Quercetin reduces expression of ATP-binding cassette transporters by regulating the PTEN/PI3K/AKT signaling pathway in breast cancer cells

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Abstract. Breast cancer is a global health challenge for women and chemoresistance is a major contributor to its high mortality rates. Quercetin (Que), a flavonoid with antioxidant, antiviral, anti-tumor and anti-inflammatory properties, sensitizes cancer cells to chemotherapy. The present study investigated the mechanism by which Que regulates ATP-binding cassette (ABC) transporter expression in MCF-7 cells using a PTEN overexpression plasmid and the PI3K inhibitor LY294002. The present study assessed cell viability via Cell Counting Kit-8 and Hoechst 33342 staining and analyzed mRNA and protein expression levels by reverse transcription-quantitative PCR and western blotting. Apoptosis was evaluated by flow cytometry and ABCG2 expression was detected by immunofluorescence. Furthermore, the present study determined the effect of Que on drug uptake using a Rhodamine 123 accumulation assay. The results of the present study demonstrated that Que suppresses cell viability and induces apoptosis in MCF-7 cells. Moreover, it enhances intracellular drug accumulation and downregulates ABC transporter expression by modulating the PTEN/PI3K/AKT signaling pathway.

Introduction

Breast cancer (BC) is a major contributor to global cancer-related mortality in women. According to the World Cancer Statistics, it was the second most prevalent cancer among women in 2022, with ~2.3 million new cases reported (~11.6% incidence) (1,2). The efficacy of BC therapy is often compromised by the development of drug resistance, a key factor associated with treatment failure and elevated mortality (3,4). Tumor cell resistance involves complex molecular mechanisms, including the inactivation of tumor suppressor genes and the overexpression of ATP-binding cassette (ABC) transporters. To date, >40 ABC transporters have been identified and their overexpression is associated with multidrug resistance (MDR) in cancer therapy (5). Key ABC transporters implicated in MDR include ABCB1 (P-glycoprotein), ABCCs (MDR-associated proteins) and ABCG2 (BC resistance protein) (6-8). Therefore, developing novel agents that inhibit ABC transporters to reduce drug efflux may enhance chemosensitivity. While synthetic inhibitors such as Verapamil exist (9), it remains unclear whether natural compounds can simultaneously target multiple ABC transporters in BC cells.

Quercetin (Que), a natural flavonoid, can inhibit proliferation and enhance the cytotoxicity of conventional chemotherapeutic agents such as doxorubicin in BC cell lines, including MCF-7 and MDA-MB-231 (10-13). It can also induce cell cycle arrest and apoptosis in drug-resistant sublines. These properties position Que as a promising candidate for overcoming chemoresistance. However, the intricate signaling networks through which Que modulates ABC transporter expression are not fully understood. The present study aims to elucidate the role of Que in regulating ABC transporters in MCF-7 cells and explore the underlying molecular mechanism.

Materials and methods

Cell culture and materials. MCF-7, a human BC cell line, was purchased from Procell Life Science & Technology Co., Ltd. and cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin (100 U/ml; Cytiva) at 37˚C in a humidified incubator with 5% CO_2 atmosphere. Cells were routinely screened for mycoplasma contamination. Que (Beijing Solarbio Science & Technology Co., Ltd.)

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was dissolved in 100% DMSO to prepare a 100 mM stock solution, which was then diluted in DMEM such that the final concentration of DMSO was maintained at 0.1% (v/v) across all working concentrations of Que (30, 60, 90 and 120 μmol/l). The pLVX-Puro-control (Plvx-con) and pLVX-Puro-human-PTEN (Plvx-PTEN, NM_000314.6) plasmids were sourced from Xiamen Life Internet Technology Co., Ltd. and LY294002, Verapamil and Rhodamine 123 was purchased from MedChemExpress.

Cell transfection. A total of $1x10^6$ MCF-7 cells were transfected with 5 μg Plvx-con or Plvx-PTEN with the Lipo8000™ Transfection Reagent (Beyotime Biotechnology) in 6-cm dishes, strictly according the manufacturer's protocol. Following transfection, the cells were treated with 60 μmol/l Que for 24 h at 37˚C before subsequent experiments.

Cell Counting Kit-8 (CCK-8) assay. For each treatment group, $5x10^3$ MCF-7 cells were plated in triplicate into 96-well plates and allowed to adhere for 24 h at 37˚C in a CO_2 incubator. Cells were treated with increasing concentrations of Que (0, 30, 60, 90 and 120 μmol/l) for 24 or 48 h at 37˚C. For combination treatments, cells were transfected with 0.2 μg Plvx-con or 0.2 μg Plvx-PTEN for 24 h at 37 ˚C, followed by treatment with 60 μmol/l Que for another 24 h at 37˚C. After that, 10 μl of CCK-8 solution (Beijing Lablead Trading Co., Ltd.) was added to each well and the cells were incubated for 2 h at 37˚C. The absorbance was measured at 450 nm using an Infinite M1000 Pro microplate reader (Tecan Trading Ag).

Apoptosis analysis by flow cytometry. A total of $1x10^5$ MCF-7 cells were cultured in six-well plates and treated with Que (0, 30, 60, 90 or 120 μmol/l) for 24 h at 37˚C. In a separate experiment, cells were treated with 60 μmol/l Que, 25 μmol/l LY294002 or a combination of both for 24 h at 37˚C. Cells were then trypsinized, and harvested via centrifugation at 800 x g (4˚C), and stained with 10 μl of Annexin V-FITC and 5 μl of propidium iodide using an apoptosis detection kit (Beyotime Biotechnology). Cells were analyzed on a Beckman Coulter (BC) FC 500 flow cytometer using the CXP software (version 2.2) for data acquisition and analysis.

Western blot analysis. MCF-7 cells were lysed in cold RIPA lysis buffer (Beyotime Biotechnology) supplemented with protease and phosphatase inhibitors (cat. no. C0104, Beijing Lanbolide Trading Co., Ltd.). The protein concentration was determined using the BCA assay according to the manufacturer's instructions (Dalian Meilun Biology Technology Co., Ltd.), and 30 μg protein samples were separated by 8-12% SDS-PAGE, transferred to PVDF membranes (Millipore Sigma) and blocked at room temperature with blocking buffer (cat. no. BM10-100; Energenesis Biomedical Co., Ltd.). The membranes were incubated with primary antibodies overnight at 4˚C. Primary antibodies included PTEN (1:5,000; cat. no. ab267787), Bcl-2 (1:6,000; cat. no. ab196495), Bax (1:6,000; cat. no. ab32503) and ABCG2 (1:5,000; cat. no. ab108312) from Abcam; p-PI3K (p85) (1:2,000; cat. no. 4228), p-AKT (Ser473) (1:2,000; cat. no. 4060), cleaved caspase-3 (1:5,000; cat. no. 9664), ABCC2 (1:1,000; cat. no. 12559) and ABCB1 (1:2,000; cat. no. 13342) from Cell Signaling Technology, Inc.; and GAPDH (1:12,000; cat. no. 60004-1-Ig), PI3K (1:3,000; cat. no. 20584-1-AP), AKT (1:3,000; 10176-2-AP), Caspase-3 (1:3,000; 19677-1-AP) and β-actin (1:10,000; cat. no. 66009-1-Ig) from Proteintech Group, Inc. After incubation with HRP-conjugated secondary antibodies (1:10,000; cat. no. SA00001-1 and SA00001-2; Proteintech Group, Inc.), protein bands were visualized using an ECL reagent (Beijing Lanbolide Trading Co., Ltd.) and imaged with a ChemiDoc XRS+ system (Bio-Rad Laboratories, Inc.).

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from MCF-7 cells using TRIzol® (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Subsequently, cDNA was synthesized from the isolated RNA using a commercial reverse transcription kit (cat. no. F0202; Beijing Lanbolide Trading Co., Ltd.). qPCR was carried out using SYBR Green master mix (Beijing Lanbolide Trading Co., Ltd.) on a Roche LightCycler® 96 System (Roche Diagnostics). The thermal cycling protocol consisted of an initial denaturation at 95˚C for 30 sec, followed by 40 cycles of denaturation at 95˚C for 5 sec and annealing/extension at 60˚C for 30 sec. GAPDH was used as the internal control and relative gene expression was calculated using the $2^{-\Delta\Delta Cq}$ method (14). Primer sequences are listed in Table I.

Hoechst 33342 staining. Transfected and Que-treated MCF-7 cells were fixed with 4% paraformaldehyde for 30 min at room temperature and stained with Hoechst 33342 solution (Beijing Solarbio Science & Technology co., Ltd.) for 30 min at room temperature. Cell nuclei were visualized and imaged under an inverted fluorescence microscope (Leica Microsystems GmbH).

Immunofluorescence analysis. A total of $1x10^5$ cells were seeded into 3.5-cm confocal dishes, transfected and subsequently treated with 60 μmol/l Que and/or 25 μmol/l LY294002 for 24 h at 37˚C. After washing with PBS, cells were fixed with 4% paraformaldehyde for 30 min at room temperature, blocked with 2% BSA (Beijing Lanbolide Trading Co., Ltd.) for 1 h at room temperature and incubated overnight at 4˚C with primary antibody against ABCG2 (1:400). Cells were then incubated with Alexa Fluor® 488-conjugated secondary antibody (1:1,000; cat. no. ab150077; Abcam) at room temperature for 1 h, and the nuclei was stained with DAPI-containing mounting medium (cat. no. ab104139; Abcam) for 10 min at room temperature. Fluorescence images were captured using a Leica SP8 confocal microscope (Leica Microsystems GmbH).

Rhodamine 123 accumulation assay. MCF-7 cells were treated as following groups: i) Control group, ii) 100 μmol/l Verapamil group, iii) 60 μmol/l Que group, iv) 25 μmol/l LY294002 group and v) 25 μmol/l LY294002 + 60 μmol/l Que group. MCF-7 cells were treated with the indicated agents and cultured for 24 h at 37˚C. Notably, Verapamil (100 μmol/l) was added to the corresponding group 1 h prior to Rhodamine 123 staining. Following the 24 h treatment, the cells were washed three times with Hanks' balanced salt solution (HBSS; Beijing Solarbio Science & Technology co., Ltd.) and incubated with 100 μmol/l Rhodamine 123 for 15 min at 37˚C (No fixation or

Table I. Reverse transcription-quantitative PCR primer sequences.

Gene name	Forward sequence (5'-3')	Reverse sequence (5'-3')
GAPDH	TGACCACAGTCCATGCCATCAC	CGCCTGCTTCACCACCTTCTT
ABCB1	CGTAGGAGTGTCCGTGGATCA	GCGAGCCTGGTAGTCAATGC
Bax	CCAAGAAGCTGAGCGAGTGTCT	AGATGGTGAGTGAGGCGGTGAG
Bcl-2	TTCGCCGAGATGTCCAGCCA	GCATCCCAGCCTCCGTTATCCT
PTEN	GCTGGAAAGGGACGAACTGGTG	ACAGGTAACGGCTGAGGGAACT
ABCG2	TCTTCTTCCTGACGACCAACCA	CACACTCTGACCTGCTGCTATG
ABCC2	TCACTTCAGCGAGACCGTATCA	ATGTCATCCTCACCAGCCAGTT

other staining steps were performed). After three additional washed with HBSS, fluorescence images were acquired using an inverted fluorescence microscope (Leica Microsystems GmbH) and fluorescence intensity was quantified to assess ABC transporter activity.

Statistical analysis. Statistical analyses were performed using the GraphPad Prism 6.0 (Dotmatics). Data are presented as the mean ± SEM from at least three independent experiments. Comparisons between groups were conducted using an unpaired Student's t-test or one-/two-way ANOVA, as appropriate. For two-way ANOVA, Bonferroni's post-hoc test was used to evaluate multiple comparisons. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Que inhibits viability, induces apoptosis and downregulates ABC transporters in MCF-7 cells. As assessed by the CCK-8 assay, Que treatment caused a marked suppression of MCF-7 cell viability, and the inhibitory effect was dependent on both the concentration and duration of exposure (Fig. 1A). Furthermore, Que downregulated the mRNA and protein expression of ABCB1, ABCC2 and ABCG2 (Fig. 1B-E). It also promoted apoptosis, as evidenced by decreased Bcl-2 and increased Bax and cleaved caspase-3 expression (Fig. 1C-D), as well as an increased percentage of apoptotic cells in flow cytometry analysis (Fig. 1F and G). Based on these results, 60 μmol/l Que was selected for subsequent experiments.

PTEN overexpression suppresses ABC transporter expression and modulates apoptosis-related proteins. PTEN inactivation or mutation is implicated in various types of cancer, including BC (15-18). Patients with PTEN mutations have a higher risk of primary and secondary types of BC (19). The present study verified successful PTEN overexpression, which significantly reduced the mRNA and protein levels of ABCB1, ABCC2 and ABCG2 (Fig. 2A-E). It also reduced the anti-apoptotic protein Bcl-2 and increased the level of the pro-apoptotic protein Bax (Fig. 2C and E), mirroring the effects of Que.

PTEN overexpression synergizes with Que to enhance anti-proliferative and pro-apoptotic effects. The combination of PTEN overexpression and Que treatment resulted in a greater suppression of cell viability compared with Que treatment alone (Fig. 3A). This combination also led to a more pronounced upregulation of PTEN and Bax and downregulation of Bcl-2 at the gene and protein level (Fig. 3B, C, E-H). Hoechst 33342 staining analysis revealed a marked reduction in viable cells in the combination group (Fig. 3D).

The combination of PTEN overexpression and Que suppresses ABC transporters via the PI3K/AKT pathway. Clinical studies have shown that overexpression of ABC efflux transporters, such as ABCB1, ABCC2 and ABCG2, contributes to MDR in cancer by reducing intracellular drug concentrations (20,21). Therefore, the present study examined whether PTEN expression influences Que-mediated regulation of ABC transporters. Analysis revealed that PTEN overexpression potentiates Que-induced downregulation of ABCB1, ABCC2 and ABCG2 mRNA and protein (Fig. 4A-E). Concurrently, the combination treatment led to a significant reduction in the levels of phosphorylated PI3K and AKT (Fig. 4D, F and G), indicating inhibition of the PI3K/AKT signaling pathway.

Inhibition of PI3K/AKT signaling enhances the effects of Que on apoptosis and ABC transporters. The PI3K/AKT signaling pathway is involved in cell proliferation and the expression of drug-resistant proteins, including ABC transporters (22-24). To explore the relationship between Que and PI3K/AKT signaling in regulating apoptosis and ABC transporters, MCF-7 cells were treated with LY294002 (a PI3K inhibitor), Que or a combination of both. Treatment with LY294002 alone enhanced the effects of cell apoptosis and inhibited ABC transporter expression. The combination of Que and LY294002 resulted in the most substantial reduction of ABCB1, ABCC2 and ABCG2 mRNA and protein, as well as the strongest inhibition of PI3K/AKT signaling (Fig. 5A-F). Immunofluorescence analysis corroborated the downregulation of ABCG2 protein (Fig. 5G) and flow cytometry revealed that the highest rate of apoptosis occurred in the combination group (Fig. 5H and I).

Que and LY294002 increase intracellular Rhodamine 123 accumulation. Rhodamine 123, a substrate for ABC transporters, is used to assess transporter activity (25). The present study investigated the functional consequence of ABC transporter downregulation using the Rhodamine 123 accumulation assay. Analysis revealed that both Que

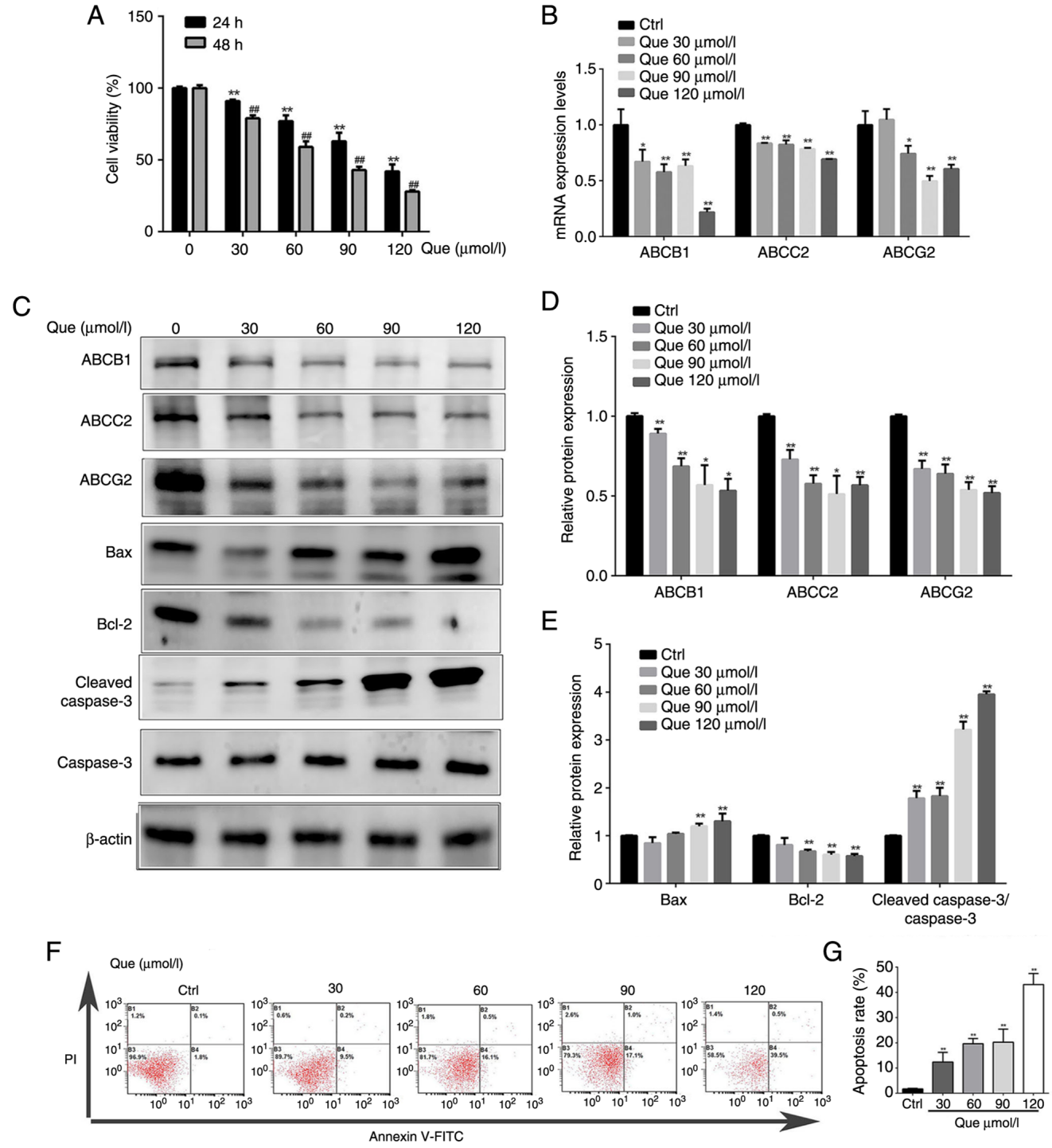

Figure 1. Effects of Que on cell viability, mRNA levels, apoptosis and protein expression in MCF-7 cells. (A) Cell viability of MCF-7 cells treated with the indicated concentrations of Que for 24 and 48 h, as determined by the Cell Cycle Kit-8 assay. (B) mRNA expression levels of ABCB1, ABCC2 and ABCG2 following Que treatment. (C) Representative western blotting images, (D) protein expression levels of ABCB1, ABCC2 and ABCG2, and (E) Bax, Bcl-2, and cleaved caspase-3 were assessed by western blotting. (F) Representative images of flow cytometry and (G) apoptosis rate was analyzed by flow cytometry after Annexin V-FITC/PI staining. Data are presented as the mean ± SEM of at least three independent experiments. Statistical significance was determined by one/two-way ANOVA followed by Bonferroni's post hoc test for multiple comparisons. *P<0.05, **P<0.01 compared with the control group, $^{\#\#}$P<0.01 compared with the Que (24 h) group. Que, Quercetin; Ctrl, control.

and LY294002 increased intracellular Rhodamine 123 retention, with the combination treatment showing the most significant effect (Fig. 6A and B), comparable to the positive control Verapamil. These results indicate that both Que and LY294002 inhibit the efflux activity of ABC transporters. The observed increase in fluorescent dye accumulation is consistent with a decrease in ABC transporter protein expression, as confirmed in Figs. 1B and C and 5A and B. This suggests that the downregulation of ABC transporters by Que and LY294002 may enhance the intracellular accumulation and efficacy of co-administered therapeutic agents.

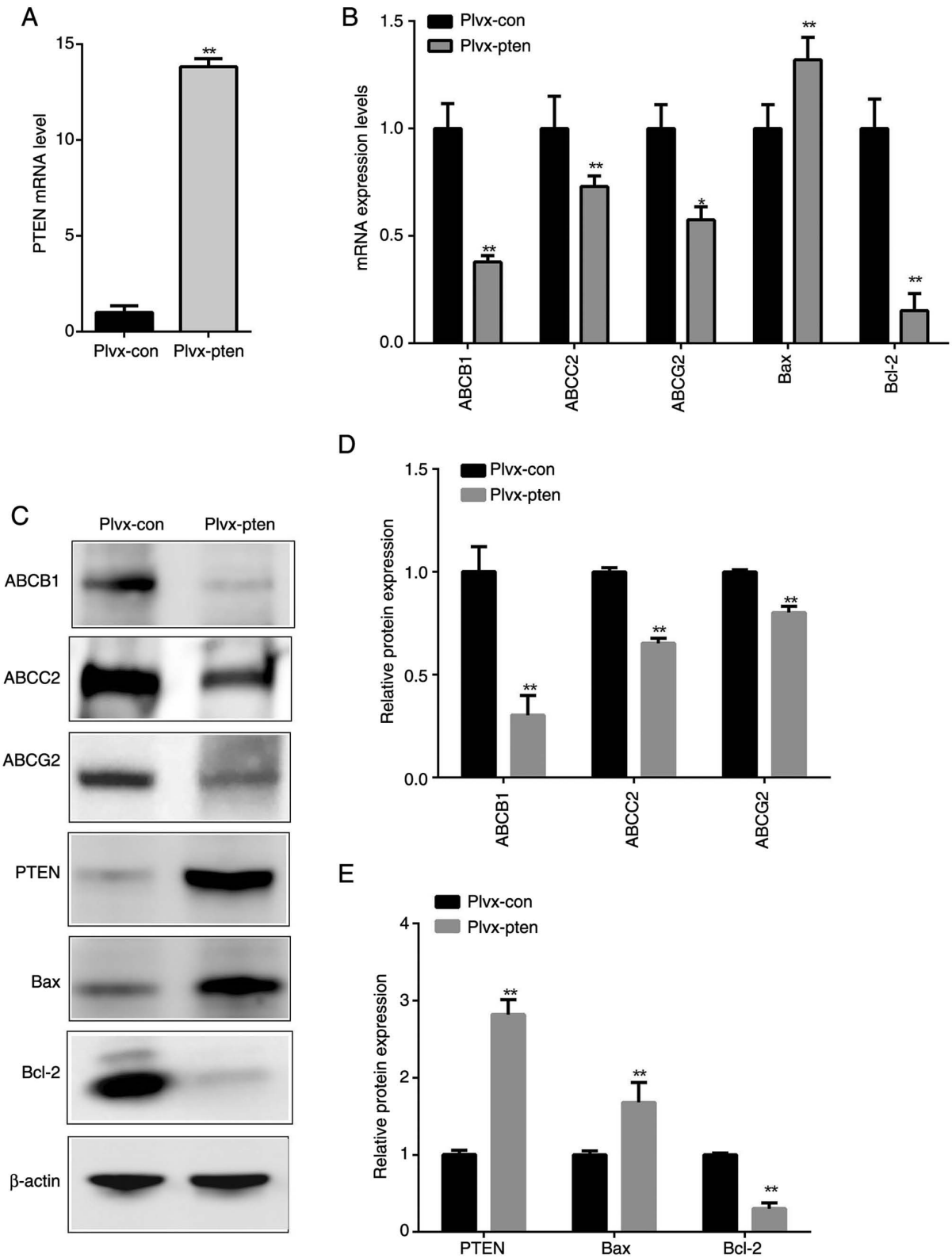

Figure 2. Effects of PTEN overexpression in MCF-7 cells. mRNA expression levels of (A) PTEN, (B) ABCB1, ABCC2, ABCG2, Bax and Bcl-2 following PTEN overexpression. (C) Representative western blotting images and protein expression levels of (D) ABCB1, ABCC2, ABCG2, (E) PTEN, Bax and Bcl-2 following PTEN overexpression. The results are presented as the mean ± SEM of at least three independent experiments and analyzed using one-way ANOVA followed by Bonferroni's post hoc test. *P<0.05, **P<0.01 compared with the Plvx-con group.

Discussion

Despite considerable advances in BC treatment, discovering effective strategies to overcome drug resistance remains important. Modern tyrosine kinase inhibitors such as lapatinib and sorafenib are effective against early-stage BC but are of limited value in advanced stages, often leading to drug resistance and side effects such as neuropathy and bone marrow

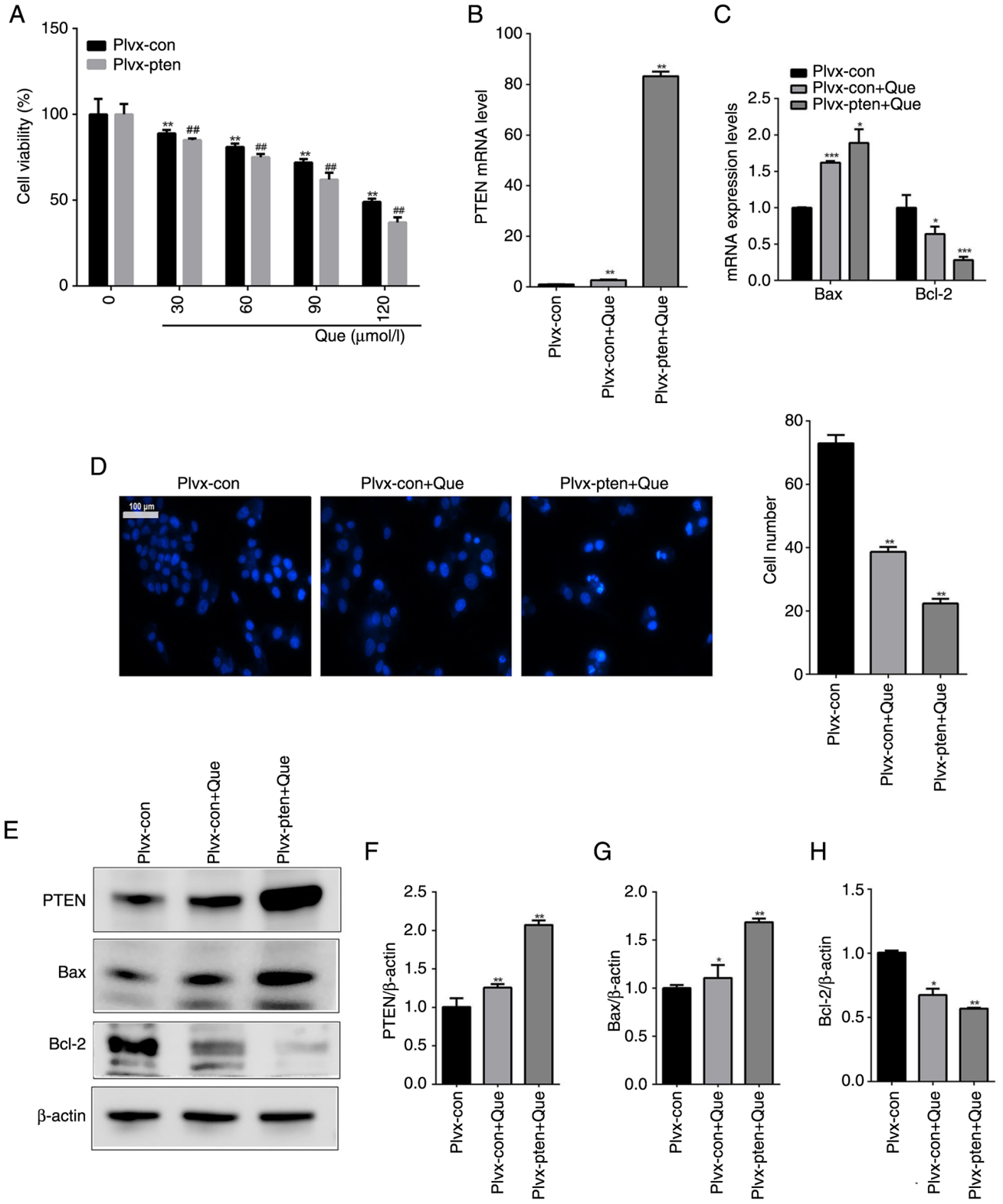

Figure 3. PTEN overexpression enhances the effects of Que on MCF-7 cells. (A) Cell viability following combined PTEN overexpression and Que (60 μM) treatment, as determined by the CCK-8 assay. mRNA expression levels of (B) PTEN, (C) Bax and Bcl-2 in different treatment groups. (D) Cell viability assessed by Hoechst 33342 staining (scale bar, 100 μm). (E) Representative western blotting images and (F) Protein expression levels of PTEN, (G) Bax and (H) Bcl-2 in different treatment groups. Data are presented as the mean ± SEM of at least three independent experiments. Statistical significance was determined by one/two-way ANOVA followed by Bonferroni's post hoc test for multiple comparisons. *P<0.05, **P<0.01, ***P<0.001 compared with the 0 μmol/l Que group, $^{\#\#}$P<0.01 compared with the Plvx-con group. Que, Quercetin; con, control.

suppression (26,27). Therefore, identifying novel therapeutic agents that mitigate side effects and prevent drug resistance is essential.

Que a naturally occurring flavonoid with anti-inflammatory and anti-aging properties, has been shown to induce autophagy and cell death in various types of cancer including myeloma,

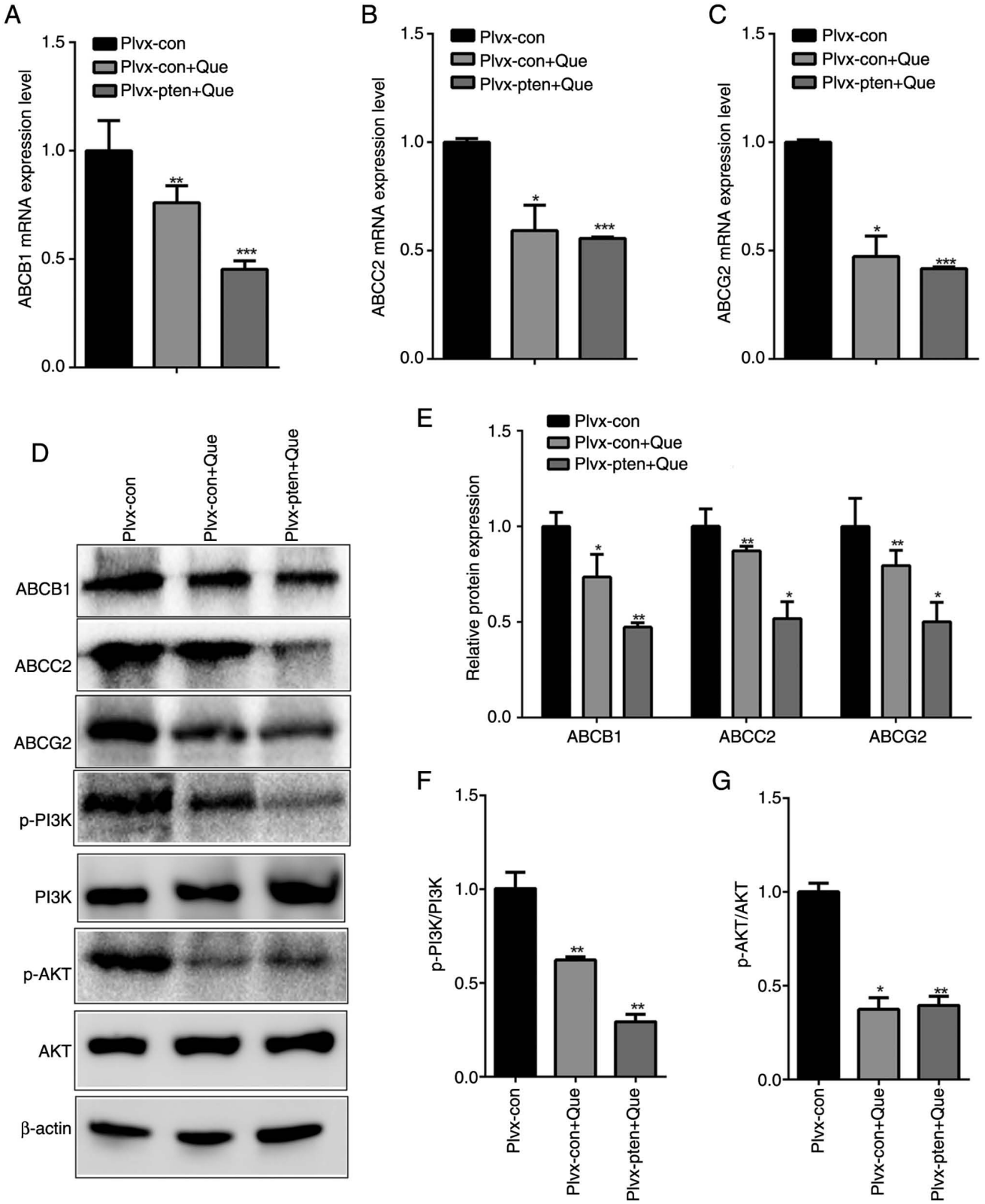

Figure 4. Effects of combined PTEN overexpression and Que treatment on ABC transporters and the PI3K/AKT signaling pathway in MCF-7 cells. mRNA expression levels of (A) ABCB1, (B) ABCC2 (C) and ABCG2 in different treatment groups. (D) Representative images and (E) protein expression levels of ABCB1, ABCC2, ABCG2, (F) p-PI3K, and (G) p-AKT were evaluated by western blotting. The results are presented as the mean ± SEM of at least three independent experiments and analyzed using one-way ANOVA followed by Bonferroni's post hoc test. *P<0.05, **P<0.01, ***P<0.001 compared with the Plvx-con group. Que, Quercetin; con, control; p, phosphorylated.

prostate cancer and lung cancer (28-30). However, its effects on drug-resistant proteins such as ABC transporters in BC cells remain poorly understood. The present study demonstrates that Que enhances drug uptake in MCF-7 cells by targeting ABC transporters through the PTEN/PI3K/AKT axis. Consistent with previous reports (10-13), the present study revealed that

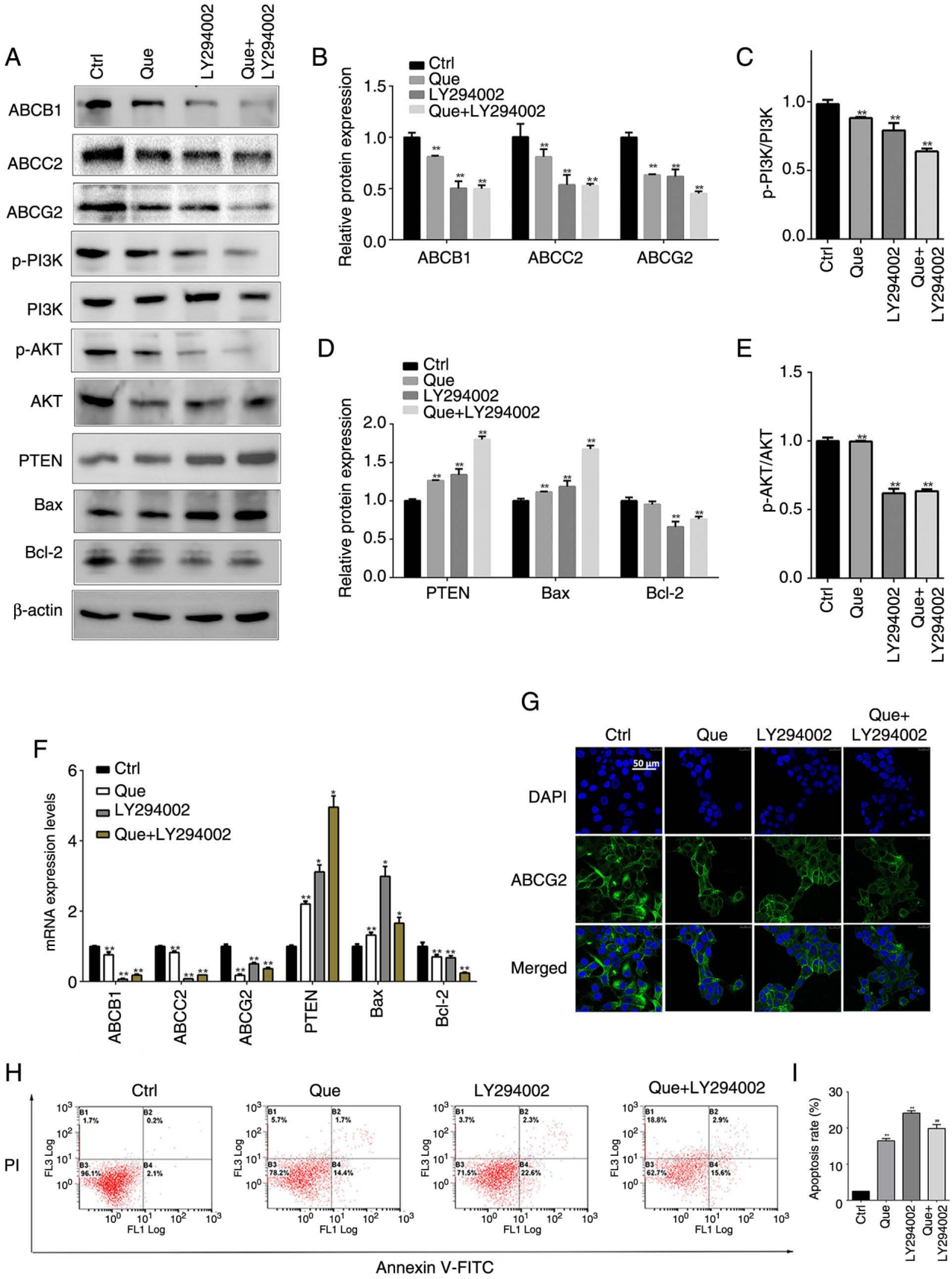

Figure 5. Effects of combined LY294002 and Que treatment on apoptosis and protein expression in MCF-7 cells. (A) Representative western blotting images and Protein expression levels of (B) ABCB1, ABCC2, ABCG2, (C) p-PI3K (D) PTEN, Bax, Bcl-2 and (E) p-AKT in different treatment groups. (F) mRNA expression levels of ABCB1, ABCC2, ABCG2, PTEN, Bax and Bcl-2 in different treatment groups. (G) Representative immunofluorescence images of ABCG2 expression (green). Nuclei were counterstained with DAPI (blue). Scale bar, 50 μm. (H) Representative flow cytometry images and (I) Apoptosis rate was analyzed by flow cytometry after Annexin V-FITC/PI staining. The results are presented as the mean ± SEM of at least three independent experiments and analyzed using two-way ANOVA followed by Bonferroni's post hoc test. *P<0.05, **P<0.01 compared with the control group; $^{\#\#}$P<0.05 compared with the Que group. Que, Quercetin; Ctrl, control; p, phosphorylated.

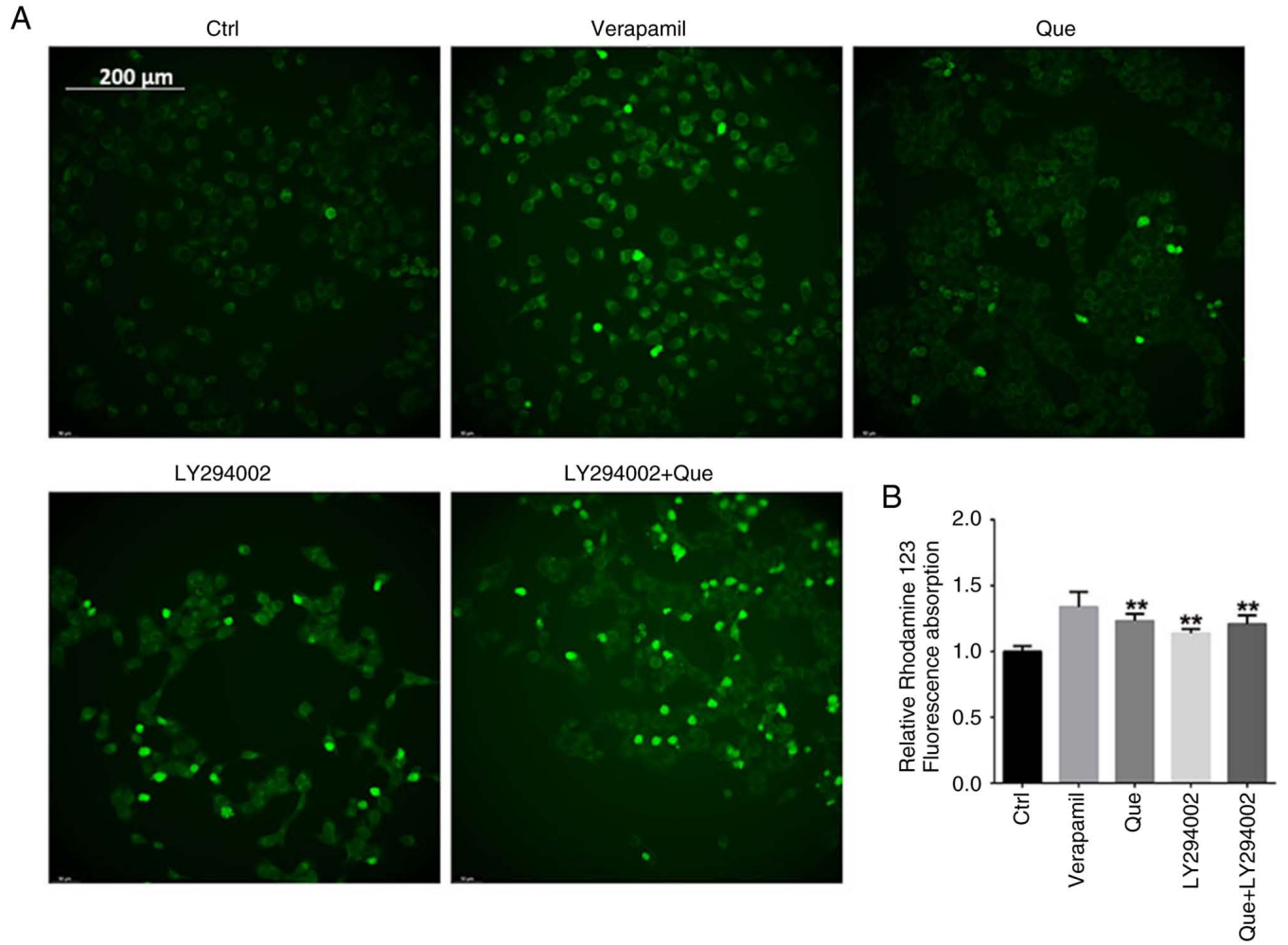

Figure 6. Effects of combined LY294002 and Que treatment on Rhodamine 123 accumulation in MCF-7 cells. (A) Representative fluorescence images of intracellular Rhodamine 123 (green). Scale bar, 200 μm. (B) Quantitative analysis of Rhodamine 123 fluorescence intensity. The results are presented as the mean ± SEM of at least three independent experiments and analyzed using one-way ANOVA followed by Bonferroni's post hoc test. **P<0.01 compared with the control group. Que, Quercetin; Ctrl, control.

Que inhibits cell proliferation and promotes apoptosis. More importantly, the present study identified a previously unreported mechanism: Que downregulates the expression of the key ABC transporters ABCB1, ABCC2 and ABCG2. This effect was functionally corroborated by enhanced intracellular accumulation of Rhodamine 123, a known substrate of these transporters (31), indicating that Que may augment drug retention in cancer cells.

While previous research has primarily focused on its pro-apoptotic role, the present study revealed a dual-acting mechanism. The present study demonstrated, for the first time: that Que not only promotes apoptosis but also concurrently overcomes a key driver of chemoresistance by transcriptionally repressing key ABC transporters. This effect may be mechanistically driven by the restoration of PTEN and subsequent inhibition of the PI3K/AKT pathway, which implicates a previously unreported signaling axis, PTEN/PI3K/AKT/ABC transporters. This repositions Que as a potential double agent that may target both cell survival and drug efflux pathways which may contribute to counteracting MDR.

To further elucidate this axis, the present study investigated the role of PTEN in more detail. As a tumor suppressor that is frequently downregulated in several types of cancer (32,33), PTEN contributes to drug resistance by regulating drug influx, efflux and anti-apoptotic processes (34,35). Results of the present study indicate that Que upregulates PTEN expression while suppressing phosphorylation of PI3K and AKT, suggesting a modulatory role in this pathway. Given that PI3K/AKT inhibition can alleviate chemoresistance (36), these findings position Que as a potential candidate for resistance prevention. This conclusion is further supported by the observation that PTEN overexpression potentiated the effects of Que and that the PI3K inhibitor LY294002 phenocopied these outcomes (37). The synergy between Que and both genetic and pharmacological inhibition of the pathway suggests the PTEN/PI3K/AKT axis as a central target of Que.

The functional outcome of targeting the PTEN/PI3K/AKT axis is the inhibition of ABC transporter activity. ABC transporters, which facilitate drug efflux, are well-established mediators of MDR (20,38). In line with this, the Rhodamine 123 accumulation assay confirmed that Que, either alone or in combination with LY294002, inhibits ABC transporter activity, leading to increased drug retention. Collectively, the results of the present study provide functional data that

Que may enhance chemosensitivity in MCF-7 cells through transcriptional and functional repression of ABC transporters, mediated by the PTEN/PI3K/AKT pathway.

The present study has limitations. The findings of the present study are completely based on *in vitro* experiments using the estrogen receptor-positive MCF-7 cell line. Consequently, the general applicability of this mechanism to other BC subtypes, particularly triple-negative BC, remains to be investigated. Furthermore, the mechanisms identified here need to be validated in more complex systems. A key future direction will be to address the lack of *in vivo* data and to assess essential translation parameters, such as a central role for the PTEN/PI3K/AKT axis in the potential of Que to prevent chemoresistance in BC treatment. Having established this core mechanism, future work will explore the broader signaling landscape and systematically investigate the interplay with other key pathways such as MAPK and Wnt/β-catenin to uncover potential synergistic nodes for overcoming multi-pathway driven resistance.

In conclusion, the present study revealed a novel PTEN/PI3K/AKT/ABC transporter axis as a mechanism through which Que may counteract MDR. By functionally associating the suppression of this pathway to the enhanced intracellular accumulation of chemotherapeutic drugs, the present study provides an explanation for the efficacy of Que. These findings highlight the dual-acting potential of Que, targeting both cell survival and drug efflux pathways, and support its further investigation as a potential adjunctive agent to re-sensitize BC cells to conventional chemotherapy.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

WF contributed to methodology and conceptualization. LL contributed to methodology and investigation. JL contributed to data curation and investigation. FQ contributed to investigation and formal analysis. CC contributed to visualization and investigation. YinCai contributed to investigation and data curation. YilCai contributed to methodology and investigation. YH contributed to methodology. WY contributed to Investigation. SZ contributed to reviewing and editing, writing the original draft, validation, methodology, data curation, conceptualization and project administration.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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