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Original Article

Human bone marrow mesenchymal stem cell-driven LncRNA PTCSC3 upregulation within lung adenocarcinoma cells reduces erlotinib resistance by mitigating Wnt/β-Catenin pathway

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Abstract: lncRNA PTCSC3, which stands for Papillary Thyroid Carcinoma Susceptibility Candidate 3, has been found to play a role in various cellular processes, including cell proliferation, apoptosis, and migration, acting as either an oncogene or a tumor suppressor depending on the context. This study investigates the influence of lncRNA PTCSC3, derived from human bone marrow mesenchymal stem cell (hBMSC), on the efficacy of erlotinib (Er)-resistant lung adenocarcinoma (LUAD) cells and elucidates underlying mechanism. The hBMSCs and LUAD (PC9 and A549) cells were employed to establish an Er-resistant LUAD cell model. It was observed that exposure to hBMSCs reduced the viability of A549-Er and PC9-Er cells and increased their rate of apoptosis. Further investigations revealed that in the presence of hBMSCs-containing medium, PTCSC3 expression was significantly upregulated, concomitantly with a suppression of the Wnt/β-Catenin pathway. Conversely, silencing PTCSC3 led to enhanced A549-Er and PC9-Er activities, reduced cell apoptosis, and activated Wnt/β-Catenin pathway. The effects of PTCSC3 modulation were also examined by transfecting LUAD cells with different PTCSC3 expression vectors and treating them with XAV939, a Wnt/β-Catenin pathway inhibitor, which similarly decreased cell viability. In the rescue experiment, the effect of hBMSCs on LUAD cells could be counteracted by down-regulation of PTCSC3, and the effect of PTCSC3 down-regulation on cells was mitigated by XAV939. This study revealed that hBMSCs promote the up-regulation of PTCSC3 in LUAD cells, thus inhibiting Wnt/β-Catenin pathway and reversing Er resistance, offering a potential novel strategy to enhance the efficacy of chemotherapy in LUAD.

Keywords: Human bone marrow mesenchymal stem cells, lncRNA PTCSC3, Wnt/β-Catenin axis, lung adenocarcinoma, erlotinib-resistance

Introduction

Lung cancer (LC) continues to be a predominant cancer globally, affecting more than 30 million patients worldwide [1]. According to the Global Cancer Statistics 2018, the morbidity and mortality of LC ranks first among malignancies in men, while it ranks third in prevalence and second in mortality after breast cancer among women [2]. In recent years, lung adenocarcinoma (LUAD) has become the most prevalent subtype of LC, accounting for 40-55% of all LC cases [3]. For advanced stages of LUAD, adjuvant chemotherapy is imperative to shrink tumor size [4]. Erlotinib (Er), an epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI), can inhibit EGFR phosphorylation and interrupt downstream survival signals, thus significantly improved survival of LUAD patients with EGFR kinase domain mutations, which establishes itself as a first-line chemotherapy drugs for LUAD [5]. However, the escalation of LUAD cases and the widespread use of Er, have highlighted drug resistance as a critical challenge in chemotherapy, leading to persistent tumor recurrence despite treatment [6, 7]. Therefore, overcoming the drug resistance in tumor cells remain a critical challenge and a focal point of contemporary clinical research.

The tumor microenvironment significantly influences chemotherapy resistance [8]. Among

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them, mesenchymal stem cells (MSCs) modulate tumor proliferation, metastasis and angiogenesis through cytokines secretion, additionally contributing to tissue regeneration [9]. Studies have shown that MSCs can impart stem-like properties to tumor cells and influence the change of their chemoresistance [10]. At present, a number of studies have reported the reversal of drug resistance in tumor cells, including those from LUAD, via MSC interventions [11-13].

Long non-coding RNAs (LncRNAs), which do not translate into proteins, are intimately associated with tumor metabolism, growth and chemoresistance [14]. For example, knockdown of lncRNA DDX11-AS1 inhibits breast cancer cell proliferation, migration and drug resistance [15], whereas lncRNA EIF3J-DT induces chemoresistance of gastric cancer through autophagy activation [16]. Additionally, Yang et al. identified abnormal expression of lncRNA PTCSC3 in a genome-wide screening of LUAD [17]. With the deepening of research, PTCSC3 has been proved to be involved in the development of tumors such as gastric and thyroid cancers [18, 19], yet its role in LUAD and chemoresistance remains unclarified. Furthermore, PTCSC3 has been linked to the differentiation of human adipose stem cells [20]. Thus, we hypothesized that MSCs might affect the Er resistance of LUAD cells through PTCSC3. However, this hypothesis awaits empirical validation.

This investigation analyzes the correlation of PTCSC3 with LUAD and evaluates the potential impact of human bone marrow mesenchymal stem cells (hBMSCs) on LUAD drug resistance through PTCSC3. The findings aim to provide a new reference for future clinical treatment of LUAD and lay a robust foundation for subsequent research.

Materials and methods

Cell culture and maintenance

hBMSCs and LUAD cells (PC9 and A549) were all obtained from the ATCC and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. The culture medium was renewed every three days, and subculturing were performed when cells attained 70-80% confluency.

hBMSCs identification

At passage four, hBMSCs were were quantified and resuspended to a density of 1×10^6/ml. Cell morphology was examined under a microscope. The cell suspension was then seeded in 6-well plates at 2 mL per well, with the culture medium was replaced bi-daily. Trypsin digestion of the cells was performed every 24 hours, and the cell growth curve was plotted after cell counting for 8 days. For phenotyping, 100 μL cell suspension was incubated with fluorochrome-conjugated antibodies: CD29-PE (Cat#303003), CD44-PE (Cat#33815), CD106-PE (Cat#305805), CD34-FITC (Cat#343503) and CD45-FITC (Cat#982316) (10 μL each) for a 30-minute at room temperature. An equal volume of cell suspension without any reagent was set as a control. Then the cells were centrifuged (1000× g, 4°C) for 5 mins and subjected for PBS rinsing. Finally, flow cytometry was performed on a FACSCalibur (Becton Dickinson, Heidelberg, Germany) to quantify the exosome biomarkers including CD29, CD44, CD106, CD34, and CD45.

Establishment of an Er-resistant LUAD cell model

Erlotinib-resistant A549 and PC9 cell models were established with incremental doses ranging from 0.01 μmol/L to 2.00 μmol/ [21]. Cells in logarithmic growth phase were trypsinized, resuspended in complete medium at a concentration of 4.5×10^4 cells/ml, aliquoted into 96-well plates (100 μL per well), and cultured at 37°C in a 5% CO_2 incubator. Post-24-hour incubation, varying concentration of erlotinib solution was introduced (0.01 μmol/L, 0.1 μmol/L, 1.0 μmol/L, and 2 μmol/L) in a final volume of 100 μL (so that the concentration of DMSO was less than 0.1%). The CCK-8 assay was conducted after 48 h of incubation: 10 μL of CCK-8 was added to each well and incubated in an incubator for 1 h. The OD value at 450 nm was measured by an enzyme counter. After model building, Er-resistant cells were routinely cultured for 2 weeks. The resistant variants were named A549-Er and PC9-Er, with phosphate buffer (PBS) treated cells serving as controls.

hBMSCs interaction with resistant cells

A549-Er and PC9-Er cells were cultured for two weeks in the conditioned medium derived from hBMSCs to evaluate the interaction effects.

Table 1. Primer sequences

	F (3'-5')	R (3'-5')
PTCSC3	GTACGGTACCCTCCTTCAGACTTCTCAGTACTC	CGACTCGAGATTGCTACTGTGAGCATAACCTAC
GAPDH*	CTCAGACACCATGGGGAGGTGA	ATGATCTTGAGGCTGTTGTCATA
β-Catenin	TGACGGTCAGTACGCCATGA	GGCATCAAACTGCGTGGAT
c-myc	AGGACACACAACGTCTTGGA	GGGCCTTTTCGTTGTTTTCC
cyclin D1	TGCGTGCAGAGGGAGATTG	AGCGGGAAGACCTCCTCTTC
GAPDH#	AGGGCTGCCTTCTCTTGTGA	AACTTGCCGTGGGTAGAGTCA

Note: GAPDH* is the internal reference of PTCSC3, GAPDH# is the internal reference of β-Catenin, c-myc, and cyclin D1.

CCK-8 assay

After the culture, cells were seeded into a 96-well plate and treated with CCK-8 solution (10 μL). After a 24-hour incubation period, the OD value at the wavelength of 450 nm was recorded by microplate reader to determine cell viability and growth kinetics.

Cell cloning assay

Cells were evenly distributed in a 6-well plate and allowed to grow for 10 days. The culture was stopped when obvious colonies were visible to the naked eyes. Thereafter, visible colonies were fixed with paraformaldehyde, stained with 0.1% crystal violet, and imaged to analyze clonal expansion and to calculate cloning efficiency.

Flow cytometry analysis

Cell apoptosis was detected by using Annexin V-(FITC)/PI Apoptosis Detection Kit (Beyotime) and Annexin V-APC (eBioscience) kit. In Brief, cells were collected and rinsed by PBS. Then, cells were resuspend in binding buffer and then incubated with Annexin V-FITC (15 mins) and later with PI (5 mins). Analysis was conducted using a flow cytometry (Beckman Coulter, Inc.) and the data processing was performed using FlowJo 10.0 software.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from cultured cells using TRIzol, followed by RNA purification and reverse transcription into cDNA for qRT-PCR. qRT-PCR was performed with AQQ SYBR Master Mix kit and custom-designed primers (**Table 1**). GAPDH served as internal reference and the relative gene expression was calculated by $2^{-\Delta\Delta Ct}$.

Northern blotting (NB)

For Northern blotting, RNA was separated on 15% polyacrylamide-urea gels, transferred to positively charged nylon membranes (Millipore), and then cross-linked by UV irradiation. The membranes were hybridized to 100 pmol 3'-digoxin (DIG)-labeled probe overnight at 43°C and detected using the DIG Luminescence Detection Kit (Sigma) following the manufacturer's instructions.

Western blotting (WB)

Total protein was lysed in RIPA buffer on ice, and the mass of total protein was measured by the BCA kit. Following separation by 10% SDS-PAGE, protein samples with an equal amount were transferred onto membranes for incubating with primary antibodies overnight at 4°C. The membranes were washed after secondary antibody incubation. Detection was performed using enhanced chemiluminescence (ECL), for final gray value analysis of the target bands.

Transient transfection procedures

Cells in the logarithmic growth phase were seeded into 6-well plates with 3×10^5 cells/well. At 80% confluence, transfection was carried out with Lipofectamine 3000 according to the manufacturer's protocol. Vectors for overexpression (PTCSC3-pcDNA3.1 vector, named pcDNA3.1 group), targeted silencing (PTCSC3-targeted silencing vector, named PTCSC3-si group) and control (the corresponding empty vector, named PTCSC3-NC group) were transfected into cells, and transfection efficiency was confirmed by PCR. PTCSC3 siRNA-1 (si-PTCSC3-1): 5'-GCACCACCTTCATTATCCAAAGTTA-3'; siRNA-2 (si-PTCSC3-2): 5'-CACCACCTTCATTATCCAAAGTTAT-3'.

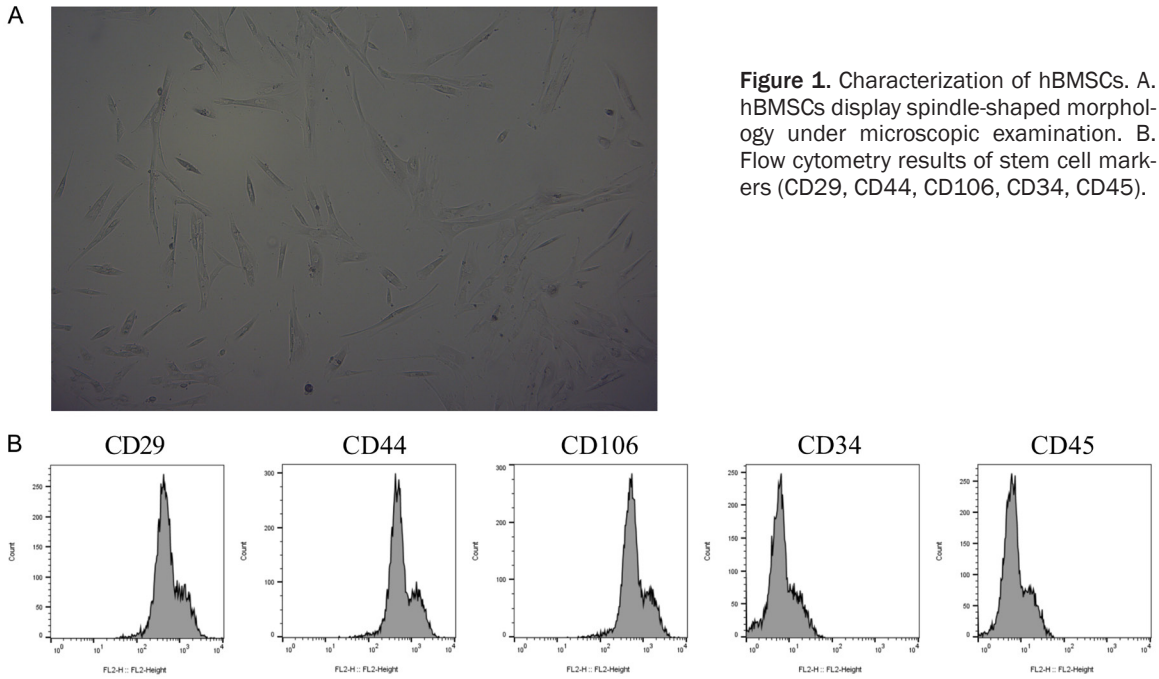

Figure 1. Characterization of hBMSCs. A. hBMSCs display spindle-shaped morphology under microscopic examination. B. Flow cytometry results of stem cell markers (CD29, CD44, CD106, CD34, CD45).

Modulation of Wnt/β-Catenin pathway

To investigate whether ER resistance in LUCA cells is mediated through the Wnt/β-Catenin pathway, we employed the Wnt/β-Catenin inhibitor XAV939, sourced from Sigma Aldrich. A549-Er and PC9-Er cells were divided into inhibition group and blank group. Cells were treated with XAV939 (10 μmol/L) in inhibition group while the equivalent volume of culture medium were added in blank group. Both sets of cells were cultivated in 6-well plates and allowed to reach 60% confluence over 24 hours in preparation for subsequent analyses.

Statistics and methods

Results were expressed as mean ± standard deviation ($\bar{x}\pm s$) and subjected to statistical evaluation by SPSS22.0 software after three repeated measurements of each test. Intergroup differences were assessed using independent samples t-test, while multiple group comparisons employed one-way analysis of variance (ANOVA) followed by a Bonferroni post-hoc test. A *P* values of <0.05 was considered to indicate statistical significance.

Results

hBMSCs identification

Microscopic examination revealed hBMSCs to be spindle-shaped. These cells entered the logarithmic growth phase after 3 days in culture and progressed to plateau and declining phases after 6 days and 7 days, respectively. Flow cytometry analysis showed confirmed successful hBMSC cultivation, showing positive markers for CD29, CD44 and CD106, while CD34 and CD45 were negative (**Figure 1**).

Effect of hBMSCs on drug-resistant cells

The CCK-8 assay revealed that both A549-Er and PC9-Er cells exhibited significantly reduced growth after 24-72 h cultivation compared to their non-resistant counterparts ($P<0.05$), with no significant differences observed from 96 h onwards ($P>0.05$). This confirms successful establishment of erlotinib resistance. Co-cultivation with hBMSCs led to a significant reduction in proliferation and clonogenicity ($P<0.05$), and an increase in apoptosis in both A549-Er and PC9-Er cells compared to culture alone, suggesting an inhibitory effect of hBMSCs on the proliferation of Er-resistance LUAD cells and an enhancement of apoptosis (**Figure 2**).

Effect of hBMSCs on PTCSC3 and Wnt/β-Catenin pathway in drug-resistant cells

In order to investigate whether co-culture with hBMSCs can regulate the expression of PTCSC3 in A549-Er cells, we performed PCR to determine the expression level of PTCSC3. The results indicated that PTCSC3 expression in hBMSCs-A549-Er was (3.83±0.21) higher than

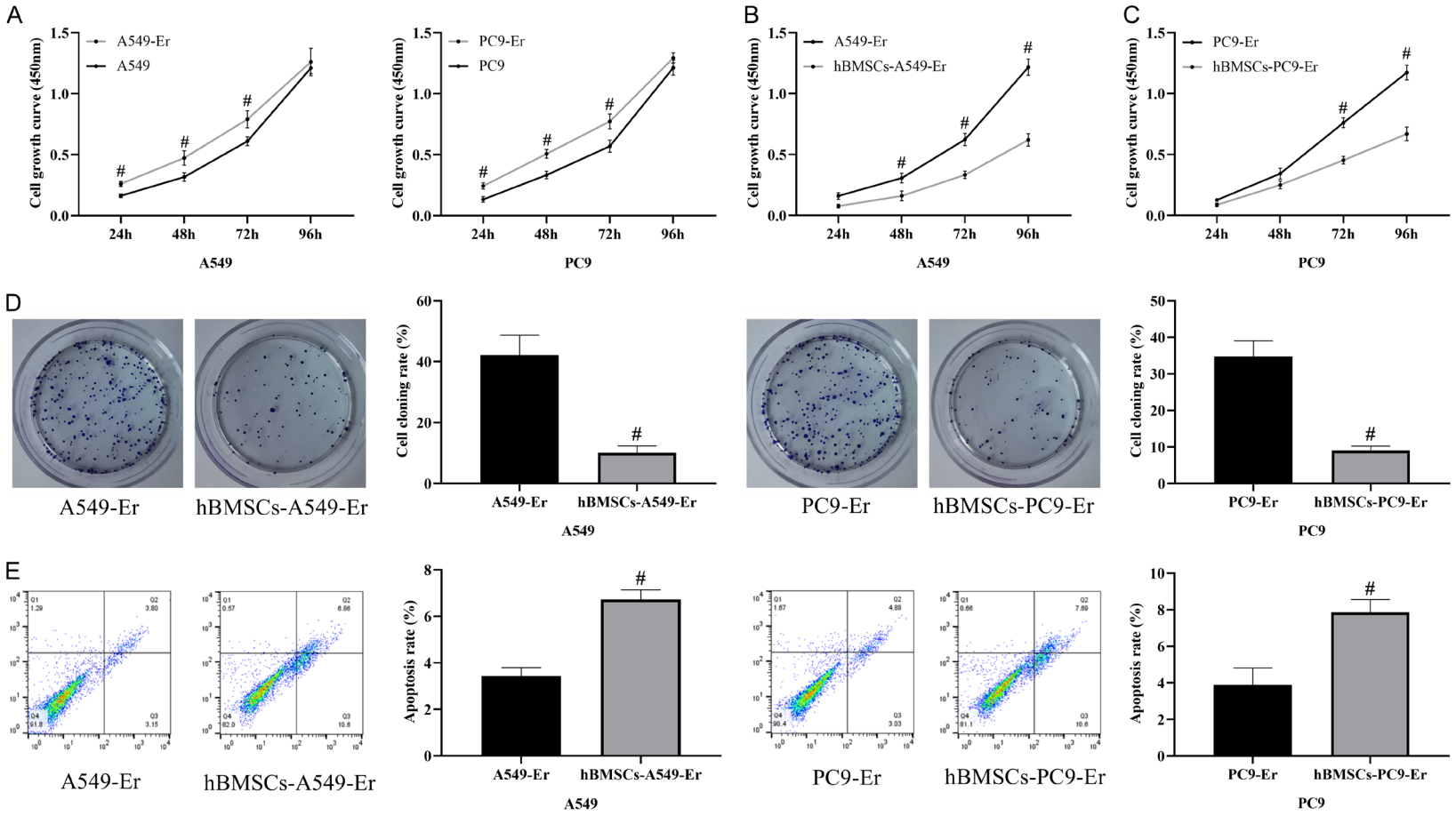

Figure 2. Evaluating the impacts of hBMSCs on drug-resistant cells. A. CCK-8 assay indicated the viability of Er-resistant LUAD cells at 450 nm. # represents P<0.05 compared to resistant cells. B. CCK-8 assay showed the influence of hBMSCs on A549-Er cell proliferation at 450 nm. C. CCK-8 assay indicated the influence of hBMSCs on PC9-Er proliferation at 450 nm. D. Cell cloning assay measured the impact of hBMSCs on the clonogenic capacity of A549-Er and PC9-Er. E. Flow cytometry quantified the effect of hBMSCs on A549-Er and PC9-Er apoptosis. # represents P<0.05 compared to resistant cells after hBMSCs intervention (P<0.05).

that in A549-Er cells (P<0.05). Similarly, PTCSC3 expression was higher in hBMSCs-PC9-Er (3.57±0.31) than in PC9-Er (P<0.05). Furthermore, the impact of hBMSCs on Wnt/β-Catenin pathway was assessed by examining the gene expression levels associated with the pathway. Reduced mRNA levels of β-catenin, c-myc, and cyclin D1 were determined in both hBMSCs-A549-Er and hBMSCs-PC9-Er cells. Western blot assays corroborated these findings, showing decreased protein expression of the same markers, suggesting that hBMSCs co-culture can suppress the Wnt/β-Catenin pathway (**Figure 3**).

Influences of PTCSC3 on drug-resistant cells

To investigate the role of PTCSC3 on drug-resistant cells, we transfected the cells with vectors to either knockdown or overexpress PTCSC3. The results showed that post-transfection, a significant modulation of PTCSC3 mRNA was observed in both A549-Er and PC9-Er cells (**Figure 4A**), which was supported by western blot analyses confirming siRNA transfection efficiency (**Figure 4B**). These results suggested that PTCSC3 silencing cell model were established. Subsequent experiments utilized si-PTCSC3-1, the more efficient siRNA, referred to as PTCSC3-si. The CCK-8 assay demonstrated that the pcDNA3.1 group had reduced proliferation capacity and the lowest clonogenic rates compared to the PTCSC3-si and PTCSC3-NC groups (**Figure 4C**, P<0.05). Conversely, the PTCSC3-si group exhibited higher proliferation and clonogenic rates than the PTCSC3-NC group (**Figure 4D**, P<0.05). The pcDNA3.1 group also exhibited the highest levels of apoptosis, while the PTCSC3-si group showed the lowest (P<0.05), suggesting that upregulating PTCSC3 inhibits growth and promotes apoptosis in LUCA drug-resistance cells, whereas silencing PTCSC3 contributed to the opposite results (**Figure 4E**).

Impacts of PTCSC3 on Wnt/β-Catenin pathway

To investigate whether PTCSC3 modulates drug-resistant cells by mediating the Wnt/β-Catenin pathway, we performed a series of experiments. PCR and WB analysis showed that overexpression of PTCSC3 (pcDNA3.1) significantly decreased the expression of β-catenin, c-myc and cyclin D1, while knockdown of PTCSC3 (PTCSC3-si) significantly increased the expression of these markers, compared with the control group (PTCSC3-NC) in both A549 and PC9 cells. The results indicated that PTCSC3 may exert its effects on LUCA cells by mediating the Wnt/β-Catenin axis (**Figure 5**).

Wnt/β-Catenin pathway's role on drug-resistant cells

To discern the role of the Wnt/β-Catenin pathway in drug-resistant cells, A549-Er and PC9-Er cells were subjected to a 24-hour treatment with XAV939 at a concentration of 10 μmol/L. Subsequent analyses of cell proliferation and clonogenic capabilities indicated that XAV939 intervention significantly curtailed both measures compared to the control group. Furthermore, a notable increase in apoptosis rates was observed (P<0.05). These findings suggesting that inhibiting Wnt/β-Catenin pathway can promote LUCA drug-resistant cell apoptosis (**Figure 6**).

hBMSCs reverses Er resistance of LUCA cells via PTCSC3/Wnt/β-Catenin pathway

The findings indicate that hBMSCs potentially suppress proliferation and promote apoptosis in erlotinib-resistant LUAD cells. To further investigate the role of PTCSC3 and Wnt/β-Catenin pathway, we employed hBMSCs with a PTCSC3-silencing vector (PTCSC3-si) with the Wnt/β-Catenin pathway inhibitor XAV939 in A549-Er and PC9-Er cells. Meanwhile, A549-Er and PC9-Er cells without any treatments as the control group. Clonogenic and apoptosis assays revealed no significant differences across the treatments (P>0.05). These results suggested the proliferation induced by PTCSC3 silencing can be mitigated by either co-culture with hBMSCs or the addition of XAV939 (**Figure 7**).

Discussion

The development of tumor cell drug resistance is a multifaceted process influenced by factors such as epigenetics, genetic disorders, signal transduction and cell metabolism. Recent research has highlighted the pivotal role of MSCs on drug resistance of tumor cells, demonstrating that MSCs revolutionized the treatment of neoplastic diseases in the future [22, 23]. hBMSCs, a subgroup of bone marrow stroma cells, have diverse differentiation potentials including forming bone, cartilage, fat, nerve and myoblasts [24]. They play a critical role in

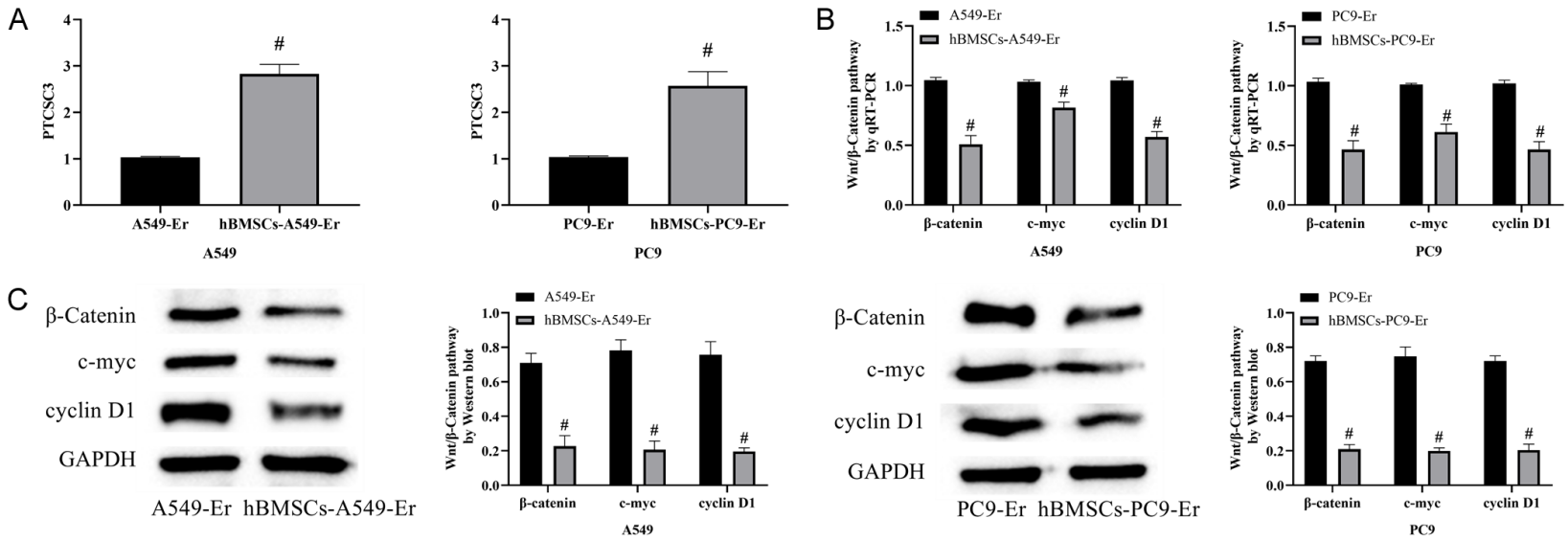

Figure 3. Impacts of hBMSCs on PTCSC3 expression and Wnt/β-Catenin pathway in drug-resistant cells. A. Quantitative RT-PCR assessment of PTCSC3 mRNA expression. B. Evaluation of mRNA levels for Wnt/β-Catenin pathway-associated markers via qRT-PCR. C. Analysis of Wnt/β-Catenin signaling pathway-associated protein expression levels by Western blot. # represents $P<0.05$ compared to resistant cells.

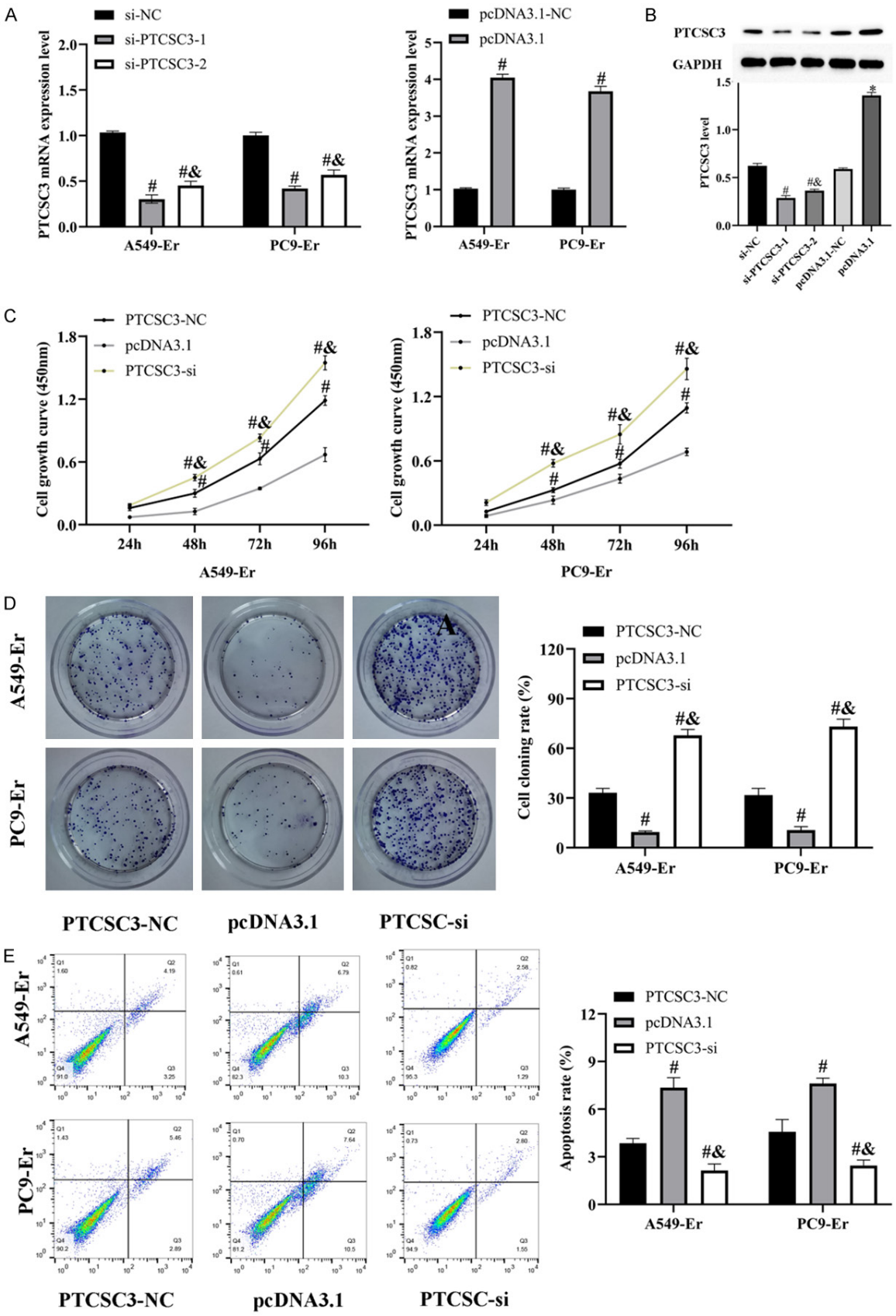
A
si-NC
si-PTCSC3-1
si-PTCSC3-2
PTCSC3 mRNA expression level
A549-Er
PC9-Er
pcDNA3.1-NC
pcDNA3.1
B
PTCSC3
GAPDH
PTCSC3 level
si-NC
si-PTCSC3-1
si-PTCSC3-2
pcDNA3.1-NC
pcDNA3.1
C
PTCSC3-NC
pcDNA3.1
PTCSC3-si
Cell growth curve (450nm)
24h
48h
72h
96h
D
A549-Er
PC9-Er
PTCSC3-NC
pcDNA3.1
PTCSC-si
Cell cloning rate (%)
E
Apoptosis rate (%)

Figure 4. Effect of PTCSC3 on drug-resistant cells. A. qRT-PCR evaluation of PTSCS3 mRNA to verify the efficiency of transfection. B. Protein expression level of PTSCS3 verified by WB. C. LUCA cell growth curve determined by CCK-8 assay at 450 nm. D. LUCA cell cloning rate accessed via clone formation. E. Analysis of LUCA cell apoptosis. # represents P<0.05 compared to PTCSC3-NC group, & represents P<0.05 compared to pcNDA3.1 group (P<0.05).

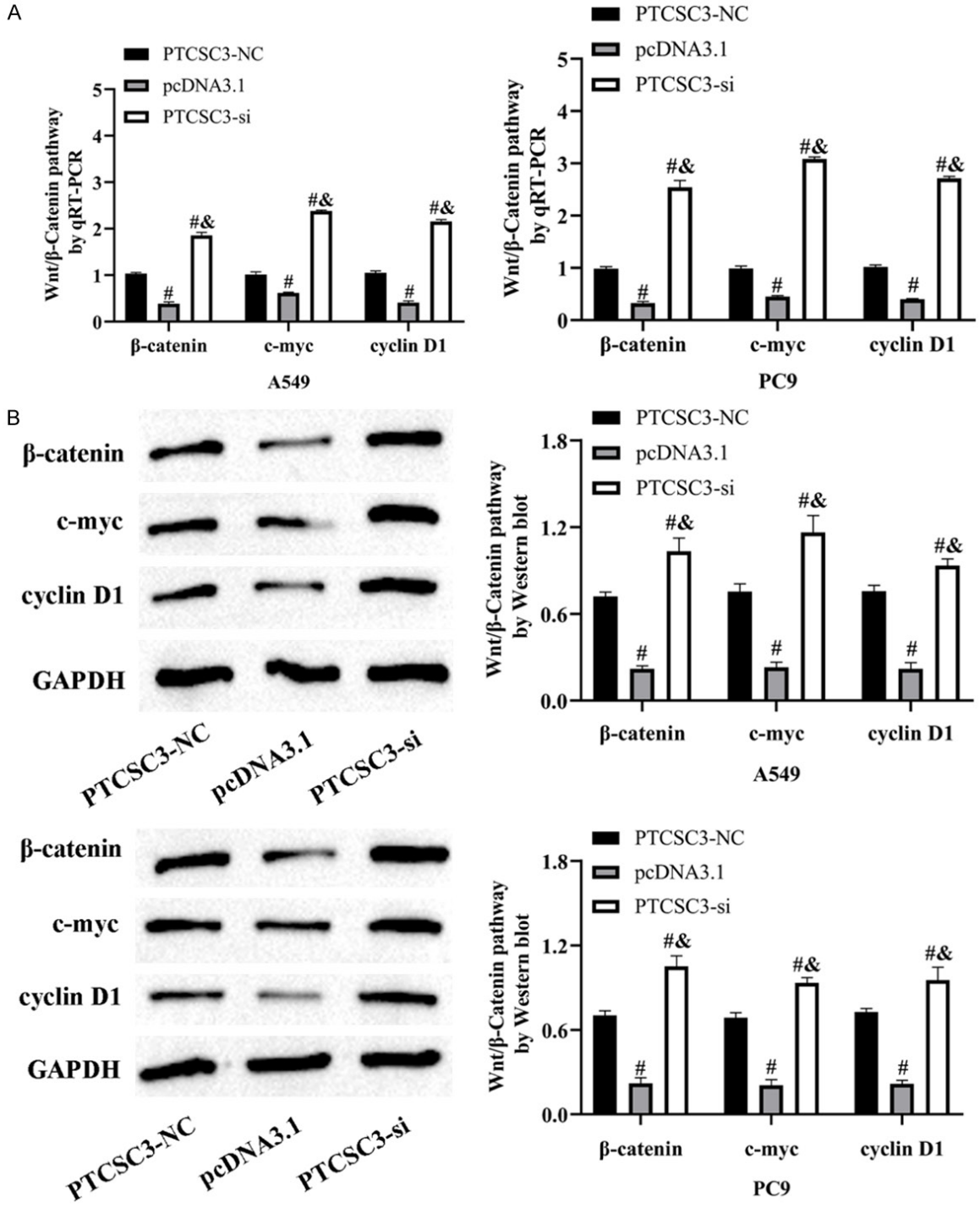

Figure 5. The Role of PTCSC3 on Wnt/β-Catenin pathway. A. Assessment of Wnt/β-Catenin signaling pathway related mRNA via qRT-PCR. B. Analysis of Wnt/β-Catenin signaling pathway protein levels through Western blot. # represents P<0.05 compared to PTCSC3-NC group (P<0.05), & represents P<0.05 compared to pcNDA3.1 group (P<0.05).

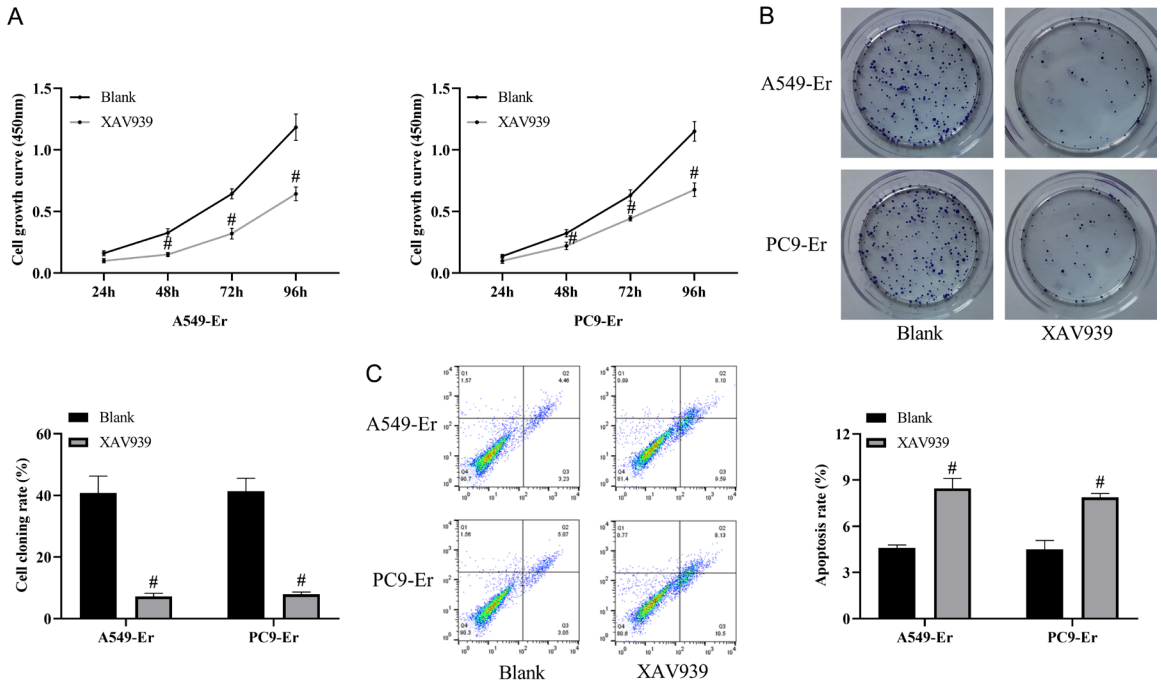

Figure 6. Influence of Wnt/β-Catenin pathway on drug-resistant cells. A. Growth curve of LUCA cells. B. Clonogenic potential of LUCA cells. C. Apoptotic rates of LUCA cells. # represents compared to blank group (P<0.05).

providing mechanical support to hematopoietic stem cells (HSCs) in bone marrow and secreting growth factors (IL-6, IL-11, LIF, etc.) that support hematopoiesis [25]. hBMSCs have been widely used in tissue engineering, cell therapy and gene therapy because of their robust proliferative and immunomodulatory properties [26, 27]. Notably, they have also been proved to exert promising intervention effects on drug resistance across various tumor types, including diffuse large B-cell lymphoma and glioblastoma [28, 29]. Therefore, this study undertakes a preliminary analysis of LUCA drug-resistant cells utilizing hBMSCs.

We began by confirming the phenotype of hBMSCs, noting positive expression of CD29, CD44 and CD106, and negative expression of CD34 and CD45. This is consistent with previous studies that hBMSCs have stem cell characterization, stable morphology, and cell vitality. In addition, CCK-8 assay revealed that the Er drug-resistant cell model was successfully established by the gradient induction method. However, the cell growth rate was obviously inhibited after hBMSCs culture, suggesting that the use of hBMSCs can ameliorate the drug resistance of LUAD cells to Er and enhance the cytotoxic impact. Prior work has shown hBMSCs reversing cisplatin resistance in non-small cell lung cancer cells [30], which can also testify the accuracy of our research results.

However, the exact mechanism by which hBMSCs influence Er resistance is currently unclear. We proposed the involvement of PTCSC3 and found its increased expression in hBMSCs-cultured A549-Er cells. In contrast to earlier findings by Yang et al. and other groups which reported PTCSC3 was under-expressed in tumor diseases [17, 31-33], our results suggested that the effect of hBMSCs on LUAD drug-resistant cells was related to PTCSC3. Although the role of PTCSC3 in other cancers remains largely unknown, our study shows that modifying PTCSC3 expression impacts A549-Er and PC9-Er cell growth and apoptosis. Up-regulation of PTCSC3 expression correlates with reduced cell growth and increased apoptosis, a pattern was reversed by PTCSC3 silencing. The results were consistent with the abovementioned situation after the intervention of hBMSCs, indicating that hBMSCs did exert an anti-tumor role via increasing PTCSC3 expression. Additionally, previous studies reported that up-regulating PTCSC3 can accelerate the apoptosis of breast cancer and cervical cancer cells [34, 35] and PTCSC3 overexpression trig-

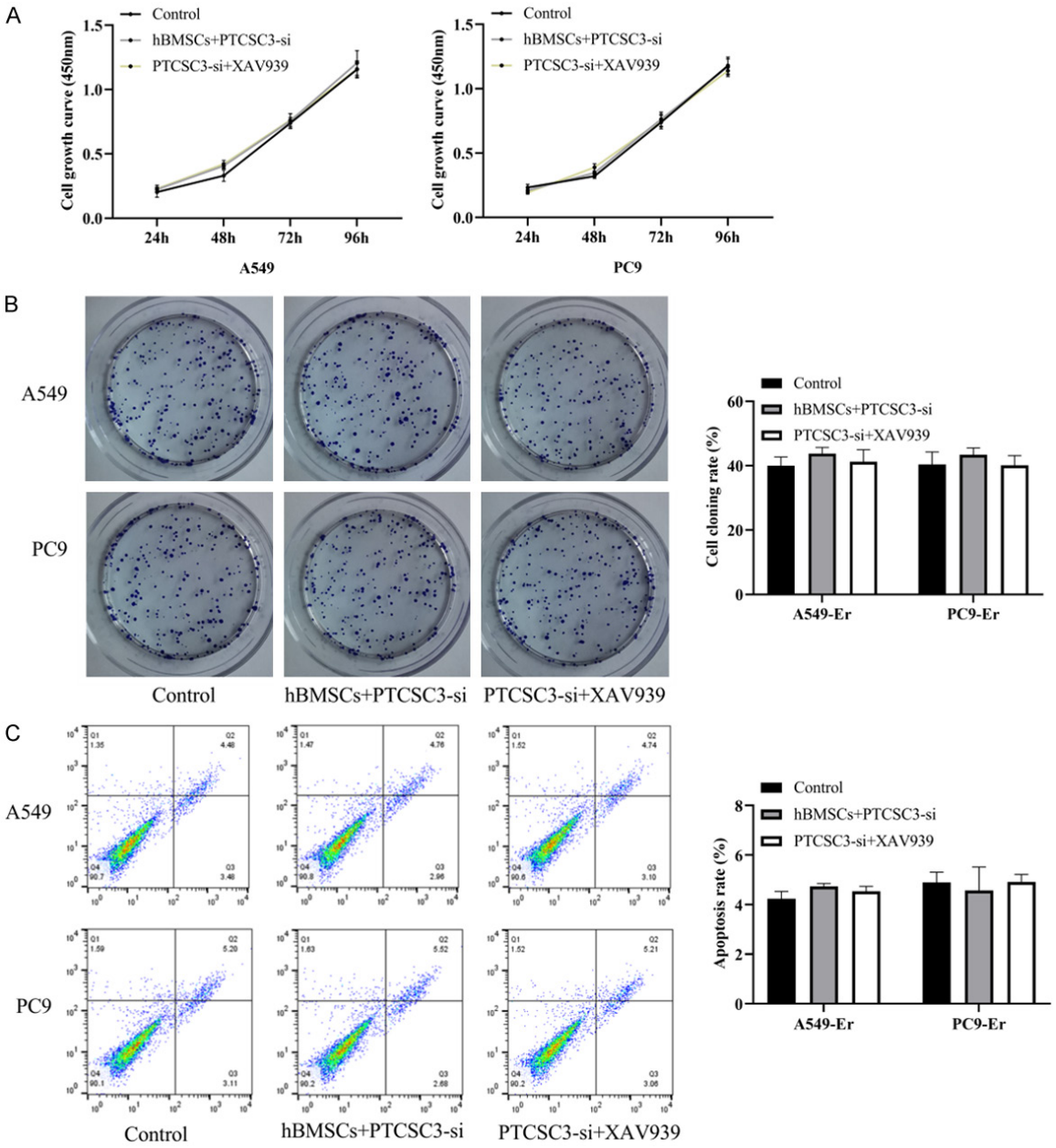

Figure 7. hBMSCs reverses Er resistance of LUCA cells through PTCSC3/Wnt/β-Catenin Pathway. A. Growth curve of LUCA cells. B. The clonogenic potential of LUCA cells. C. Apoptotic rates LUCA cells.

gered both apoptosis and autophagy in human oral cancer cells [32], further corroborating our findings.

The pathway through which PTCSC3 influences tumor cells is indicated to be closely linked to the Wnt/β-Catenin signaling [36]. This association has been recently observed in glioblastoma, where PTCSC3 inhibits the Wnt/β-Catenin signaling cascade to suppress the growth and invasion of glioblastoma [37]. Given the established role of Wnt/β-Catenin signalling in tumor, including verified influence on LUCA [38, 39], PTCSC3 may exert its effects on LUCA cell activity through this pathway. Our analysis indicates that the Wnt/β-Catenin pathway expression in hBMSCs-A549-Er and hBMSCs-A549-Er was obviously inhibited by hBMSCs. PTCSC3 upregulation expression inversely related to Wnt/β-Catenin expression. Consistently, previous studies have shown that Wnt/β-Catenin, which is activated in LUAD, can promote tumor

growth, invasion and metastasis [40, 41], reinforcing the hypothesis that hBMSCs influence LUCA via Wnt/β-Catenin. The application of the pathway inhibitor XAV939 further supports this link, showing decreased LUCA activity and increased apoptosis.

In the final rescue experiment, co-treatment LUAD cells with hBMSCs + PTCSC3 silencing expression vector, as well as PTCSC3 silencing expression vector + XAV939, revealed that the cell proliferation, cloning and apoptosis were completely consistent with control group observations. This suggests that the effect of hBMSCs on LUCA cells was reversed by PTCSC3 down-regulation, and conversely, the influence of PTCSC3 down-regulation on cells could be mitigated by XAV939, confirming our initial hypothesis that hBMSCs counteract Er resistance in LUCA cells through the PTCSC3/Wnt/β-Catenin pathway.

However, this study has limitations that require addressing, such as the need for *in vivo* experiments validations and assessments of LUCA cells biological behavior via scratch assays. The underlying mechanisms of hBMSCs' influence are yet to be fully elucidated, as other transcription factors or changes in cell activities might contribute to the modulation of PTCSC3 or Wnt/β-Catenin pathway. Future research will aim to fill these gaps and also explore the impact of hBMSC-derived exosomes on LUAD cells to solidify findings for clinical application.

Conclusion

This study revealed a novel role for hBMSCs in inducing the up-regulation of PTCSC3 within LUCA cells, offering a potential mechanism for reversing erlotinib resistance by targeting the Wnt/β-Catenin pathway. This discovery opens up promising avenues to enhance chemotherapy efficacy for LUCA, potentially representing a significant advancement in therapeutic strategies.

Disclosure of conflict of interest

None.

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