tabersonine; CDDP: cisplatin.

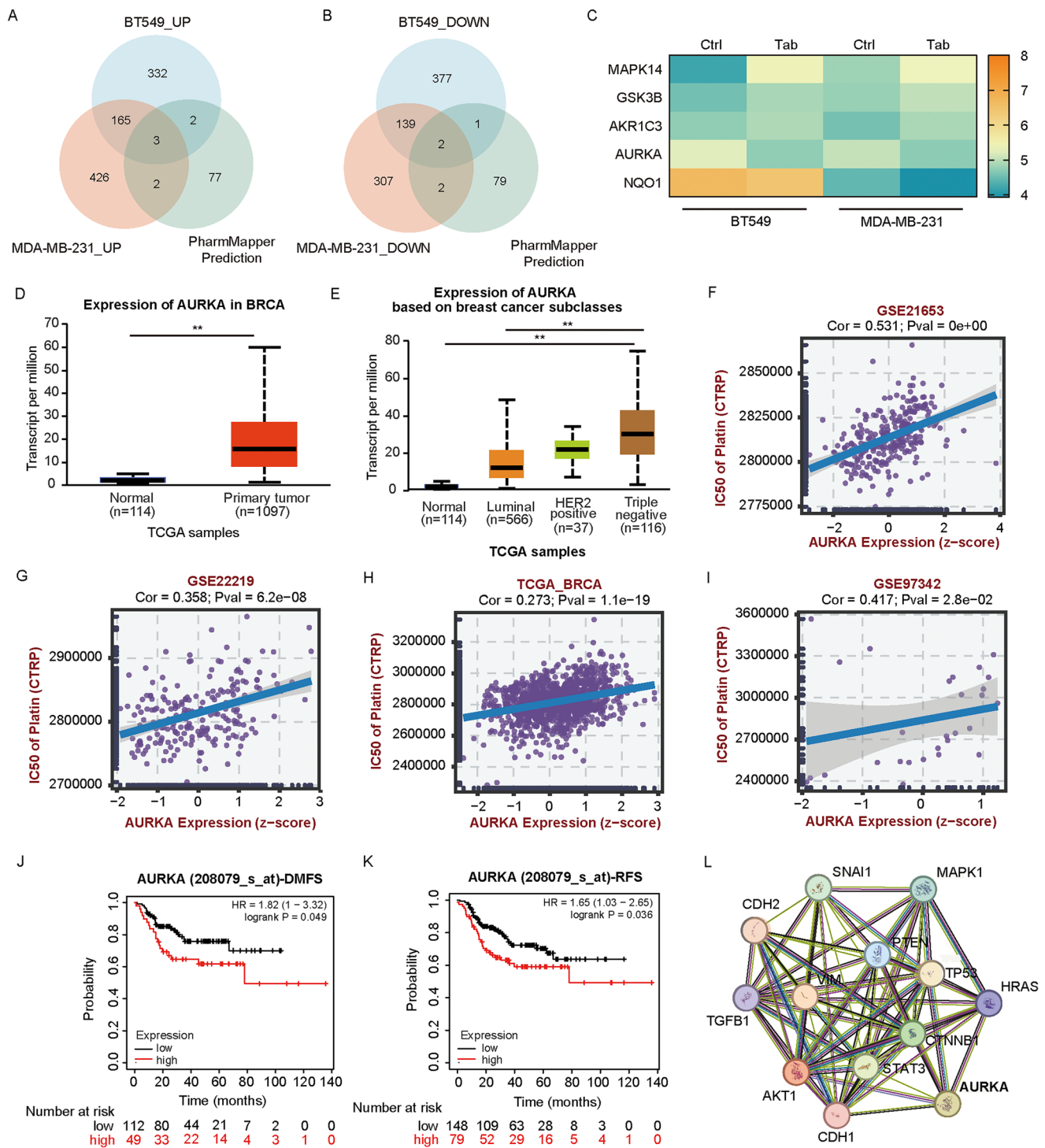


Figure 4. AURKA is a potential target of tabersonine in TNBC. (A,B) Venn diagram to exhibit the intersection between predicted targets (from PharmMapper Server) and DEPs (from quantitative proteomic analysis). (C) The heatmap showing the expression of five overlapping proteins in BT549 and MDA-MB-231 cells. (D–E) Comparison of AURKA transcriptional expression between BC and control tissues (D), as well as in different subtypes of BC (E). (F–I) Scatter plots of the association between AURKA expression and platinum IC_{50} values in the BC (GSE21653, GSE22219 and TCGA_BRCA) and TNBC (GSE97342) datasets. (J–K) Kaplan–Meier plotter revealed the association of AURKA expression with DMFS and RFS in TNBC patients receiving chemotherapy. (L) PPI network of AURKA and EMT-related genes, performed by the STRING database. ** $p < 0.01$.

Further exploration using the UALCAN database revealed a higher transcriptional expression of AURKA in BC tissues than in normal samples, with elevated TNBC tissue levels (Figure 4(D,E)). The correlation between AURKA expression and platinum sensitivity was analysed using the BEST database, showing a positive association with platinum IC_{50} values in BC

(GSE21653, GSE22219, TCGA_BRCA; $p < 0.05$) and TNBC data sets (GSE97342; $p < 0.05$) (Figure 4(F–I)).

Kaplan–Meier plotter analysis indicated that high AURKA expression was correlated with poorer DMFS and RFS in TNBC patients undergoing chemotherapy (Figure 4(J–K)). Previous research has highlighted the critical role of AURKA in regulating

migration and invasion by promoting EMT in various cancers (Liu X et al. 2016; Chen C et al. 2017). We further analysed AURKA and EMT-related genes using the STRING database, building a protein–protein interaction (PPI) network as shown in Figure 4(L). These findings suggest that AURKA may be a potential target for tabersonine and could be positively correlated with platinum resistance in TNBC.

The modulation of tabersonine on AURKA expression in TNBC cells

To investigate how tabersonine regulates AURKA, we assessed the expression of AURKA protein in TNBC cells treated with varying concentrations of tabersonine. Tabersonine treatment led to a significant decrease in AURKA expression in BT549 and MDA-MB-231 cells (Figure 5(A)). Additionally, a molecular docking study was conducted to evaluate the potential interactions between AURKA and tabersonine, using the SwissDock tool. Given previous reports of tabersonine interaction with TAK1, identified through a biotinylated compound–protein interaction pull-down assay (Chen, Lin, Y et al. 2023), TAK1 was used as a positive control in this docking prediction.

The 3D structures of TAK1 (PDB: 4GS6) and AURKA (PDB: 2X6D, 2J50, 2X6E) were retrieved from the PDB database (Fancelli et al. 2006; Bavetsias et al. 2010; Wu J et al. 2013) and are shown in Figure 5(B–E). The molecular docking results, including the interaction modes of tabersonine with TAK1 and AURKA, are presented in Figure 5(F–I). The 2D interaction map indicated potential interactions of tabersonine with AURKA at amino acid residues such as Val 147, Leu 139 and Arg 220 (Figure 5(J–L)). Furthermore, the binding free energies were evaluated by negative Delta G values. The binding prediction results revealed that the lowest Delta G values for the interaction between tabersonine and AURKA were -8.32 kcal/mol (PDB: 2X6D), -7.90 kcal/mol (PDB: 2J50) and -7.85 kcal/mol (PDB: 2X6E). In comparison, the lowest Delta G value for the interaction between tabersonine and TAK1 was -7.48 kcal/mol (PDB: 4GS6). These findings suggest that tabersonine suppresses AURKA expression in TNBC cells and implies a potential binding affinity between tabersonine and AURKA.

Discussion

Tabersonine, mainly extracted from *Catharanthus*, is widely used as a biosynthetic precursor in the synthesis of several alkaloids, including vindoline, a notable chemotherapeutic agent for treating various tumours (Zhang et al. 2018; Lemos Cruz et al. 2021). In addition to its role in chemotherapy, multiple studies have highlighted the anti-inflammatory properties of tabersonine in various diseases such as liver injury (Zhang et al. 2018), osteoporosis (Sun et al. 2020) and atherosclerosis (Shi et al. 2020). However, the specific mechanisms of the tumour-inhibitory effects of tabersonine require further elucidation. In this study, we have demonstrated significant antitumor properties of tabersonine in two TNBC cell lines. When combined with CDDP, tabersonine effectively reduced cell proliferation, invasion and migration, primarily by inhibiting EMT phenotypes in these TNBC cells. These findings suggest that tabersonine can enhance CDDP sensitivity, underscoring its potential for further exploration in cancer treatment.

EMT promotes metastasis and drug resistance in various cancer types (Xu et al. 2022). Research has shown that EMT

contributes to CDDP resistance in BC, a process regulated by the focal adhesion kinase (FAK)/phosphatidylinositol 3-kinase (PI3K)/AKT signalling pathway activated by 14,15-epoxyeicosatrienoic acid (Luo et al. 2018). Similarly, Rab31 is known to mediate CDDP resistance and metastasis in stomach adenocarcinoma through Twist1-induced EMT (Chen, Xu, K et al. 2023). Various studies have identified antitumor compounds that can reverse EMT phenotypes, potentially leading to reduced tumour invasion and increased chemosensitivity (Hashemi et al. 2022). Our study used quantitative proteomic analysis to identify DEPs regulated by tabersonine, followed by functional enrichment analysis.

These DEPs were predominantly associated with EMT-related pathways. Experimental validation confirmed that tabersonine, when combined with CDDP, synergistically inhibited EMT phenotypes in TNBC cells. These findings further underscore the importance of targeting EMT to enhance CDDP sensitivity in TNBC.

We propose that tabersonine may exert its antitumour effects on TNBC by modulating the activity of AURKA. AURKA belongs to the serine/threonine kinase family and is critical to coordinate cell division by regulating mitotic processes. Increasing evidence supports the oncogenic role of AURKA, particularly its role in facilitating EMT and chemoresistance to CDDP in various malignancies (Du et al. 2021). For example, a study reported that AURKA overexpression promotes EMT *via* the PI3K/AKT pathway in hepatocellular carcinoma (Chen et al. 2017). AURKA has also been shown to induce TGF- β -mediated chemoresistance by enhancing SNAIL expression in TNBC cells, with both TGF- β and SNAIL being key regulators of EMT (Jalalirad et al. 2021). Pharmacological targeting of TGF- β and AURKA simultaneously has increased sensitivity to docetaxel *in vitro* and inhibited tumour relapse and metastasis *in vivo* (Jalalirad et al. 2021).

AURKA knockdown in CDDP-resistant gastric cancer cells reduced cell proliferation and was associated with down-regulation of p-eIF4E, HDM2 and c-MYC expression (Wang L et al. 2017). In our study, an analysis of the BEST database observed a significant positive correlation between CDDP resistance and AURKA expression. Furthermore, the high expression of AURKA was correlated with poorer survival outcomes in TNBC patients receiving chemotherapy, as shown by Kaplan–Meier plotter analysis. These findings indicate that targeting AURKA with tabersonine may be a promising strategy to enhance antitumor therapy and overcome CDDP resistance in TNBC.

The multifaceted oncogenic role of AURKA has sparked considerable interest in developing inhibitors targeting this kinase. Recent studies have highlighted a growing array of natural products capable of down-regulating AURKA (Huang et al. 2022). For example, tanshinone has been shown to inhibit cell proliferation and induce apoptosis in non-small cell lung cancer by down-regulating AURKA (Liu X et al. 2020). Similarly, curcumin has demonstrated the ability to decrease AURKA activity, enhancing chemosensitivity to CDDP in TNBC cells (Ke et al. 2014). Our research contributes to this field by confirming a significant decrease in AURKA expression in TNBC cells after treatment with tabersonine. Additionally, our molecular docking assays have provided insight into the binding mode of tabersonine with AURKA, indicating a potential binding affinity. These findings suggest that tabersonine treatment could be a viable strategy against TNBC through its modulation of AURKA.

Our findings indicate that tabersonine may increase sensitivity to CDDP by inhibiting EMT and down-regulating AURKA expression in TNBC cells. However, further research is essential to overcome certain limitations. First, while our *in vitro* models have shown the antitumor properties of tabersonine, its efficacy

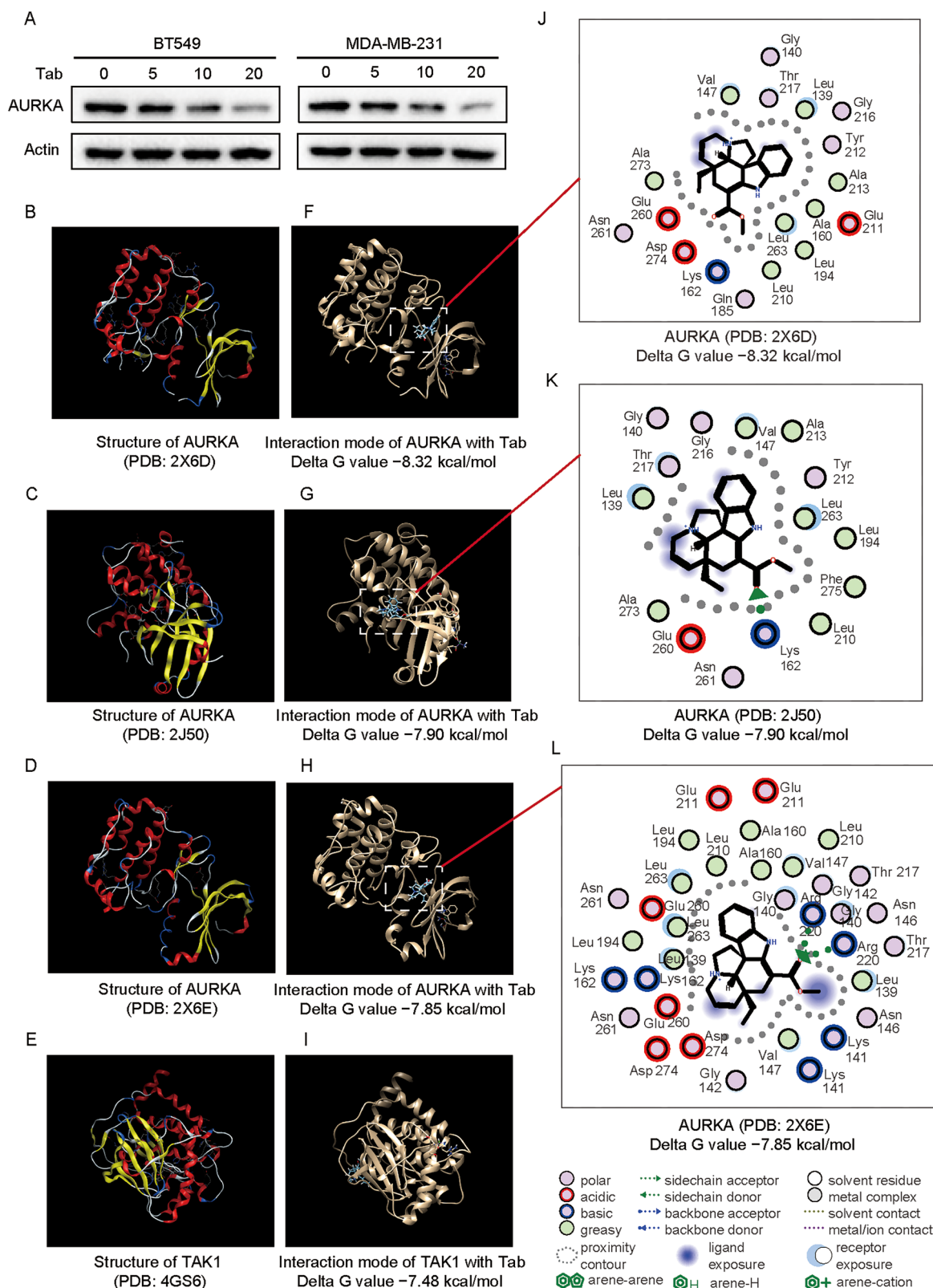


Figure 5. Combined experimental analysis and molecular docking assays identify the relationship between tabersonine and AURKA. (A) Expression of AURKA in BT549 and MDA-MB-231 cells incubated with a specified concentration of tabersonine (0, 5, 10 and 20 μ M). (B–E) the 3D structure of AURKA (PDB: 2X6D; PDB: 2X6E) and TAK1 (positive control, PDB: 4GS6), downloaded from the PDB database. (F–I) Predictions of tabersonine binding to AURKA or TAK1. (J–L) The interaction map (2D) between tabersonine and AURKA (PDB: 2X6D; PDB: 2J50; PDB: 2X6E).

in *in vivo* models remains to be investigated. Additionally, considering the critical role of AURKA in regulating EMT and chemoresistance in various cancers (Chen C et al. 2017;

Dawei et al. 2018), a more detailed investigation of how AURKA-mediated EMT is affected after tabersonine treatment is necessary. Furthermore, the possibility that tabersonine affects

EMT through other significant regulators remains to be explored. Totally, these findings underscore the potential of natural small molecules in cancer therapy and offer a novel approach to enhance the therapeutic efficacy of CDDP in TNBC cells.

Conclusions

Our study demonstrates that tabersonine significantly inhibits cell proliferation, invasion and migration in TNBC cells. Tabersonine increases the sensitivity of TNBC cells to CDDP by suppressing the EMT phenotypes. Through target prediction and experimental validation, tabersonine has been identified as a regulator that decreases the expression of the oncogenic protein AURKA in TNBC. Molecular docking assays have elucidated the binding interaction between tabersonine and AURKA. These findings position tabersonine as a potential therapeutic inhibitor in TNBC treatment and suggest its use in a promising combination therapy strategy for TNBC.

Author's contributions

Zhijie Xu conceived and designed the study. Xi Chen performed the experiments and wrote the manuscript. Yuanliang Yan, Yuanhong Liu, and Qiaoli Yi performed data analysis. All authors read and approved the final manuscript.

Disclosure statement

The authors declare no conflicts of interest.

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Data availability statement

The data sets used and/or analysed during this study are available from the corresponding author on reasonable request.

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